National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

UNIVERSITY OF ALBERTA

ADRENERGIC REGULATION OF THERMOGENESIS DURING FEEDING AND THERMAL EXPOSURE IN SHEEP AND CATTLE: THE MODULATORY ROLE OF α_2-, β_1- AND β_2-ADRENOCEPTORS

BY

JACOB OLONGIDA OLE MIARON

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY**.

IN

ANIMAL PHYSIOLOGY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1994

ISBN 0-315-95243-1

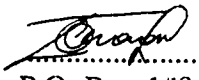
UNIVERSITY OF ALBERTA
RELEASE FORM

NAME OF AUTHOR: **Jacob Olongida Ole Miaron**

TITLE OF THESIS: **Adrenergic Regulation of Thermogenesis During Feeding and Thermal Exposure in Sheep and Cattle: The Modulatory Role of α_2-, β_1- and β_2-Adrenoceptors**

DEGREE: **Doctor of Philosophy**

YEAR THIS DEGREE GRANTED: **FALL 1994**



P.O. Box 158,
Loitokitok, Kenya.

DATE 30/6/94

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the faculty of graduate studies and research for acceptance, a thesis entitled **ADRENERGIC REGULATION OF THERMOGENESIS DURING FEEDING AND THERMAL EXPOSURE IN SHEEP AND CATTLE: THE MODULATORY ROLE OF α_2-, β_1- AND β_2-ADRENOCEPTORS** submitted by **JACOB OLONGIDA OLE MIARON** in **ANIMAL PHYSIOLOGY.**

R.J. CHRISTOPHERSON
Supervisor

V.B. BARACOS
Committee Member

R.J. HUDSON
Committee Member

W. C. SAUER
Committee Member

L.C. WANG
Committee Member

A.D. Kennedy
External Examiner

Date 28/6/94

ABSTRACT

A series of experiments was conducted to study the adrenergic regulation of thermogenesis in sheep and cattle. Propranolol, a nonselective ß-blocker, metoprolol, a $ß_1$-blocker and ICI 118551, a $ß_2$-blocker were used to investigate the β-adrenoceptor mediated whole body and organ oxygen consumption ($\dot{V}O_2$) in sheep during adrenaline infusion, acute cold exposure and feeding. The α_2-adrenoceptor mediated thermogenic effect was examined in steers kept at either -9°C, 11°C or 28°C and fed restricted diet and in animals acclimated to -19°C and 22°C and fed ad libitum, as well as in fasted and fed sheep.

In sheep, adrenaline increased (P<0.05) whole body and portal drained viscera $\dot{V}O_2$ by 30 and 40% respectively. The adrenaline-induced increase was reduced (P<0.05) by the nonselective, β_1- and β_2-blockers respectively. However, in the portal drained viscera, the $ß_2$- but not the $ß_1$-blocker effects were significant. The adrenaline-induced increase in the hindquarter $\dot{V}O_2$ was nonsignificantly decreased by the ß-blockers. Acute cold exposure increased (P<0.05) whole body and hindquarters $\dot{V}O_2$ by about 60 % but, not that of the portal drained viscera. Feeding induced a 41 % elevation (P<0.05) in whole body and a non-significant increase in the portal drained viscera $\dot{V}O_2$. The ß-adrenoceptor blockers suppressed the increase in whole body $\dot{V}O_2$ associated with acute cold exposure but, only the reduction induced by the $ß_2$-adrenoceptor antagonist (ICI 118551) was significant (P=0.02) during the 2ndhour of infusion. The response to feeding was not altered by the ß-blockers.

Guanfacin (80 µg/kg body weight) an α_2-adrenoceptor agonist, when compared to control (vehicle) reduced (P<0.05) the heat production in steers kept in the -9°C and 11°C thermal environments by 23 and 20 % respectively. In steers at 28°C environment the reduction (P<0.1) in heat production was only 8 %. The rectal temperature was lower (P<0.05) at -9°C, unchanged at 11 but elevated (P<0.05) at 28°C in response to guanfacin. In steers fed ad libitum and exposed to -19°C and 20°C, guanfacin reduced (P<0.05) the heat production by 12%. Guanfacin (0.8, 1.6 and 2.4 mg) when compared to vehicle, reduced (P<0.05) the heat production of fasted sheep and doses of 1.6 and 2.4 mg lowered (P<0.05) the heat production of the fed

sheep.

The adrenaline infusion with and without the nonselective and selective ß-blockers demonstrates that whole body, portal drained viscera and hindquarter metabolic rate are modified by the ß-adrenergic system. However, during acute cold exposure, the metabolic rate of the whole body but not that of the portal drained viscera is modulated by ß-adrenoceptors. On the other hand, the α_2-adrenoceptor stimulation by guanfacin suppresses the heat production of steers in the -9°C and 11°C but not 28°C thermal environments. The elevated rectal temperature at 28°C may have prevented a significant decrease in heat production due to guanfacin. Guanfacin was also antithermogenic in steers fed ad libitum as well as in fasted and fed sheep. However, in the later, guanfacin had no effect on the heat increment of feeding. These studies suggest that, the stimulation of α_2-adrenoceptors by guanfacin and the blockade of whole body and portal drained viscera $ß_1$- and $ß_2$-adrenoceptors by propranolol, metoprolol and ICI 118551 results in reduced heat production which could be involved in energy conservation in ruminants. Consequently, cattle and sheep have the potential to improve energy conservation either by activating α_2-adrenoceptor-mediated processes or by suppressing the $ß_1$-, and $ß_2$-adrenergic pathways in response to thermal and nutritional stimuli.

ACKNOWLEDGMENTS

This study was partly supported by the Natural Sciences and Engineering Research Council (NSERC), Canada, the Farming for the Future program of the Alberta Agricultural Research Institute, and the Norwegian Agency for Overseas Development (NORAD). Guanfacin was generously donated by Sandoz Canada, Inc., Dorval, Quebec and ICI 118551 was donated by ICI Pharmaceutical (Wilmington, DE and Macclesfied, England).

I wish to thank the staff of the Laird McEllroy Environmental and Metabolic Centre of the Department of Animal Science for their excellent help and management of the animals used in this study. I am particularly grateful to Sarah Cosgrove for her assistance with animal handling, sampling and sample preparation. The assistance of Dr. R.T. Hardin and Mr. Ray Weingardt in the statistical analysis of the data is highly appreciated. I thank Lori Mathison and Margita Fergel (Dr. Christopherson's summer students) for their assistance in sample analysis.

Finally I would also like to thank my supervisor Dr. R.J. Christopherson for his support throughout the entire study and for editing, constructive and critical reading of the manuscript.

Table of Contents

List of Tables

List of Figures

1. INTRODUCTION

In accordance with the first law of thermodynamics, energy consumed as food by an animal must balance that lost as heat if body weight and energy balance are to be maintained. When the energy intake exceeds output then weight gain/growth ensues, however, if energy ingested is less then output, then body energy stores are mobilized. Therefore, external events such as thermal and nutritional stress influence the animal's energy balance. Thermal exposure, especially cold but not long-term heat exposure, induces an increase in heat production. This thermic effect of cold exposure is usually associated with an increased energy requirement to maintain thermostability. The latter could incur high nutritional costs resulting partly from increased food intake necessitated by reduced digestive efficiency coupled with a proportionately higher energy loss as heat. The thermic effect of feeding is also associated with increased energy dissipation as the "heat increment of feeding."

To improve efficiency of energy utilization in livestock production systems, there is considerable interest in identifying new techniques, based on physiological principles, that will allow for the manipulation of thermogenesis at rest, as well as the thermic effects of thermal exposure and feeding in favour of reduced maintenance energy costs. This approach may provide a potential means of improving energy balance in animals (Hunter et al. 1993). The regulatory mechanisms underlying resting metabolism and the thermic effects of temperature exposure and feeding may be investigated at the whole body, organ, cellular or molecular levels (Figure 1.1). Studies at the organ and whole body level are particularly important for assessing quantitative

responses to endocrine, pharmacological or other management treatments and, therefore, provide an indication of the scope for potential impact on production efficiency.

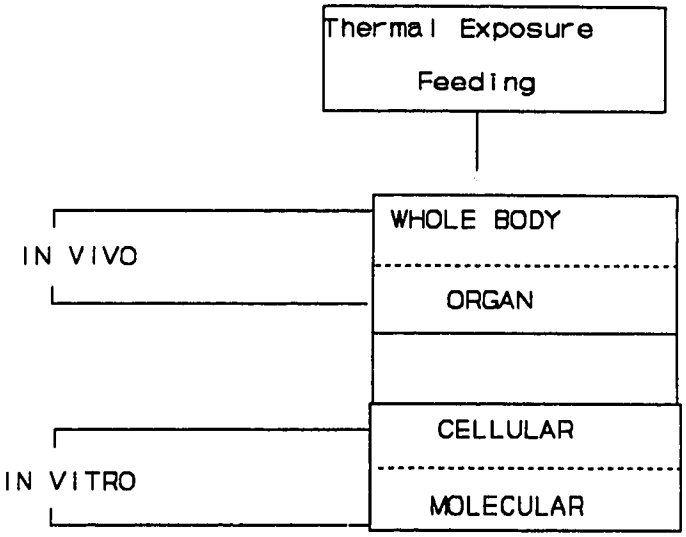


Figure 1.1. The whole body, organ and cellular/molecular approach

At the level of the whole body and individual organs, thermal- and feed-induced thermogenic processes are modulated, in part, by the adrenoceptor system (Webster et al. 1974, Schwartz et al. 1988, Christopherson and Brockman 1989, Astrup et al. 1990). In this thesis, the whole body and organ level approaches in vivo (Figure 1.1) were used to investigate the β-adrenoceptor mediated thermogenic and metabolic responses. The α-adrenergic effects were examined on the whole body only.

1.1. The Sympathoadrenal System

Feeding and exposure to cold usually increases heat production (thermogenesis).

During feeding, heat production is associated with the energy expended for digestion, absorption, conversion and storage. The thermic effect of thermal environment is best illustrated by the relationship between heat production and ambient temperature (Giradier 1977), a modification of which is shown in Figure 1.2.

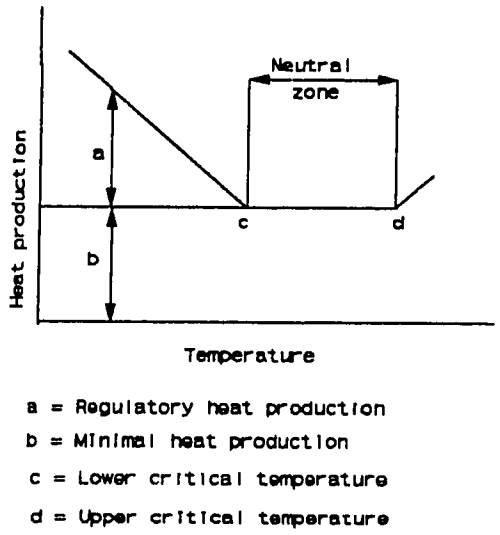


Figure 1.2. The heat production curve.

In Figure 1.2, as temperature increases above the thermoneutral zone, i.e. above "d" the increase in heat production could be fatal. It is not known exactly what causes this effect which appears to be regulatory. The term regulatory is used in relation to changes that depend on ambient temperature. The cost of thermoregulation e.g. increased respiration or sweating rate could be a contibuting factor and the modulatory role of the adrenoceptors could not be ruled out (Alveras and Johnson 1973). Previous suggestions that the heat induced increase in heat production is due to overheating and death of cells has been re-enforced by recent discovery of proteins associated with thermotolerance (Welch 1992). When a family of these proteins, commonly known as

heat shock proteins are being synthesized, the synthesis of all other proteins ceases (Welch 1992). Heat shock proteins are synthesized very rapidly for about 10 - 60 minutes. Recent preliminary data show that, although cattle breeds differ in resistance to heat shock, there is no differential synthesis of the most common heat shock protein by bovine lymphocytes (HSP70) (Kamwanja et al. 1993). At the moment there is not enough data to link the synthesis of this protein which may confer thermotolerance, to the thermic changes at temperatures above "d" in Figure 1.2. On the other hand, as the temperature decreases below the lower critical temperature "c" in Figure 1.2, the regulatory component of the thermic effect of cold exposure has been attributed to changes in thermic processes which include shivering and various non-shivering processes such as ion transport, and substrate cycles. In addition, the adrenal gland and the sympathetic nervous system appear to be instrumental in regulating substrate mobilization and may regulate some of the thermogenic processes. Therefore, thermal exposure (cold and hot), feeding and basal metabolic rate have regulatory components (Astrup et al. 1990). Cold- and feed- induced thermogenic responses could be abolished by ß-adrenergic blockade (Webster 1974, Astrup et al. 1990) thus, implying a role for the sympathoadrenal system (SAS). The SAS consists of two components namely; the sympathetic nervous system (SNS) and the adrenal medulla.

The sympathetic nerves and the adrenal medulla have been recognized as distinct neuroendocrine units (Blaak et al. 1993). Both act via the sympathoadrenoceptor system. These adrenoceptors mediate the diverse metabolic and neuroendocrine actions of catecholamines (adrenaline and noradrenaline). There are

two major types of adrenoceptors designated α and β receptors (Ahlquist 1948). On the basis of their different responses to various pharmacological agents, the adrenoceptors are further subdivided into α_1, α_2, β_1 and β_2 subtypes (Ariens and Simonis 1983, Lefkowitz and Carron 1988). The ß-adrenoceptors activate stimulatory pathways while the α_2-receptors tend to activate the inhibitory ones. At the cellular level, the β_1- and β_2-adrenoceptors activate adenylate cyclase. The α_2-adrenoceptors inhibit adenylate cyclase while the α_1-adrenoceptor activates the phospholipase C signal transduction pathway.

Recently, genes for the adrenoceptors subtypes have been cloned, providing opportunity for direct analysis of structural features required for agonist/hormonal receptor interaction (Nijkamp et al. 1992). Cloning studies suggest that the β_1 and β_2 and also α_1 and α_2 are products of distinct genes with different tissue distributions (Dixon et al. 1986, Kobilka et al. 1987). Each of the α- and β-adrenoceptors consists of a single polypeptide chain with seven transmembrane domains spanning the lipid bilayer. With the use of site-directed antipeptide antibodies prepared against the hydrophillic segments of the receptor, the precise topography for the β_2-adrenoceptor (Hsiu-Yu Wang et al. 1989) and the α_2-adrenoceptor (Kobilka et al. 1987) has been demonstrated. There are several subtypes of the α_2-adrenoceptors which appear to have different tissue distribution (Uhlen and Wikberg 1991).

1.1.1. Hormone/agonist adrenoceptor interaction

Sutherland and Rall (1957) were the first to show that catecholamines acting on

adrenergic cell surface receptors activated the adenylate cyclase enzyme. When the adrenoceptor system is stimulated, a sequence of events is set into motion that leads to a large variety of cellular metabolic responses. The 3'5'-cyclic adenosine monophosphate (cAMP), a known second messenger discovered by Sutherland and Rall (1957), is responsible for the diversified metabolic responses. Briefly, binding of a hormone/agonist to the adrenoceptor results in the interaction of the other two main components, namely, a guanine nucleotide-binding protein commonly known as the G-protein (Collins et al. 1992) and the adenylate cyclase enzyme. The latter, when stimulated, leads to the conversion of adenosine triphosphate to cAMP which stimulates a cAMP-dependent protein kinase. The stimulated protein kinase, in turn, phosphorylates a variety of protein substrates (Baxter and Finder 1979), thus accounting for the diversity of metabolic responses observed at the whole body and cellular levels. The catecholamines increase cAMP when acting on ß-adrenoceptors, decrease cAMP when acting on α_2 receptors but alter calcium-mediated intracellular events when acting on α_1-adrenergic receptors.

1.2. Sympathoadrenal System And Cold Thermogenesis

1.2.1. β-adrenoceptor

Cold exposure induces an increase in heat production, which is associated with increased catecholamine excretion (Webster et al. 1969). Other evidence for the role of catecholamines in cold thermogenesis in ruminants include elevated plasma catecholamine concentrations (Christopherson et al. 1978) and the observation that

adrenaline infusion is calorigenic in sheep (Graham and Christopherson 1981). On the other hand, injection of noradrenaline (sympathetic neurotransmitter) is preferentially calorigenic in rodents and newborn animals which possess brown adipose tissue (BAT; Webster 1974). In these animals, the regulatory component of thermogenesis is closely linked to the presence of BAT and is abolished by ß-adrenoceptor blockade (Webster 1974). However, adult sheep and other ruminants which lack BAT, increase their heat production in response to cold and feeding and this increase could be influenced by ß-blockade. For example, propranolol (non-selective ß-blocker) causes a significant reduction in metabolic heat production in sheep exposed to severe but not mild cold stress (Webster et al. 1969, Webster 1974).

The ß-adrenergic receptors participate in muscle metabolism (Meyer and Stull, 1971) with the $ß_2$-adrenoceptor subtype having a dominant role in skeletal muscle of most mammals (Ijzerman et al. 1984, Elfellah et al. 1988). Astrup et al. (1989) suggest that the mammalian thermogenic responses takes place in skeletal muscle, liver and white adipose tissue and may be mediated by plasma catecholamines. Thorin et al. (1986) reported that the adrenoceptor mediating thermogenesis in muscle is of the $ß_1$-subtype. This suggestion has not been supported by the work of Fagher et al. (1986, 1988) which shows a major thermogenic role of the $ß_2$-receptors. Nevertheless, the thermogenic behaviour following activation of these adrenoceptor subtypes in response to physiological stimuli, i.e., food, cold, heat stress and exercise, has not been thoroughly investigated.

The role for the SNS normally involves release of noradrenaline near its target

cells, while the adrenal medulla releases adrenaline plus a small amount of noradrenaline into the blood to be delivered by blood to tissues. Currently, we employ selective ß-adrenergic blocking agents to characterize ß-adrenoceptor responses and assess their peripheral adrenaline-induced metabolic roles. Noradrenaline tissue turnover has traditionally been used to estimate SNS mediated effects. However, under certain conditions these effects could be confounded with noradrenaline secreted by the adrenal medulla (Young et al. 1984). Adrenergic agents, e.g., clonidine and guanfacin, that selectively suppress the SNS by inhibiting central and peripheral noradrenaline release, could potentially be used in studies to estimate the noradrenaline mediated SNS component of the sympathoadrenal system during thermal exposure or in response to other physiological states such as feeding.

1.2.1.1. β-adrenergic blockers: a historical perspective

The origin of this group of drugs could be traced to the work of Ahlquist (1948) and Shanks (1984). Prior to Alquist's report, it was known that adrenaline relaxed the human uterus, an organ now known to have predominantly β_2-adrenoceptors (Ariens and Simonis 1983). It was also known that some drugs did antagonize the adrenaline-induced responses. The first synthetic antagonist was dichloroisoprenaline (DCI; Shanks 1984). This compound was reported to block the cardiac effects of adrenaline, noradrenaline, isoprenaline and cardiac nerve stimulation. From earlier studies, Moran and Perkins (1961) used the term 'β-adrenergic blocking drug' for the first time. The side effects of DCI led to the discovery of yet another blocker known as pronethalol.

Pronethalol was quickly withdrawn when it was discovered to be cancinogenic but not before the now popular nonselective β-blocker propranolol was developed (Black et al. 1964). Propranolol inhibits the responses of adenylate cyclase to catecholamines. This was followed by a discovery of selective β-blocking agents now used as therapeutic agents as well as research tools. Amongst the most common research tools are metoprolol (a selective β_1-blocker) and ICI 118551 (a selective β_2-blocker) which, together with propranolol, have been used in the present study to assess the adrenoceptor mediated metabolic responses in sheep.

1.2.2. α_2-adrenoceptors

Several pharmacological agents that act on α_2-adrenoceptors may suppress the sympathetic outflow and thus SNS activity. Cold pressor test (usual test for sympathetic response) studies have confirmed that guanfacin, a novel long acting α_2-agonist with both peripheral and central effects, causes an inhibition of the sympathetic outflow (Koshiji et al. 1992). This effect is thought to be associated with a reduction in peripheral noradrenaline release and a consequent decrease in catecholamine induced thermogenesis in peripheral tissues. In a thermoneutral environment, clonidine, an α_2-agonist, reduces basal metabolic rate in humans (Thompson et al. 1984) and, in cattle, Hunter (1992) reported a 20% decrease in fasting heat production in response to a novel α_2-agonist, guanfacin. This could indicate that the basal metabolic rate in humans and fasting heat production in steers is

influenced by the sympathoadrenal system via α_2-adrenoceptor stimulation. On the other hand, peripheral α_1-adrenoceptor vasoconstrictor effects could be associated with energy conservation in cold acclimated and cold exposed animals (Hidari et al. 1991). In the latter study, blockade of α-adrenoceptors with phentolamine was associated with a 30% increase in heat production. Therefore, both α-adrenoceptor subtypes are likely important in the modulation of whole body cold thermogenesis and heat exchange in most species including the small and large ruminants, resulting in energy conservation.

1.3. Adrenoceptor-Mediated Thermic Effect Of Feeding

1.3.1 β-Adrenoceptors

Diet-induced thermogenesis mediated via the sympathoadrenal system is a major controlling mechanism in the regulation of energy balance in the rat (Rothwell and Stock 1979). In the latter study, elevated diet-induced thermogenesis in cafeteria fed rats was inhibited by propranolol, a nonselective ß-blocker. In sheep, Christopherson and Brockman (1989) have shown that, the thermic effect of feeding is associated with the ß-adrenoceptor system. These authors, reported an increased metabolic activity of portal drained viscera following feeding in sheep kept in a thermoneutral environment. The metabolic responses were partially reversed by propranolol. Therefore, ß-adrenoceptors in tissue of the portal drained viscera may account for a portion of a food-induced increase in oxygen consumption. Propranolol a nonselective β-blocker causes a much smaller but significant reduction in resting energy expenditure (Blaak et al. 1993). The latter is evidence for a regulatory role of the ß-adrenoceptor

sympathoadrenal system in resting metabolic rate. Since resting energy expenditure includes the thermic effects of feeding, perhaps propranolol suppressed the latter in the study of Blaak et al. (1993).

1.3.2. α-Adrenoceptors

There is much less information on the role of α_2-adrenoceptor stimulation in the modulation of thermic effects of feeding. In man, the thermic effect of feeding has been shown to be influenced by the sympathoadrenal system (Schwartz et al. 1988) . These authors reported a marked reduction in the heat production associated with feeding in humans in response to α_2-adrenoceptor stimulation with clonidine an α_2-agonist. Guanfacin, a long acting α_2-adrenergic agonist, resulted in a 26 and 28% reduction in heat production in underfed and ad libitum fed rats, respectively (Sillence et al. 1992). Information about the modulatory role of the α_2-adrenoceptors in the thermic effect of feeding in ruminants is not available.

1.4. Adrenoceptors and Animal Growth

Dietary ß-adrenergic agonists have been recognized as energy repartitioning and anabolic agents and in animals, these agents acting via ß-adrenoceptors promote growth by increasing muscle protein accretion (Reeds et al. 1988, MacRae et al. 1988, Choo et al. 1992 and Dawson et al. 1993). In contrast, the ß-adrenoceptor antagonists have not been used as dietary supplements but long term treatment of cardiovascular

conditions in humans with these antagonists result in fat deposition (Astrup et al. 1990). Therefore, the ß-adrenoceptor antagonists are likely to result in the storage of energy in the form of fat. This could be particularly important in seasonal energy biotransformation of wild and domestic ruminants. The animals may deposit energy in times of plentiful supply in anticipation of times when food is scarce, e.g., during winter and dry season. It might be possible that animals are able to suppress the ß-adrenoceptors during lipogenesis by unspecified neuroendocrine mechanisms.

On the other hand, dietary α_2-agonists have not been used successfully in animal production (Onischuk and Kennedy 1986, Schaefer et al. 1990). Clonidine, an α_2-agonist, given as a single injection at 7 µg/kg, did not improve growth performance in lambs (Onischuk and Kennedy 1986) and oral administration of clonidine showed no energy repartitioning properties in steers (Schaefer et al. 1990). However, continuous administration of clonidine to lambs decreased renal fat content and influenced growth rate after a prolonged period of drug administration (Kennedy and Belluck 1987). In earlier phases of drug administration, the decrease in growth rate was ascribed to effects of environmental stressors (Kennedy and Belluk 1987). In their studies (Kennedy et al. 1988, Schaefer et al. 1990), clonidine was hyperglycaemic and hyperinsulinaemic in steers and lambs. This effect is consistent with reduced fuel utilization and hence energy conservation attributable to α_2-adrenoceptor stimulation (Hunter et al. 1993). In addition, the contradictory effects of clonidine on plasma growth hormone, i.e., a decrease (Kennedy et al. 1988) and an increase (Schaefer et al. 1990), may not necessarily influence growth rate since circulating growth hormone

concentration is not always correlated to growth performance in animals (Dawson et al. 1993). Guanfacin, a novel α_2-agonist, increased feed conversion efficiency in steers (Hunter 1992) but when given to rats at higher doses it had a negative effect on growth (Sillence et al. 1992). It is more likely that, the metabolic effect of α_2-agonists is dose- and breed-dependent as described by Kennedy and Belluk (1987) and may be influenced by ambient temperature (Maskrey et al. 1970). However, the effect of the interaction between α_2-stimulation with guanfacin and ambient temperature on thermogenesis has not been examined previously.

1.5. Thermogenic Process, Substrates, and The Adrenoceptor System

In animals with brown adipose tissue, the mechanisms involved in thermoregulatory nonshivering thermogenesis are similar to those associated with diet-induced thermogenesis and both may result in part from sympathoadrenal activation of the brown adipose tissue (Rothwell and Stock 1979, Trayhurn 1990). Since adult ruminants lack brown adipose tissue, the mechanism described for brown adipose tissue may not explain thermogenic responses in these animals.

Mammals exposed to cold temperatures increase their total body oxygen consumption. The actual tissues involved in the increased oxygen consumption are not well defined. Tissues with high capacities for oxidative metabolism (Jansky 1973, Grav and Blixen 1979) may contribute significantly to thermoregulatory thermogenesis. Elevated cytochrome oxidase activity of skeletal muscle has been reported in cold-acclimated piglets (Herpin 1989). In muscle of this species, the actual

location of a portion of increased heat production and thus oxygen consumption is thought to be the subsarcolemmal mitochondria (Herpin 1989). The role of other tissues is elusive. In recent experiments with laboratory animals (cats), Loncar et al. (1986) have shown that white adipose tissue after prolonged exposure to cold, is structurally (vascularized) similar to brown adipose tissue. The involvement of the apparent transformed white adipose tissue in thermoregulatory thermogenesis has not been investigated. On the other hand, oxygen consumption of the liver but not the portal drained viscera has been shown to increase during cold exposure (Thompson et al. 1975).

1.5.1. Thermogenic Processes

The biochemical events at the cellular level responsible for heat production in animals have been reviewed by Summers et al. (1988). These cellular events include protein turnover, ion transport, substrate cycles, vital organ function and work of skeletal muscle. The activity of the sodium-potassium pumps present in cells could be stimulated by the catecholamines via ß-receptors (Chinet and Clausen 1984 and see a review by Clausen (1986)) and cold stress (Gregg and Milligan 1982). The adrenaline-induced hypokalaemia involves the interaction of ß-adrenoceptors ($ß_2$) and the Na^+-K^+ pumps in skeletal and cardiac muscle muscle in rabbits (Elfellah and Reid 1990). The pumps are involved in ion transport. McBride and Kelly (1988) reviewed the significance of the contribution of gastrointestinal tract biochemical process such as ion transport, towards whole body energy transformations. Kelly et al. (1993) showed

that the portal drained viscera contributes about 22-47 % towards whole body oxygen consumption. Of this total, the ouabain sensitive and hence the Na^+/K^+ ATP-ase associated consumption ranged from 3-18%. The tissues of the GIT account for about 23% of total oxygen consumption and hence ATP utilization. A possible mediator of thermogenesis in skeletal muscle is the ß-adrenoceptor system (Thorin et al. 1986, Fagher et al. 1986, 1988). It is suggested that these receptors mediate stimulation of substrate cycles, which result in heat production by hydrolysis of ATP. Substrate cycles may be involved in heat generation in ruminants (Lobley 1990), however, only the acetate/acetyl-CoA cycle has been investigated in sheep (Crabtree et al. 1987). Crabtree et al. (1987) showed that this cycle accounts for only about 0.5% of whole body heat production of sheep in a thermoneutral environment. The role that it might play in cold exposed or postprandial sheep is not known.

1.5.2. Energy Substrates as Fuels

The major fuels for thermogenesis include lipids, glucose and amino acids. In both the thermoneutral environment and in cold stress, glucose alone may contribute about 10% towards total heat production in each case (Tsuda et al. 1979). In another study, Tsuda et al. (1984) suggested that both glucose and lipid metabolism together may account for 50% of total heat production in these circumstances (thermoneutral and cold environments). However, from these studies (Tsuda et al. 1979, 1984) the cold environment is associated with a 75% increase in glucose turnover and 50% increase in glycerol and palmitic acid concentration. In a hot environment, consistent data on

the relative contribution of the metabolic fuels towards whole body thermogenesis is not well documented.

During stressful episodes, proteins could be used as sources of metabolic fuels. Protein and amino acid metabolism may increase, remain unchanged or decrease in response to cold, feeding and other stressors. The effect of heat stress on protein and amino acid metabolism is not clearly defined in domestic ruminants.

In monogastric animals amino acid utilization for gluconeogenesis is quite substantial (Jungas et al. 1992) and in fed sheep the gut tissue oxidizes a substantial amount of glutamine (Wolff et al. 1972). Several studies showed that the hindquarters could be a major source of glutamine in sheep (Heitman and Bergman 1978) and dogs (Souba and Wilmore 1983, Muhlbacher et al. 1984). In various in vivo experiments, it has been assumed that the hindquarters of the sheep comprise largely of muscle. Perfusion studies and in vitro experiments indicate that the muscle mass is indeed a major contributor (Smith 1986), however, the contribution of glutamine by the skin, bone and particularly the adipose tissue might increase significantly in cold exposed sheep and needs to be taken into account. Glutamine flux (synthesis and efflux from the hindquarters) could be influenced by catecholamines. Serial addition of adrenalin into the perfusion medium caused a significant increase of glutamine in the perfusate of the rat hindlimb (Loweinstein and Goodman 1978). The latter might suggest that catecholamines, acting via adrenergic receptors, may promote the efflux of carbon and nitrogen skeletons from the hindquarters. This could be difficult to reconcile with the current view that beta-adrenergic stimulation augments N retention in muscle.

Additionally, the exact nature of the interaction between catecholamines and circulating amino acids is unclear. In man, epinephrine infusion caused a decrease in most plasma amino acids which was reversed by propranolol (Shamoon et al. 1980). An exception was the amino acid alanine which remained unchanged by either adrenaline or propranolol. Organ or tissue responses of individual amino acids to adrenaline infusion has not been thoroughly examined.

1.6. Summary

To summarize, an important role of the sympathoadrenal system in the regulation of energy metabolism has been established in many species. In several species including ruminants, previous studies have shown an increase in energy expenditure in response to noradrenaline and adrenaline infusions (Graham and Christopherson 1981, Staten et al. 1987). The regulatory component of thermal and diet-induced thermogenesis is mediated by an increased sympathetic tone and a possible elevation in adrenal medulla activity (Girardier 1977, Astrup 1986).

In ruminants, the nature and subtype of the adrenoceptors that mediate whole body and organ thermal- and feed-induced thermogenesis is not completely known. In addition, although β-adrenoceptor mediated processes have been reported for animals in thermoneutral environments, there is little information available for the differential roles for whole body and organ β_1- and β_2 -adrenoceptor mediated proceses in animals exposed to thermal or nutritional stress. Finally, much less is known about the role of

α_2-adrenoceptors in modulating thermal- or feed-induced thermogenesis in ruminants.

1.7. Hypotheses

A series of experiments was designed to test the hypotheses that a portion of whole body and organ oxygen consumption is mediated by the adrenoceptor system (α and ß) and that the adrenoceptor mediated thermogenic effect is differentially modulated by α_2, $ß_2$ and $ß_1$-adrenoceptor. In addition, the ß-adrenoceptor pathways are calorigenic while stimulation of α_2-adrenoceptors is anticalorigenic. Further, both α_2- and ß-adrenoceptor-mediated metabolic effects are influenced by environmental factors (cold and feed). Figure 1.3 is a diagrammatic representation of the whole body and organ approach used by the author.

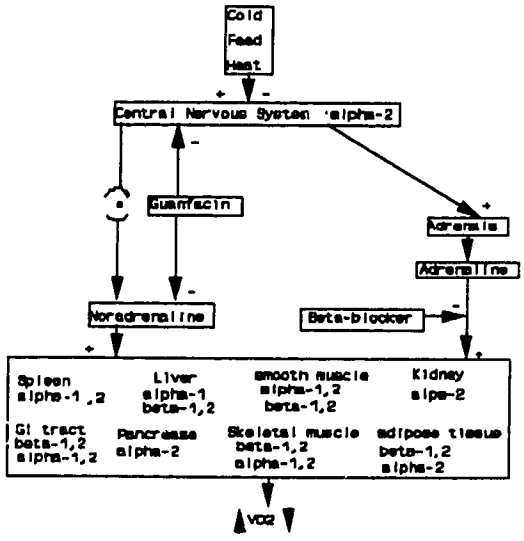


Figure 1.3. Representation of whole body and organ thermic effects of thermal exposure and feeding and possible adrenoceptor stimulatory pathways.

The "+" sign denotes stimulation and the "-" an inhibition. Oxygen consumption is an index of the rate of energy biotransformation. The arrows on either side of "VO_2" indicate an increase or a decrease in oxygen consumption. The whole body represents a black box in which some of the mechanisms that are associated with thermic effects of adrenoceptor stimulation have been identified.

1.8. Subhypotheses

In this study, the following subhypotheses were addressed:

1. β_1- and β_2-adrenoceptors differentially modulate whole body and organ thermic effects of adrenaline infusion.

2. The whole body, hindquarter and the portal drained viscera thermogenesis is elevated in response to cold exposure and a portion of the increase is mediated by the β-adrenoceptor system.

3. A portion of the whole body and the portal drained viscera thermic response to feeding is modulated by the β-adrenoceptor system.

4. Whole blood amino acid concentrations and organ fluxes of amino acids are modulated by the ß-adrenoceptors.

5. Guanfacin, an α_2-adrenoceptor agonist, is anticalorigenic in steers kept in different thermal environments.

6. Guanfacin is antithermogenic in warm- and cold-acclimated steers fed ad libitum.

7. Guanfacin suppresses fasting heat production and the thermogenic response to feeding in sheep.

1.9. REFERENCES

Ariens E.J. and Simonis A.M. 1983. Physiological and pharmacological aspects of adrenergic receptor classification. Biochem. Pharmacol. 32 (10):1539-1545.

Ahlquist R.P.A. 1948. A study of the adrenotropic receptors in sympathetically mediated thermogenesis. Am. J. Physiol. 153:586-600.

Astrup A.V. Christensen N.J., Simonsen L. and Bulow J. 1990. Effect of nutrient intake on sympathoadrenal activity and thermogenic mechanism. J. Neurosc. Methods 34:187-192.

Astrup A.V. 1986. Thermogenesis in human brown adipose tissue and skeletal muscle induced by sympathomimetic stimulation. Acta Endocrinol. 112(278):1-32.

Astrup A.V., Simonsen L., Bulow J. and Christensen N.J. 1989. The contribution of skeletal muscle to carbohydrate-induced thermogenesis in man: the role of sympathoadrenal system. In: H. Lardy and F. Stratman eds., Hormone, Thermogenesis and Obesity. Elsevier. New York pp 187-196.

Baxter J.D. and Finder J.W. 1979. Hormone receptors. New Eng. J. Med. 301(21):1149-1161.

Blaak E.E., Van Baak M.A., Kempen K.P. and Saris W.K. 1993. Role of α- and β-adrenoceptors in sympathetically mediated thermogenesis. Am. J. Physiol. 264:E11-E17.

Black J.W., Crowther A.F., Shanks R.G., Smith L.H. and Dorhorst A.C. 1964. A new adrenergic beta-receptor antagonist. Lancet i:1080-1081.

Choo J.J., Horan M.A., Horan R.A., Rothwell N.J. 1992. Anabolic effects of clenbuterol on skeletal muscle are mediated by β_2-adrenoceptor stimulation. Am. J. Physiol. 263:E50-E56.

Chinet A. and Clausen T. 1984. Energetics of active sodium-potassium transport following stimulation with insulin, adrenaline or salbutamol in rat soleus muscle. Pflugers Arch. 401:160-166.

Christopherson R.J. and Brockman R.P. 1989. Effect of feeding and a ß-adrenergic blocking agent on oxygen consumption by the portal drained viscera, liver and hindquarters of the sheep. EAAP 43:147-150.

Christopherson R.J. , Thompson J.R., Hammond V.A. and Hills G.A. 1978. Effects of thyroid status on plasma adrenaline and noradrenaline concentration in

sheep and during acute and chronic cold exposure. Can. J. Physiol. Pharmacol. 56:490-496.

Clausen T. 1986. Regulation of active Na^+-K^+ transport in skeletal muscle. Physiol. Rev. 66:542-580.

Collins S., Caron M.G. and Lefkowitz R.J. 1992. From ligand binding to gene expression: new insights into the regulation of G-protein-coupled receptors. TIBs 17:37-39.

Crabtree B., Marr S.A., Anderson S.E. and MacRae J.C. 1987. Measurement of substrate cycling between acetate and acetyl-CoA in sheep muscle in vivo: effect of infusion of acetate. Biochem. J. 243:821-827.

Dawson J.M., Craigon J., Buttery P.J. and Beever D.E. 1993. Influence of diet and ß-agonist administration on plasma growth hormone and insulin-like growth factor-1 in young steers. Br. J. Nutr. 70:93-102.

Dixon R., Kobilka B., Strader D., Benovich J., Dohlan H., Frielle I., Bolanowski M., Bennet C., Rand E., Diehl R., Mumford R., Slater E., Sigal I., Caron M., Lefkowitz R. and Strader C. 1986. Cloning of the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin. Nature Lond. 321:75-79

Elfellah M.S., Deighton N. and Reid J.L. 1988. Regulation of ß-adrenoceptors by catecholamine in the rabbit skeletal muscle. Eur. J. Pharmacol. 157:215-220.

Elfellah M. and Reid J.L. 1990. The relationship between the hypokalaemic response to adrenaline, ß-adrenoceptors, and Na^+-K^+ pumps in skeletal and cardiac muscle membranes in the rabbit. J. Card. Pharmacol. 15:62-67.

Fagher B., Liedholm H., Monti M. and Moritz U. 1986. Thermogenesis in human skeletal muscle as measured by direct microcalorimetry and muscle contractile performance during B-adrenoceptor blockade. Clin. Sci. 70:435-441.

Fagher B., Monti M. and Thulin T. 1988. Selective B_1-adrenoceptor blockade and muscle thermogenesis. Acta med. Scand. 223:139-145.

Giradier L. 1977. The regulation of the biological furnace of warm blooded animals. Experientia 33:1121

Graham A.D. and Christopherson R.J. 1981. Effects of adrenaline and noradrenaline on the heat production of warm and cold acclimated sheep. Can. J. Physiol. Pharmacol. 59:985-993.

Grav H.J. and Blixen A.S. 1979. A source of nonshivering thermogenesis in fur seal skeletal muscle. Science 204:87-89.

Gregg V.A. and Milligan L.P. 1982. Role of Na^+K^+-ATPase in muscular energy expenditure of warm- and cold-exposed sheep. Can. J. Anim. Sci. 62:123-132.

Herpin P. 1989. Oxidative capacities and mitochondrial respiration in muscle of cold-acclimated piglets. EAAP 43:179-182.

Heitmann R.N. and Bergman E.N. 1978. Glutamine metabolism, interorgan transport, and glucogenicity in sheep. Am. J. Physiol. 234: E197-E203.

Hidari H., Christopherson R.J. and Ole Miaron J.O. 1991. Effects of a cold environment and alpha and beta adrenergic blocking agents on metabolic rate in steers and beta blockade on organ and VO_2 in sheep. EAAP 58:76-78.

Hsieu-Yu Wang, Lipfert L., Malbon C.C. and Bahauths S. 1989. Site directed anti-peptide antibodies define the topography of the β-adrenergic receptor. J. Biol. Chem. 264(24):14424-14431.

Hunter R.A. 1992. The effect of α_2-adrenergic agonist, guanfacine, on the energy metabolism of steers fed on low quality roughage diets. Br. J. Nutr. 67:337-343.

Hunter R.A., Sillence M.N., Gazzola C. and Spiers W.G. 1993. Increasing animal growth rate of cattle by reducing maintenance energy requirements. Aust. J. Agric. Res. 44;579-595.

Ijzerman A.P., Butsman T., Timmermans H. and Zaagsman J. 1984. The relation between ionisation and affinity of ß-adrenoceptor ligands. Naunyn-Schmieb. Arch. Pharmacol. 327:293-298.

Jansky L. 1973. Nonshivering thermogenesis and it's thermoregulatory significance Biol. Rev. 48:85-132.

Jungas R.L., Halpern M.L. and Brosnan J.T. 1992. Quantitative analysis of amino acid oxidation and related gluconeogensis in humans. Physiol. Rev. 72(2):419-449.

Kamwanja L.A., Chase C.C., Gutierrez J.A., Gurriero V., Hammond A.C., Olson T.A. and Hansen P.J. 1993. Effect of breed and antioxidant status on responses of bovine lymphocytes to heat shock. J. Anim. Sci. 71(suppl. 1):118.

Kelly J.M., Southorn B.G., Kelly C.E., Milligan L.P. and McBride B.W. 1993. Quantification of in vitro and in vivo energy metabolism of the gastrointestinal tract of fed and fasted sheep. Can. J. Anim. Sci. 73:855-868.

Kennedy A.D. and Belluk B.M. 1987. Growth and carcass composition of ramb lambs treated with clonidine. Can. J. Anim. Sci. 67:417-425.

Kennedy A.D., Tekpetey F.R. and Onishchuk L.A. 1988. Growth hormone, insulin, prolactin, glucose and mononuclear leucocyte insulin binding response to clonidine in ram lambs. Can. J. Anim. Sci. 68:655-667

Kobilka B.K., Matsui H., Kobilka T.S., Tang-Fen T.L., Franckie U., Caron M.G., Lefkowitz R.J. and Regan J.W. 1987. Cloning, sequencing, and expression of the gene coding for the human platelet α_2-adrenergic receptor. Science 238:650-856.

Koshiji M., Ito H., Minatoguchi S., Watanabe H., Imai Y., Kakami M and Hirakawa S. 1992. A comparison of guanfacine, bunazosin, atenolol and nadolol on blood pressure and plasma noradrenaline responses to cold pressor testing. Clinical Exp. Pharmacol. and Physiol. 19:481-488.

Lefkowitz R.J. and Carron M.G. 1988. Adrenergic receptor models for the study of receptor coupled to guanine nucleotide regulatory proteins. J. Biol. Chem. 263:4993-4996.

Lobbley G.E. 1990. Energy metabolism reactions in ruminant muscle: responses to age, nutrition and hormones. Rep. Nutr. Dev. 30: 12-34.

Loncar D., Bedrica L., Mayer J., Cannon B., Nedergaard J., Afzelius A. and Snajger A. 1986. The effect of intermittent cold treatment on the adipose tissue of the cat. Apparent transformation from white to brown adipose tissue. J. Ultrastructure. Mol. Struct. Res. 97:119-129.

Lowenstein J.M. and Goodman M.W. 1978. The purine nucleotide cycle in skeletal muscle. Federation Proc. 37:2308-2312.

MacRae J.C., Skine P.A., Connel A., Buchan V. and Lobley G.E. 1988. The action of ß-agonist clenbuterol on protein and energy metabolism in fattening wether lambs. Br. J. Nutr. 59:457-465.

Maskrey M., Vogt M. and Bligh J. 1970. Central effects of clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, St 155) upon thermoregulation in the sheep and goat. Eur. J. Pharmacol. 12:297-302.

McBride B. W. and Kelly J.M. 1988. Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. J. Anim. Sci. 68:2997-3010.

Meyer S.E. and Stull J.T. 1971. Cyclic AMP in skeletal muscle. Ann. N.Y. Acad. Sci. 185:433.

Moran H.C. and Perkins M.E. 1961. An evaluation of adrenergic blockade of the mammalian heart. J. Pharmacol. Exp. Ther. 133:192-201.

Muhlbacher F., Kapadia R., Colpoys M.F., Smith R.J., and Wilmore D.W. 1984. Effects of glucocorticoids on glutamine metabolism in skeletal muscle. Am. J. Physiol. 247:E75-E83.

Nijkamp F.P., Engels F., Hendricks P.A.J. and Van Oosterhout A.J.M. 1992. Mechanisms of ß-adrenergic receptor regulation in lungs and its implications for physiological responses. Physiol. Rev. 72(2):323-362.

Onischuk L.A. and Kennedy A.D. 1986. The effect of clonidine on weight gain, feed efficiency, serum insulin and plasma glucose concentration in ewe lambs. Can. J. Anim. Sci. 66:1206.

Reeds P.J., Hay S.M., Dorward P.M. and Palmer R.M. 1988. The effect of ß-agonists and antagonists on muscle growth and body composition of young rats (Rattus sp). Br. Comp. Biochem. Physiol. Vol. 89c(2):337-341.

Rothwell N.J. and Stock M.J. 1979. A role for brown adipose tissue in diet-induced thermogenesis. Nature 281:31-35.

Schaefer A.L., Jones S.D.M., Kennedy A.D., Tong A.K.W. and Onischuk L.A. 1990. The effect of the alpha adrenergic agonist clonidine on the growth, carcass yield, meat quality and physiological-endocrinological response in steers. Can. J. Anim. Sci. 70:857-866.

Schwartz R.S., Jaeger L.F., and Veith R.C . 1988. Effect of clonidine on the thermic effect of feeding in humans. Am. J. Physiol. 254:R90-R94.

Shamoon H. Jacob R and Sherwin R.S. 1980. Epinephrine-induced hypoaminoacidemia in normal and diabetic human subjects: Effect of beta blockade. Diabetes 29:875-881.

Shanks R.G. 1984. The discovery of beta-adrenoceptor blocking drugs. TIPs 5(10):405-409.

Sillence M.N., Tudor G.D., Mathews M.L. and Lindsay D.B. 1992. Effects of the α_2-adrenoceptor agonist guanfacine on growth and thermogenesis in mice. J. Anim. Sci. 70:3429-3434.

Smith R.J. 1986. Role of skeletal muscle in interorgan amino acid exchange. Fed. Proc. 45: 2172-2176.

Souba W.Y. and Wilmore D.W. 1983. Postoperative alteration of arteriovenous exchange of amino acids across the gastrointestinal tract. Surgery 94:342-350.

Staten M.A., Mathews D.E., Cryer P.E. and Bier D.M. 1987. Physiological increments in epinephrine stimulated metabolic rate in humans. Am. J. Physiol. 235:E322-E328.

Summers M., McBride B.W., and L. P. Milligan. 1988. Components of basal energy expenditure. In A. Dobson and M.J. Dobson eds. Aspect of Digestive Physiology in Ruminants. Comstock Publishing Associates Ithaca and London.

Sutherland E.W. and Rall T.W. 1957. The properties of an adenine ribonucleotide produced with the cellular particles, ATP, Mg^{2+} and epinephrine or glucagon. J. Am. Chem. soc. 79:3608.

Thompson D.A., Penicaud L. and Welle S.L. 1984. Alpha-2 adrenoceptor stimulation inhibits thermogenesis and food intake during glucoprivation in humans. Am. J. Physiol. 247:R560-R566.

Thompson G.E., Gardner J.W. and Bell A.W. 1975. The oxygen consumption, fatty acid and glycerol uptake of the liver on fed and fasted sheep during cold exposure. Quart. J. Expt. Physiol. 60:107-121.

Thorin D., Golay A., Simonsen D.C., Jequier E., Felber J.P. and DeFronzo R.A. 1986. The effect of selective Beta adrenoceptor blockade on glucose-induced thermogenesis in man. Met. 35(6):524-528.

Trayhurn P. 1990. Energy expenditure and thermogenesis: Animal studies on brown adipose tissue. Intern. J. Obesity 14(suppl.1):17-29.

Tsuda T., Fujita M. and Ambo K. 1979. Metabolism of glucose in cold exposed sheep. Ann. Rech. Vet. 10(2/3):373-375.

Tsuda T., Ambo K., Shoji Y., Fujita M. and Sunagawa K. 1984. Distribution of energy source expenditure in warm- and cold-exposed sheep. Can. J. Anim. Sci. 64(Suppl.) 265-266.

Uhlen S. and Wikberg J.E.S. 1991. Delineation of rat kidney α_{2A}- and α_{2B}-adrenoceptors with [^{3}H]RX821002 radioligand binding:computer modelling reveals that guanfacine is an α_{2A}-selective compound. Eur. J. Pharmacol. 202:235-243.

Webster A.J.F. 1974. Physiological effects of cold exposure. In MTP International Review pp 33-69.

Webster A.J.F., Heitman J.H., Hays F.L. and Olynyk G.P. 1969. Catecholamines and cold thermogenesis in sheep. Can. J. Physiol. Pharmacol. 47:719-724.

Welch W.J. 1992. Mammalian stress response: cell physiology, structure/function of stress proteins and implication for medicine and disease. Physiol. Rev.72(4):1063-1077.

Wolff J.E., Bergman E.N., and Williams H.H. 1972. Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep 223:438-446.

Young J.B., Rosa R.M. and Landsberg L. 1984. Dissociation of sympathetic nervous system and adrenal medullary responses. Am. J. Physiol. 247:E35-E40.

2. Whole Body and Organ Thermic and Metabolic Responses to Adrenaline Infusion: Effects of Nonselective and Selective ß-Adrenoceptor Blockade

2.1. INTRODUCTION

Studies on energy metabolism have traditionally focused on the effects of various physiological and pharmacological stimuli on whole animal energy balance, with little emphasis on contribution of the organs/tissues to whole body metabolism. Today, there is considerable interest in identifying organs/tissues, cell types and physiological mechanisms involved in energy metabolism (Lobley 1991). Several organs/tissues, including the portal drained viscera and the hindquarters of the sheep, have been implicated as major contributors to energy metabolism (Christopherson and Brockman 1989) including the observation that the portal drained viscera could be influenced by ß-adrenoceptor processes (Christopherson and Brockman 1989). The adrenoceptors are classified, according to responses to different catecholamines, into α and ß receptors which are further divided into $ß_1$- and $ß_2$-adrenoceptors (Ariens and Simonis 1983). Although the physiological responses to β-adrenoceptor stimulation are well known for several organs, e.g., bronchioles and cardiovascular system (Nijkamp et al. 1992), responses in gut tissue are incompletely understood.

β-adrenoceptor mediated pathways are known to modulate upper gastrointestinal nutrient transit in humans (O'Brien et al. 1989, McIntyre et al. 1992). In sheep both β_1- and β_2-adrenoceptors are involved in the regulation of the reticulorumen and upper gut motor activity (Brikas et al. 1989) and the food-induced

portal drained viscera thermogenesis appears to be partly blocked by propranolol a ß-adrenoceptor antagonist (Christopherson and Brockman 1989). In vitro studies with guinea pig gut smooth muscle cells suggest that the predominant β_2-adrenoceptors interact with β-agonists to increase intracellular cAMP and hence ATP hydrolysis (Zhang et al. 1990). There is little evidence in the literature for differential roles for β_1- and β_2-adrenoceptors in the modulation of whole body and organ oxygen consumption in vivo.

A decrease in whole blood amino acid concentration has been reported during fasting (Ericksson et al. 1988) and lower plasma levels are associated with trauma- and cold-related stressors (Souba and Douglas 1983, Kelly 1987). Additionally, epinephrine infusion caused a reduction of most plasma amino acids in normal and diabetic man (Shamoon et al. 1980) which was reversed by propranolol, thus suggesting an effect of the β-adrenoceptor system in protein and amino acid metabolism. ß-adrenoceptor processes mediate protein accretion in animals (Choo et al. 1992) and, in ruminants, stimulation by ß-adrenergic agonists results in increased thermogenesis and protein accretion (MacRae et al. 1988). The blockade of ß-adrenergic receptors may reduce metaboli ate and, therefore, maintenance energy requirements, which may be beneficial for energetic efficiency. However, if ß-blockers also reduce protein anabolism, they might exert detrimental effects on lean tissue growth. In one study (Reeds et al. 1988), however, ß-agonists were shown to increase protein accretion without increasing thermogenesis, suggesting that it may be possible to dissociate the thermogenic and the protein accretion responses. It is, therefore, important to gain

insights into effects of ß-blockade on indices of protein and amino acid metabolism.

In the present study the differential effects of ß-adrenoceptor stimulation on whole body, portal drained viscera and hindquarters oxygen consumption of the sheep during adrenaline infusion was examined with the use of nonselective and selective beta antagonists. In addition, blood parameters that could be associated with adrenoceptor mediated metabolic responses including [K^+] and whole blood amino acids were determined in sheep during adrenaline infusion with or without the the ß-adrenergic blockers.

2.2. MATERIALS AND METHODS

2.2.1. Animals

Five whether sheep weighing 63±2 kg were kept in metabolism crates in a controlled environment (16±3 °C, 12 h light-dark cycle). The animals were fed 1 kg/d of alfalfa pellets (crude protein = 16%, DM = 93% and gross energy 4.4 Kcal/g DM) and 0.5 kg/d of alfalfa cut hay (crude protein = 11% and DM = 90%) daily. Water and salt were available ad libitum.

2.2.2. Implantation Of Blood Flow Probes

Anaesthesia was induced with intraval (Thiopentone Sodium, M.I.T.C. Pharmaceutical, Cam., ON) and maintained by halothane (2%) administered via endotracheal intubation. The sheep was then placed in lateral recumbency and the abdominal cavity

was opened at the paralumbar fossa. The portal vein was carefully separated from the bile duct by blunt dissection to create an opening approximately 1 cm in length. The silastic-coated metal loop of the blood flow probe was threaded through this opening for placement around the vein. The hepatic artery, which adhered to the portal vein, was also included in the sensing window of the transit time probe resulting in the measurement of total hepatic blood flow. The iliac artery in the caudal abdominal region was accessed through the same incision. The 24S and 12S transit time ultrasonic blood flow probes (Transonic systems, Inc., Ithaca, NY) were placed around the portal vein and iliac artery, respectively. The exposed common trunk of the portal vein was catheterized via a branch of the mesenteric vein. The position of the silastic catheter (id 0.06 and 0.125 in. od; Dow Corning Corp. Michigan USA) in the portal vein was confirmed by palpation. Care was taken to ensure that the portal sampling catheters did not extend through the window of the flow probe. Additional sampling catheters (silastic) were placed into the iliac vein and mesenteric artery.

The catheters were flushed daily with heparinized sterile saline solution. Long acting terramycin and analgesic (Butorphanol, Ayerst, Winipeg, MB) were given i.v. at 0.1 mg/kg on the day of surgery and analgesic was given on the first day post-operation. The animals recovered quickly from surgery and were feeding within 3-4 h. Fourty millilitres of 5 % dextrose (Baxter Corp. Toronto, ON) was administered slowly (over a period of 10 min) intravenously immediately after surgery and at 2 h postsurgery. The sheep were allowed 10 d recovery period before any measurements were made. During this period the animals were accustomed to the experimental

manipulations and were given a period of training in the metabolic hoods. Treatments were randomly applied as shown under the experimental protocol. Twenty four hours before the start of an infusion experiment polyvinyl catheters (id 0.04 and 0.07 in OD; Norton Performance Plastic, Akron, Ohio) were inserted into both jugular veins of each animal. The sheep were always kept in pairs throughout the experiment. The jugular vein, sampling catheters (4 m in length) and the blood flow probes extended to a sampling station located outside the room in which the sheep were kept. The 4 m length for the probe extension cord was the maximum length recommended by the manufacturer. Therefore, infusions, blood flow monitoring and sampling were done from the sampling station. This procedure allowed the study to be conducted under relatively nonstressful conditions.

2.2.3. Experimental Protocol and Treatments

The infusion treatments were adrenaline (Sigma Chemical Co., St. Louis, Mo), adrenaline with propranolol (Sigma Chemical Co., St. Louis, Mo) a nonselective ß-blocker, adrenaline plus metoprolol (Sigma Chemical Co., St. Louis, Mo) a selective β_1 blocker and adrenaline plus ICI 118551 (ICI Pharmaceuticals, Macclesfield, England) a selective β_2 blocker. The control (vehicle; sterile 0.9% saline + 0.01% ascorbic acid as an antioxidant) was made up in dark bottles and adrenaline was added to yield a final concentration that would satisfy an infusion rate at 0.3 $\mu g\ kg^{-1}\ min^{-1}$ (Graham and Christopherson 1981) for 3 h. A priming dose of propranolol (1 mg kg^{-1}), metoprolol and ICI 118551 at 100 $\mu g\ kg^{-1}$ followed by a continuous infusion of 5

(Christopherson and Brockman 1989), 1.5 (Thorin et al. 1986) and 2.5 (Harmon 1992) $\mu g\ kg^{-1}\ min^{-1}$ respectively via the contralateral jugular vein was initiated 10 minutes after adrenaline infusion. The infusions were designed such that each animal acted as it's own control. All the infusions including control (saline) were given for a period of 3 h and a 2 day break was allowed between each infusion treatment. All the infusates were prepared ex tempore.

2.3. MEASUREMENTS

2.3.1. Oxygen Consumption

Whole body oxygen consumption was determined by an open-circuit calorimetry apparatus (Young et al. 1975) connected to a ventilated hood to which the sheep were previously accustomed. Ventilation rate of the head hood was read from a flowmeter (Rotometer, Fisher and Porter, Warminster, PA) and the oxygen concentration difference between incoming and outgoing respired air, from a single channel oxygen analyzer (Servomix 540A Sussex, England). The data was acquired every 10 seconds and averaged over 3 minutes by a computerized data acquisition program developed in our laboratory (Godby and Gregory 1992). The three minutes readings were averaged over hour. The oxygen consumption was the product of ventilation rate ($mL\ min^{-1}$) at STP and oxygen decrement. The measuring system was calibrated with nitrogen as a zero gas and by the iron burn method of Young et al. (1984).

2.3.1. Blood flow

Since we had only one blood flow meter, values for each treatment were determined on separate days. Blood flow was measured with transit time ultrasonic blood flow probes connected to a blood flow meter (Transonic Systems, INC., Ithaca NY). The blood oxygen saturation and haemoglobin was determined in duplicate samples with a OSM 2 hemoximeter (Radiometer, Copenhagen, Denmark). Prior to infusion a pre-infusion zero time blood sample was collected and, thereafter samples were collected at scheduled intervals of 1 h. The flowmeter was set up to allow easy switching from the portal probe to the iliac probe at alternating 10 minute interval. Blood flow was measured continuously during each 10 minutes interval and averaged over each hour. Since the hepatic artery was inside the portal vein probe's measuring window, we assumed the hepatic arterial flow to be 5 % of the total flow (Barnes et al. 1984). Therefore, the portal blood flow in this study was estimated by subtracting 5 % from total probe flow. The oxygen concentration was calculated using the following equation: O_2 mL.dL^{-1} = Hb g.dL^{-1} * 1.38 mL.gHb^{-1} * %saturation/100. Organ (portal drained viscera and hindquarter) oxygen consumption was the product of blood flow and the A-V oxygen difference. Additionally, in a pilot study, blood flow was estimated from p-aminohippuric acid dilution (PAH). The PAH (1.5%) was infused into the mesenteric vein at 1 mL/min and concentration in blood was determined by a spectrophotometer (Milton Roy 3000) according to the procedure of Katz and Bergman (1969). In this study with 2 sheep, the portal blood flow measurements estimated by the transit time method compared favourably with and were not different

from the values for blood flow measured by the p-aminohippurate (PAH) dilution procedure, (see appendix 2, Figure A2.1). Arterial and iliac vein blood plasma [K^+] were determined by atomic absorption spectrophotometry (Model 400, Perkin Elmer Corp., Nowark Conn. 06856, USA).

2.3.2. Amino Acid Analysis

The whole blood was deproteinized on ice with 3 % trichloroacetic acid solution. The whole blood amino acids were then derivatized using the o-phthaldialdehyde method (Jones and Gilligan 1983) and quantified using a Varian 5000 high perfomance liquid chromatograph and a Shimadzu RF-535 fluorescence detector (Shimadzu Scientific Instruments, Inc. Columbia MD. USA). Samples were injected using a varian 9090 autosampler (Varian Associates, Inc. 1985). A supelcosil 3 micron LC-18 reverse phase column (4.6 x 150 mm; Oakville, ONT) equipped with a guard column (4.6 X 50 mm) packed with Supelco LC-18 reverse phase packing (20-40 micron) was used to separate the amino acids. The data was acquired and processed by the Shimadzu Ezchrom Chromatography Data System (Shimandzu Scientific Instruments, Inc.).

2.4. DATA ANALYSIS

Data for responses to infusion treatments were divided into preinfusion and infusion (average of the last 2 h of infusion) periods for the purpose of the analysis. ANOVA

for the randomized block design, where animals were blocks, was then computed on the change from pre-infusion values by the general linear model procedure of SAS (SAS Inc., Cary, NC.). Treatment was tested against animal by treatment interaction. When ANOVA was significant the change from pre-infusion values means for the last 2 h of infusion were compared by Student's Newman Keuls (Steel and Torrie 1980). Means for blood and plasma parameters are absolute values. The other values are presented as a change from the preinfusion values. The preinfusion values were used as a covariate because the measurements were made on different days. For the amino acid data, the p values for treatment and treatment*time are shown.

2.5. RESULTS

The whole body, portal drained viscera and hindquarters control (preinfusion only) values (means±SE) for oxygen consumption were 236±7.4, 61±6.0 and 13±3.1 mL min^{-1} respectively. Adrenaline increased whole body oxygen consumption by 30 % (Figure 2.1). This increase was completely prevented by the nonselective (propranolol) and selective (β_1- and β_2-) blockers.

Due to loss of catheter patency, data for portal drained viscera and hindquarter oxygen consumption was successfully collected from 4 and 3 animals, respectively. A 40% increase in adrenaline-induced portal drained viscera oxygen consumption was completely suppressed and even reversed by the nonselective (propranolol) and β_2 (ICI 118551) blockers (Figure 2.2). The β_1-blocker (metoprolol) did not significantly reduce

the response to adrenaline. The hindquarter oxygen consumption responses (a 62% increase, Figure 2.3) appeared to show similar trends to the whole body and portal drained viscera responses to adrenaline and the β-blockers, although these effects were not statistically significant.

The preinfusion blood flow values were 1780 and 240 $mL.min^{-1}$ for portal vein and iliac artery respectively. Adrenaline infusion caused a significant ($P<0.05$) increase in blood flow from the portal drained viscera and to the hindquarters (Figure 2.4, Figure 2.5). The portal blood flow affected the portal drained viscera oxygen consumption response. Adrenaline plus metoprolol differed ($P<0.05$) and approached significance ($P<0.1$) when compared to adrenaline plus ICI 118551 but not different from control or adrenaline plus propranolol. In the iliac artery, the adrenaline-induced increase was significantly ($P<0.05$) and differentially reduced by the nonselective (propranolol) as well as the selective β_1- (metoprolol) and β_2- (ICI 118551) blockers. The β_1- (metoprolol) blocker response, though significant, was less than the β_2-(ICI 118551) response.

Blood haemoglobin concentration was significantly ($P<0.05$) elevated in response to adrenaline infusion (Table 2.1), and the response was reduced by a selective β_1-blocker metoprolol. The arterial oxygen content was slightly but not significantly higher after adrenaline infusion compared to vehicle. This effect was significant ($P<0.05$), however, when ICI 118551 was infused together with adrenaline. As a consequence of an increase ($P<0.05$) in iliac vein oxygen saturation, there was an increase in iliac vein oxygen content which resulted in changes in the calculated

parameters, namely, the hindquarter A-V oxygen difference and the hindquarter oxygen extraction. The latter was reduced ($P<0.05$) in response to adrenaline infusion. The β-blockers except for metoprolol abolished this effect.

In order to standardise a dilution error associated with determination of the blood plasma [K^+], it was necessary to use a plasma volume correction factor which was based on the average change in hemoglobin concentration. The arterial blood plasma [K^+] was not affected by adrenaline, however, the value was elevated ($P<0.05$) in response to adrenaline plus $ß_2$-adrenoceptor blocker. The venous plasma [K^+] was reduced ($P<0.05$) by adrenaline and the blockers restored it to normal values with the $ß_2$-adrenoceptor antagonist showing an increase above basal values.

Adrenaline infusion was accompanied by a generalized tendency towards a small but nonsignificant ($P>0.05$) decrease in whole blood arterial amino acid concentration (Table 2.2). This tendency was pronounced and significant ($P<0.05$) for arterial asparagine and citrulline. Several amino acids showed a significant treatment x time interaction (Table 2.2) which were difficult to interpret. For some amino acids e.g. serine, tryptophan, methionine, valine, and phenylalanine the treatment responses approached significance ($P<0.1$). There were no consistent responses for the portal and hindquarter amino acid A-P, A-V differences and net fluxes to either adrenaline alone or in combination with the ß-adrenergic blockers. The amino acid fluxes and A-V differences were highly variable partly because calculations were based on only 3 sheep. This data is presented in Appendix 4 (Table A4.1-A4.6). In this appendix, the net fluxes of amino acids were the product of arterio-venous differences and blood

flow (Appendix 6; Table 6.1a). The positive sign denotes a removal or uptake of the amino acids by the tissue and a negative sign denotes a release of the amino acid from the tissue into the blood.

2.6. DISCUSSION

Preliminary data for whole body, portal drained viscera and hindquarters oxygen consumption from the present study have been published (Ole Miaron and Christopherson 1992[1]). The whole body oxygen consumption values are in agreement with published data (Schaefer et al. 1982, Christopherson and Brockman 1989, Harris et al. 1989). Surprisingly, there was no differential inhibition by the β-blockers. However, this data is consistent with that of Harman (1990) in which a differential effect for ß-adrenoceptor-mediated lipolysis but not thermogenesis was observed. The complete inhibition of both $ß_1$- and $ß_2$-adrenoceptor-mediated whole body oxygen consumption in response to adrenaline infusion in sheep could result from cross-reactivity of the ß-blockers. Unlike for the α-adrenoceptors, subtype-selective radioligands for ß-adrenoceptors subtypes are not available, thus, all the current ß-adrenoceptor ligands do bind to $ß_1$- and $ß_2$-adrenoceptors although with different affinities (Motulsky and Insel 1987). Other factors including species differences effects and presence of atypical ß-adrenoceptors could be involved. In man, metoprolol infused continuously over a period of three hours at a rate of 1.5 $\mu g.kg.min^{-1}$ (a dose similar to that used in the present study) was found to be devoid of any $ß_2$-

[1]A version of this chapter has been published as an abstract. Ole Miaron J.O and Christopherson R.J. 1992. Can. J. Anim. Sci. 72:987

adrenoceptor antagonist activities and reduced heat production by 64% (Thorin et al. 1986). In the latter study, $ß_2$-antagonist effects were detectable when plasma concentration of metoprolol was above 100 $ng.mL^{-1}$. A $ß_3$-adrenergic receptor has been postulated to be involved in the catecholamine-mediated metabolic response (Emorin et al. 1989). These authors have shown that, the isoproterenol-induced increase in cAMP in vitro was completely abolished by ICI 118551 and metoprolol in chinese hamster cell lines transfected with $ß_3$-adrenoceptor genes while the response of the nontransfected cells was unchanged. Therefore it is possible that a combination of these factors could be responsible for complete inhibition of the adrenaline-induced increase in whole body oxygen consumption.

The oxygen consumption by the portal drained viscera reflected the blood flow pattern. This may suggest that there are no major differences in selectivity between the hemodynamic and metabolic effects of adrenaline. Therefore, both adrenergic receptor types are present in the heterogenous organs and tissues of the portal drained viscera, a view consistent with earlier in vivo observations (Brikas et al. 1989). The adrenaline-induced increase in the portal drained viscera oxygen consumption is most likely associated with the β_2-(hormonal, extrajunctional) adrenoceptors known to be predominant in the smooth muscle cells (Ariens and Simonis 1983, Zhang et al. 1990). Moderate $ß_1$-adrenoceptor mediated effects cannot be ruled out, since metoprolol partially suppressed the portal drained viscera oxygen consumption. The portal drained viscera met it's increased oxygen requirements by increased blood flow since there were no significant changes in the arteriovenous oxygen difference. Bearn et al. (1951)

showed that the adrenaline-induced increase in the splanchnic oxygen consumption in man was associated with a significant increase in blood flow while the A-V difference remained unchanged. Therefore, the increased blood flow and hence oxygen delivery, in response to stimulation by adrenaline is a reflection of the increased oxygen consumption by some tissues of the portal drained viscera. In the dog, the adrenaline-induced increase in oxygen consumption of the jejunum is associated with elevated glucose uptake (Grayson and Oyebola 1983). Additionally, the portal drained viscera metabolic response to adrenaline observed in this study is consistent with functional hyperaemia reported previously by Eldostone and Holzman (1981) and Huntington et al. (1990). Eldostone and Holzman (1981) showed that the neonatal gastrointestinal tract of sheep meets its oxygen demand with a comparatively large blood flow and oxygen delivery after a mild stress induced by starvation.

The lack of significance of the changes in hindquarter oxygen consumption after adrenaline infusion could be partly attributed to largely, between animal variability. However, the decrease in oxygen extraction in response to adrenaline and the subsequent inhibition of the decrease by ß-adrenoceptor antagonist is consistent with ß-adrenoceptor mediated changes in metabolic activity of the hindquarter of the sheep. It appears that a ß-adrenergically-induced vasodilatation in the hindquarters together with a probable increase in cardiac output was responsible for a proportionately larger increase in blood flow to the hind limb than was needed to meet the increased metabolic demand of the tissue. Therefore, oxygen extraction by the hindquarter was accordingly decreased.

The ß-adrenoceptor mediated processes can modulate metabolism in the whole body, organs of the portal drained viscera and hindquarters. The specific thermogenic mechanisms that are activated by the β_2-adrenoceptors in the portal drained viscera are uncertain but could involve adrenaline-induced β_2-adrenoceptor mediated relaxation (inhibition) of the gut smooth muscle. This inhibitory mechanism is ATP-dependent (Schneid et al. 1979, Zhang et al. 1990) and hence might increase oxygen consumption. Additionally, the ion transport across the mucosal epithelia and the triacylglycerol/fatty acid cycle could be influenced by the β_2-adrenoceptors (Smith 1984, Brooks et al. 1983). Recently Kelly et al. (1993) have shown that, in the gut of the sheep, the ouabain sensitive (hence Na^+/K^+-ATPase activity) metabolic activity may account for 18% of the oxygen consumption of fasted sheep. Although, the adrenergic receptor type that mediates the lipolytic action of adrenaline remains controversial (Raptis et al. 1981), Ole Hansen et al. (1990) showed that propranolol (nonselective) and ICI 118551 (β_2 blockers) inhibited adrenaline-induced increases in serum free fatty acids. It is possible that the increased portal drained viscera metabolic activity observed in this study is associated with adrenaline-induced β_2-adrenoceptor mediated omental fat lipolysis. Lipolysis from other peripheral fat depots could account for a significant portion of the adrenaline- and ß-blockers-induced changes in whole body oxygen consumption. In addition, the hypokalaemia reported in this experiment could indirectly be linked to the activity of the Na^+-K^+ pump (Elfellah and Reid 1987, Everts et al. 1988, Clausen 1990) and hence ion transport. Adrenaline results agreed with that of Bolton and Weekes (1986) in which adrenaline infused at

0.25 $\mu g.kg^{-1}.min^{-1}$ caused a marked reduction in serum $[K^+]$ in sheep. The ß-blockade response in sheep is in agreement with that reported in humans (Struthers and Reid 1984). In both cases the blockers abolished the adrenaline-induced hypokalaemia. The significant increase in $[K^+]$ during ICI 118551 (selective $ß_2$-blocker) infusion compared to control is also consistent with earlier observations (Struthers and Reid 1984). The latter may be evidence for a tonal effect of the sympathoadrenal system on the basal plasma K^+ levels exerted via the $ß_2$-adrenoceptors. Everts et al. (1988) suggests that in skeletal muscle the activation of the Na^+-K^+ pump by adrenaline may be associated with a significant clearance of K^+ from the plasma. Although, adrenaline may stimulate the Na^+-K^+ pump (Chinet and Clausen 1984, Everts et al. 1988, Clausen 1990) and the Na^+-K^+-ATPase activity is associated with oxygen consumption, there are no in vivo data that link K^+ uptake to increased oxygen consumption. Elfellah and Reid (1987) suggested that the fate of plasma K^+ may include uptake by cells, tissues or organs and in sheep cortisol has been shown to enhance kaliuresis (Bolton and Weekes 1986).

The blood flow values from this study are comparable to those in literature (Naylor et al. 1985, Christopherson and Brockman 1989). Additionally, portal blood flow measured by PAH and transit time probe are in good agreement (see Appendix 1, table A1.1). However, my values differ somewhat from a study with cattle (Huntington et al. 1990) in which a poor correlation between PAH dilution technique and transit time was observed. In the latter study, the lack of agreement between the two methods was attributed to anatomical structure of the portal vasculature of cattle and inadequate

space to accommodate the probe (Huntington et al. 1990). In sheep, the anatomy of the portal vasculature allowed for a comfortable placement of the probe. We concur with the suggestion that the portal vein blood flow in sheep could be determined accurately electronically (Bergman E.N., cited by Huntington 1990). Moreover, the ultrasonic transit time blood flow probes have been used successfully to validate the measurement of the portal vein blood flow by the magnetic resonance imaging technique in dogs (Pelc et al. 1992).

In the present study, the selective β_2-blocker (ICI 118551) was more effective in abolishing the adrenaline-induced hemodynamic effects. Since the blood vessels are associated with the junctional (neuronal) β_1-adrenoceptors (Ariens and Simonis 1983), the lack of a β_1-adrenoceptor hemodynamic advantage over the β_2 receptors (Ole Hansen et al. 1990) was unexpected.

The arterial whole blood amino acid concentrations for the thermoneutral control compare favourably with published data (Heitmann and Bergmann 1980a 1980b, Thompson et al. 1978). Adrenaline infusion was accompanied by a generalized tendency towards a small but nonsignificant decrease in whole blood arterial amino acid concentration. The β-blockers had no apparent effects on the responses to adrenaline. Therefore, these responses are only partially consistent with those of Shamoon et al. (1980). The explanations for these changes are uncertain. However, the change in citrulline could be associated with arginine and ornithine metabolism (Jungas et al. 1992). The latter are important constituents of the urea cycle in sheep (Heitmann and Bergmann 1980a). The lack of the inhibitory effects on the altered

amino acid responses by the blockers is unexpected in view of a previous study with humans (Shamoon et al. 1980) in which propranolol reversed the adrenaline-induced decrease in all amino acids except alanine. Perhaps the doses used in the present study where only three sheep were used were not optimal. Although the doses of ß-blockers used in this study were effective for reducing thermogenic responses, effective doses of β-adrenoceptor blockers have not been determined for amino acid responses in sheep. The data on amino acid changes show that adrenaline infusion exerts relatively small effects on whole blood arterial amino acid concentrations. However, those changes that were significant or approached significance in response to adrenaline were always a reduction in plasma concentrations suggesting a generalized increase in uptake of amino acid by tissues.

In conclusion, the data from this study suggests that functional hyperaemia was largely associated with the adrenaline-induced increase in portal drained viscera oxygen consumption. Further, both β_1- and β_2-adrenoceptors mediate a portion of whole body adrenaline-induced oxygen consumption in sheep. Additionally, propranolol (nonselective β-blocker), the selective ICI 118551 (β_2-blocker) and metoprolol ($ß_1$-blocker) effectively inhibited adrenaline-induced changes in the portal drained viscera of sheep, suggesting a role for both β-adrenoceptors in the mediation of thermogenic and metabolic processes in the gut.

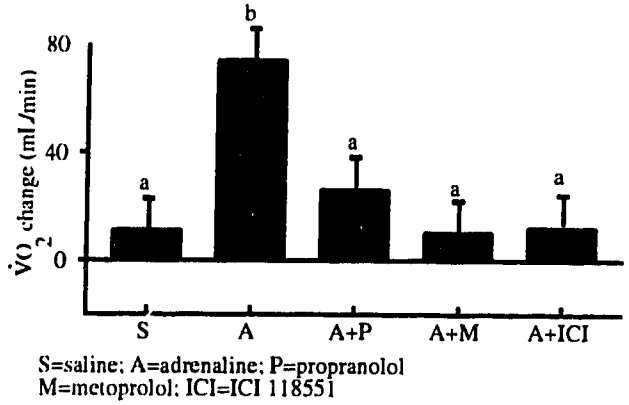
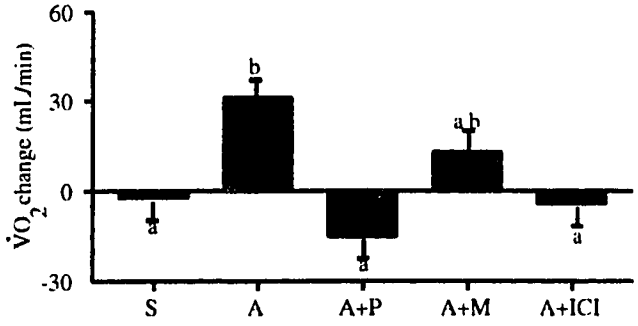


Figure 2.1. The effect of adrenaline infusion and beta-blockade on whole body oxygen consumption of the sheep. The basal $\dot{V}O_2$ was 236 mL/min (SE = 7.4). Means with similar letters were not different ($P<0.05$).



S=saline; A=adrenaline; P=propranolol
M=metoprolol; ICI=ICI 118551

Figure 2.2. The effect of adrenaline infusion and beta-blockade on the portal drained viscera oxygen consumption of the sheep. The basal $\dot{V}O_2$ was 61 mL/min (SE = 6). Means with similar letters were not different ($P<0.05$).

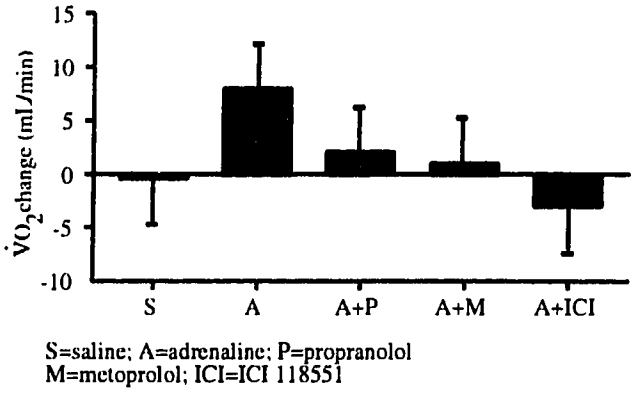


Figure 2.3. The effect of adrenaline infusion and beta-blockade on the hindquarter oxygen consumption of the sheep. The basal $\dot{V}O_2$ was 13 mL/min (SE = 3.1). Means with similar letters were not different ($P<0.05$).

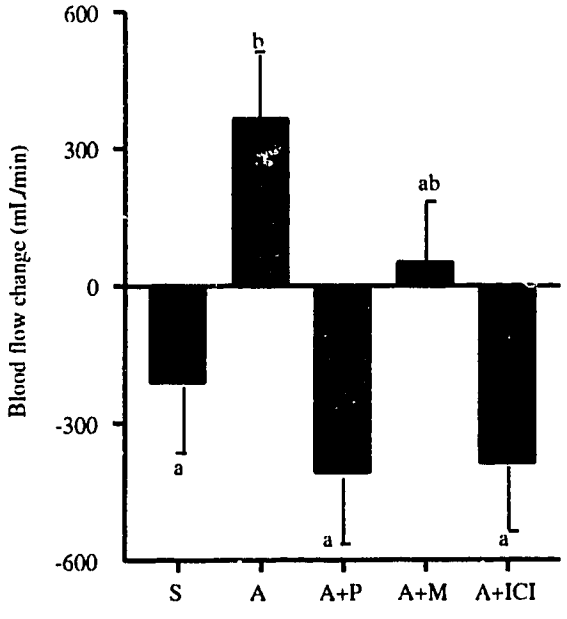


Figure 2.4. The effect of adrenaline infusion and beta-blockade on the portal vein blood flow of the sheep. The basal flow was 1780 mL/min (SE = 88). Means with similar letters were not different ($P<0.05$).

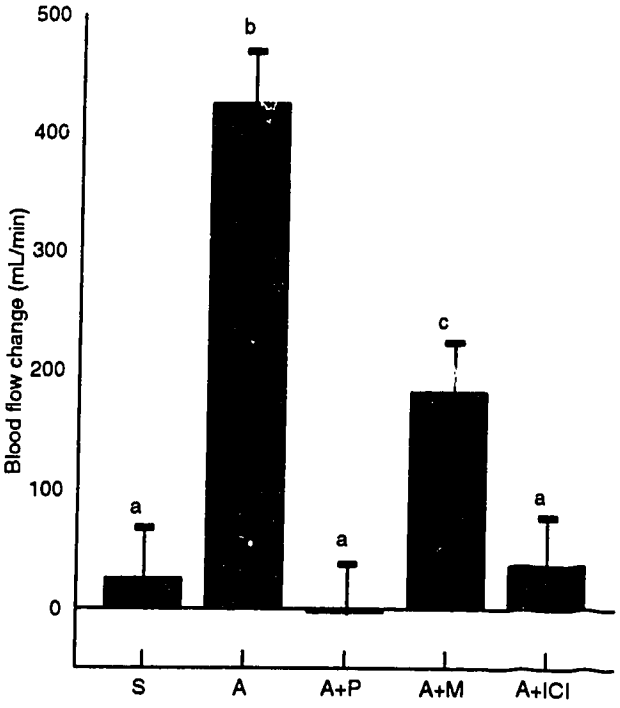


Figure 2.5. The effect of adrenaline and beta-blockade on the iliac artery blood flow of the sheep. The basal flow was was 240 mL/min (SE = 39). Means with similar letters were not different (P<0.05).

Table 2.1. Effect of adrenaline infusion and beta-blockers (P=propranolol a nonelective, M=metoprolol a β_1-blocker, ICI=ICI 118551 β_2-blocker) on O_2 blood parameters of sheep.

Item	S[1]	A	A+P	A+M	A+ICI	SE[4]
Haemoglobin						
g/dl.	8.7^{c}	9.4^{a}	9.2^{ab}	9.1^{bc}	9.5^{a}	0.2
Arterial O_2						
Saturation (%)	92.9	91.6	92.4	93.2	93.6	0.6
portal vein O_2						
Saturation (%)	63.6	67.1	62.5	68.5	62.5	3.7
Iliac vein O_2						
Saturation (%)	47.6^{bc}	63.6^{a}	48.7^{bc}	55.1^{b}	43.4^{c}	2.7
Arterial O_2						
content(mL/dL)	11.0^{b}	11.7^{ab}	11.6^{ab}	11.6^{ab}	11.9^{a}	0.2
portal vein O_2						
content(mL/dL)	7.2	8.2	7.9	8.7	8.2	0.4
Iliac vein O_2						
content(mL/dL)	5.8^{b}	7.8^{a}	6.4^{b}	7.2^{ab}	5.6^{b}	2.7
PDV[2] O_2 A-P						
(mL/dL)	3.7	3.6	3.8	2.9	3.6	0.3
HQ[3] O_2 A-V						
(mL/dL)	5.4^{ab}	3.9^{b}	5.4^{ab}	4.5^{b}	6.4^{a}	0.5
PDV O_2						
Extraction(%)	33.8	30.5	32.7	25.3	30.6	2.7
HQ O_2						
Extraction(%)	47.8^{a}	29.9^{c}	43.1^{ab}	33.0^{bc}	44.7^{a}	5.2
Plasma [K^+] mmols/l.[5]						
Arterial	5.10^{b}	4.73^{b}	5.06^{b}	4.79^{b}	5.36^{a}	0.29
Venous	4.77^{b}	3.75^{c}	4.58^{b}	4.68^{b}	5.33^{a}	0.33
K^+ A-V	0.34	0.98	0.46	0.13	0.04	0.44

[1]Saline (Control)
[2]Portal drained viscera
[3]Hindquarter
[4]Pooled SE
[abc]Within rows values with different superscript are different (P<0.05).
[5]Plasma volume correction based on hematocrit and average change in haemoglobin.

Table 2.2. Effect of adrenaline infusion and ß-adrenoceptor blockade (P=propranolol a nonselective ß-blocker; metoprolol a selective $ß_1$-blocker; ICI 118551 a selective $ß_2$-blocker) on arterial whole blood amino acid ($\mu M.L^{-1}$) profile of sheep.

AMINO							P>F	
ACID	[1]C	A	A+P	A+$ß_1$	A+$ß_2$	SE[2]	TRT	TRT*T[3]
ASP	26.2	23.6	18.9	23.4	21.4	7.6	.603	.615
GLU[4]	307.0	282.2	210.1	281.2	300.9	85.0	.269	.112
ASN	40.2	31.7	29.8	29.3	32.0	4.0	.014	.554
SER	49.5	23.1	13.8	24.4	17.5	17.8	.071	.912
HIS	89.0	64.3	65.3	67.8	87.1	13.2	.219	.270
GLY	720.1	533.9	542.8	543.8	588.2	261.7	.228	.036
THR	70.8	50.3	45.7	53.1	83.2	3.3	.381	.872
CIT	240.3	188.3	141.8	154.0	129.2	48.8	.039	.446
ARG	155.2	155.9	97.1	127.9	83.9	73.6	.155	.313
TAU	72.9	88.6	100.5	85.6	91.8	79.9	.584	.862
ALA	158.8	191.2	167.1	215.3	152.3	79.5	.263	.529
TRY	88.0	75.4	44.1	57.7	82.9	16.1	.546	.125
TRP	25.9	13.7	17.8	18.2	24.4	3.7	.104	.052
MET	12.8	8.1	7.0	12.7	13.8	3.2	.102	.200
VAL	203.9	182.8	136.9	155.5	154.9	15.4	.089	.454
PHE	49.5	42.6	33.7	45.1	53.5	8.6	.109	.081
ILE	86.0	79.4	55.7	68.3	68.4	8.2	.083	.135
LEU	130.2	125.8	98.4	125.2	124.6	17.9	.461	.177
ORN	158.1	140.3	120.4	159.8	126.9	36.0	.764	.060
LYS	157.6	120.7	101.6	148.5	129.2	31.5	.550	.214
TOTALS	2842.2	2421.3	1934.7	2397.5	2165.6	406.7	.115	.082
TOTAL[4]								
BCAA	420.1	387.3	291.0	348.9	347.9	35.8	.131	.236

[1]C = control (saline)
[2]SE = pooled standard error
[3]LSmeans and SE not shown
[4]Include values for glutamine
[4]Total branched chained amino acids

2.7. REFERENCES

Ariens E.J. and Simonis A.M. 1983. Physiological and pharmacological aspects of adrenergic receptor classification. Biochem. Pharmacol. 32 (10):1539-1545).

Barnes R.J., Comline R.S. and Dobson A. 1986. Control of splanchnic blood flow. In L.P. Milligan, W.L. Grovum and A. Dobson, eds. Control of Digestion and Metabolism in Ruminants. Prentice-Hall Englewood Cliffs, NJ. pp 41-59.

Bearn A.G., Billing B. and Sherlock S. 1951. The effect of adrenaline and noradrenaline on hepatic blood flow and splanchnic carbohydrate metabolism in man. J. Physiol. 115:430-441.

Bolton S.C. and Weekes T.E.C. 1986. The response of plasma minerals, free fatty acids and glucose to adrenaline and cortisol infusion in sheep. J. Agric. Sci. 106:209-217.

Brikas P., Bueno L. and Fioramonti J. 1989. Central and peripheral beta-adrenergic influences on reticulo-rumen and upper-gut myoelectrical activity in sheep. J. Vet Pharmacol. Therap. 12:430-437.

Brooks B.J., Arch J.R.S. and Newsholme E.A. 1983. Effect of some hormones on the rate of the triacylglycerol/fatty-acid substrate cycle in adipose tissue of the mouse in vivo. Biosc. Reports 3: 263-267.

Chinet A. and Clausen T. 1984. Energetics of active sodium-potassium transport following stimulation with insulin, adrenaline or salbutamol in rat soleus muscle. Pflugers Arch. 401:160-166.

Choo J.J., Horan M.A., Horan R.A., Rothwell N.J. 1992. Anabolic effects of clenbuterol on skeletal muscle are mediated by β_2-adrenoceptor stimulation. Am. J. Physiol. 263:E50-E56.

Christopherson R.J. and Brockman R.P. 1989. Effect of feeding and a beta-adrenergic blocking agent on oxygen consumption by the portal drained viscera, liver and hindquarter of sheep. EAAP 43:147-150.

Clausen T. 1990. Significance of Na^+-K^+ pump regulation in skeletal muscle. NIPs Vol.5:148-151.

Eldostone D.I. and Holzman I.R. 1981. Oxygen consumption by the gastrointestinal tract and liver in conscious newborn lambs. Am. J. Physiol. 240:G297-G304.

Elfellah M.S. and Reid J.L. 1987. The role of skeletal muscle ß-adrenoceptors in the regulation of plasma potassium. J. Auton. Pharmac. 7:175-184.

Emorine L.J., Marullo S., Brient-Sutren M.M., Patey G., Tate K., DeLavier-Klutctko C., and Strosberg A.D. 1989. Molecular characterization of the human $ß_3$-adrenergic receptor. Science 245:1118-1121.

Eriksson L.S., Olsson M. and Bjorkman O. 1988. Splanchnic metabolism of amino acids in healthy subjects: effects of 60 hours fasting. Metabolism 37(12): 1159-1162.

Everts M.E., Retterstol K. and Clausen T. 1988. Effects of adrenaline on excitation-induced stimulation of the sodium-potassium pump in rat skeletal muscle. Acta Physiol. Scan. 134:189-198.

Godby G. and Gregory P. 1992. Datagrabber. Department of Animal Science, University of Alberta.

Graham A.D. and Christopherson R.J. 1981. Effects of adrenaline and noradrenaline on the heat production of warm- and cold-acclimated sheep. Can. J. Physiol. Pharmacol. 59:985-993.

Grayson J. and Oyebola D.D.O. 1983. The effect of catecholamines on intestinal glucose and oxygen uptake in the dog. J. Physiol. 343:311-322.

Harris P.M., Garlick P.J. and Lobley G.E. 1989. Interactions between energy and protein metabolism in the whole body and hind limb of the sheep in response to intake. EAAP 43:167-170.

Harmon M.C. 1992. The effects of beta-antagonist and epinephrine infusion on warm- and cold-acclimated sheep. M.Sc. Thesis, University of Alberta.

Heitmann R.N. and Bergman E.N. 1980a. Transport of amino acids in whole blood and plasma of sheep. Am. J. Physiol. 239:E242-E247.

Heitmann R.N. and Bergman E.N. 1980b. Integration of amino acid metabolism in sheep: effects of fasting and acidosis. Am. J. Physiol. 239:E248-E253.

Hidari H., Christopherson R.J. and Ole Miaron J.O. 1991. Effects of a cold environment and alpha and beta adrenergic blocking agents on metabolic rate in steers and beta blockade on oxygen consumption in sheep. EAAP 58:76-79.

Huntington G.B., Eisemann J.H. and Whitt J.M. 1990. Portal blood flow in beef steers: comparison of techniques and relation to hepatic blood flow, cardiac output and

oxygen uptake. J. Anim. Sci. 68:1666-1673.

Jones B.N. and Gilligan J.P. 1983. o-Phthaldehyde precolum derivatization and reverse phase high perfomance liquid chromatography of polypeptide hydrosylates and physiological fluids. J. Chromatog. 226:471-482.

Jungas R.L., Halpern M.L. and Brosnan J.T. 1992. quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. Physiol. Rev. 72(2):419-449.

Katz M.L. and Bergman E.N. 1969. Simultaneous measurements of hepatic and portal venous blood flow in the sheep and the dog. Am. J. Physiol. 216:946.

Kelly J.M., Southorn B.G., Kelly C.E., Milligan L.P. and McBride B.W. 1993. Quantification of in vitro and in vivo energy metabolism of the gastrointestinal tract of fed and fasted sheep. Can. J. Anim. Sci. 73:855-868.

Kelly J.M. 1987. Digestibility of amino acids in sheep in cold . Msc. Thesis, University of Alberta.

Lobley G.E. 1991. Organ and tissue metabolism: present status and future trends. EAAP 58:80-87.

MacRae J.C., Skine P.A., Connel A., Buchan V. and Lobley G.E. 1988. The action of ß-agonist clenbuterol on protein and energy metabolism in fattening wether lambs. Br. J. Nutr. 59:457-465.

McIntyre A.S., Thompson D.G., Days S., Durnham W.R. and Walker E.R. 1992. Modulation of human upper intestinal nutrient transit by a beta adrenoceptor mediated pathway. Gut 33: 1062-1070.

Motulsky H.J. and Insel P.A. 1987. In vitro methods for studying human adrenergic receptors: Methods and applications. In P.A. Insel (ed). Adrenergic Receptors in Man. pp 139-160, Marcel Dekker, Inc. NY.

Naylor J.M., Freeman D.E., Richardson D. and Kronfeld D.S. 1985. Measurement of portal and hepatic blood flows using a modified method for surgical placement of catheters in the major splanchnic vessels of sheep. Am. J. Vet. Res. 46:202.

Nijkamp F.P., Engels F., Hendricks P.A.J. and Van Oosterhout A.J.M. 1992. Mechanisms of ß-adrenergic receptor regulation in lungs and its implications for physiological responses. Physiol. Rev. 72(2):323-362.

O'Brien J.D., Thompson D.G., Days S.J., Burnham W.R. and Walker E. 1989. Perturbation of upper gastrointestinal transit and antroduodenal motility by

experimentally applied stress: The role of beta-adrenoceptor mediated pathways. Gut 30:1530-1539.

Ole Hansen, Johansson B.W. and Nilsson-Ehle P. 1990. Metabolic, electrocardiographic and hemodynamic responses to increased circulating adrenaline: Effects of selective and nonselective beta adrenoceptor blockade. Angiology 41(3):175-188.

Pelc L.R., Pelc N.J., Rayhill S.C. Castro L.J, Glover G.R., Herfkens R.J. Miller D.C. and Jeffrey R.B. 1992. Arterial and venous blood flow: Noninvasive quantitation with MR imaging. Radiology 185: 809-812.

Prewitt L.R., Jacobson D.R., Hemken R.W. and Hatton R.H. 1975. Portal blood flow as measured by the doppler shift technique in sheep fed chopped forages. J. Anim. Sci. 41:596.

Raptis S., Rosenthal J. and Welzel D. 1981. Effect of cardioselective and non-cardioselective beta blockade on adrenaline-induced metabolic and cardiovascular response in man. Eur. J. Clin. Pharmacol. 20:17-22.

Reeds P.J., Hay S.M., Dorward P.M. and Palmer R.M. 1988. The effect of ß-agonists and antagonists on muscle growth and body composition of young rats (Rattus sp). Comp. Biochem. Physiol. 89c:337-341.

Schaefer A.L., Young B.A. and Turner B.V. 1982. The effect of cold exposure on blood flow distribution in sheep J. Therm. Biol. 7:15-21.

Schneid C.R., Honeyman T.W. and Fay F.S. 1979. Mechanism of ß-adrenergic relaxation of smooth muscle. Nature Lond. 277:32-36.

Shamoon H., Jacob R. and Sherwin R.S. 1980. Epinephrine-induced hypoaminocidemia in normal and diabetic man: effect of β-blockade. Diabetes 29:875-881.

Souba W.W. and Douglas W.D. 1983. Postoperative alteration of arteriovenous exchange of amino acids across the gastrointestinal tract. Surgery 94(2):342-350.

Steel R.G.D. and Torrie J.H. 1980. In Principles and Procedures of Statistics, McGraw-Hill Book Co., New York, N.Y.

Smith P.A. 1984. Does the electrogenic Na^+/K^+ pump play a role in the neuronal effects of catecholamines? TIPs October: 422-425.

Struthers A.D. and Reid J.L. 1984. Adrenaline causes hypokalaemia in man by $ß_2$-

adrenoceptor stimulation. Clin. Endocrinol. 20:409-414.

Thorin D., Golay A., Simonsen D.C., Jequer E., Felber J.P. and DeFronzo R.A. 1986. The effect of selective beta adrenoceptor blockade on glucose-induced thermogenesis in man. Metabolism 35(6):524-528.

Thompson G.E., Manson W., Clarke P.L. and Bell A.W. 1978. Acute cold and metabolism of glucose and some of it's precursors in the liver of the fed and fasted sheep. Quart. J. Expt. Physiol. 63:189-199.

Young B.A., Kerrigan B. and Christopherson R.J. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55:17-22.

Young B.A., Fenton T.W. and McLean J.A. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol. 56(4):1120-1125.

Zhang L., Jensen R.T. and Maton P.N. 1990. Characterization of ß-adrenoceptors on smooth muscle cells from guinea pig stomach. Am. J. Physiol. 259:G436-G442.

3. Whole Body and Organ Thermic Effect of Acute Cold Exposure and Feeding in Sheep: Effects of Nonselective and Selective ß-Adrenoceptor Blockade[2]

3.1. INTRODUCTION

Increased thermogenesis in response to cold exposure (Graham and Christopherson 1981) and feeding (Christopherson and Brockman 1989) have been demonstrated in sheep. A portion of the increased metabolic activity in the cold could involve β-adrenoceptor processes (Webster 1969). Infusion of catecholamines (adrenaline or noradrenaline) and cold exposure causes an increase in heat production in sheep (Graham and Christopherson 1981). In sheep, propranolol a nonselective β_1- and β_2-adrenoceptor antagonist inhibits whole body thermogenic responses associated with severe, but not mild, acute cold exposure (Webster 1974). This elevation in thermogenesis that occurs in response to both catecholamines and cold exposure is evoked by β-adrenoceptor agonists (Graham and Christopherson 1981, Chapter 2) and may be blocked by β-adrenoceptor antagonists (Webster et al. 1969, Chapter 2), and hence constitutes a basis for β-adrenoceptor system involvement.

Ingestion of food results in elevated heat production. In rats, the feed-induced increase in thermogenesis is inhibited by the β-blocker propranolol (Rothwell et al. 1980), thus suggesting an involvement of the β-adrenoceptors. Webster and Hays (1968) did not observe any significant suppression of the energy cost of eating in sheep by the ß-blocker propranolol. However, Dauncy and Ingram (1979) reported a

---

[2] Version of this chapter has been published as an abstract. Miaron J.O. and Christopherson R.J. 1993. J.Anim. Sci. 71(suppl. 1):118.

significant propranolol-induced reduction in the post-feeding heat production of the pig. Christopherson and Brockman (1989) showed that the β-adrenoceptor mediated processes modulates post-prandial thermogenesis in the whole body, portal drained viscera and other tissues in sheep. Thus, there is not complete agreement in the literature with respect to the role of β-adrenoceptors in regulating the heat production response to feeding.

Little information exists on the portal drained viscera and hindquarter tissue metabolic responses to cold and feeding. In addition, to the roles of the β-adrenoceptors and the adrenoceptor subtypes that may mediate these responses have not been examine in sheep. Hence, in this study, the differential roles of β_1- and β_2-adrenoceptors in modulating the whole body and organ cold- and feed-induced thermogenesis were investigated. Blood parameters that could be associated with cold-induced thermogenesis including whole blood amino acid concentration were also measured since a decrease in whole blood amino acid concentration has been reported during fasting (Ericksson et al. 1988) and lower plasma levels are associated with trauma- and cold-related stressors (Souba and Dauglas 1983, Kelly 1987). Additionally, epinephrine infusion caused a reduction of most plasma amino acids in normal and diabetic man (Shamoon et al. 1980) which was reversed by propranolol and in some amino acids in sheep (see Chapter 2), thus suggesting an effect of the β-adrenoceptor system in protein and amino acid metabolism.

3.2. MATERIALS AND METHODS

3.2.1. Animals

Four whether sheep weighing (60±2 Kg) were kept in metabolism stalls in a thermoneutral controlled environment (16±3 °C, 12 h light-dark cycle). The animals were fed 1.0 kg/d of alfalfa pellets (crude protein = 16%, DM = 93% and gross energy 4.4 Kcal/g DM) and 0.5 kg/d of alfalfa cut hay (crude protein = 11% and DM = 90%). Prior to experimentation the coolers were turned on and the sheep were exposed to a mild cold (4±2 °C) for 24 hours. Measurement of whole body and organ oxygen consumption were made for a period of 4 h commencing approximately 18 h after the previous feeding and 24 h after the start of cold exposure. For the feeding experiment, animals were exposed to a thermoneutral environment (16±3 °C) and fed 1.0 kg of the alfalfa pellets (crude protein = 16%, DM = 93% and gross energy 4.4 Kcal/g DM). Whole body and portal drained viscera oxygen consumption was recorded for 1 h before feeding starting approximately 18 h after the last feed and for 4 h after feeding. Water and salt were available ad libitum.

3.2.2. Implantation Of Blood Flow Probes

Anaesthesia was induced with intraval (Thiopentone Sodium, M.I.T.C. Pharmaceutical, Cam., ON) and maintained by halothane (2%) administered via endotracheal intubation. The sheep was then placed in lateral recumbency and the abdominal cavity was opened at the paralumbar fossa. The portal vein and iliac artery were visually identified and a 24S and 12S Transit time ultrasonic blood flow probes (Transonic

systems, Inc., Ithaca, NY) were respectively placed around them as described in chapter 2. The exposed common trunk of the portal vein was catheterized via a branch of the mesenteric vein. The position of the silastic catheter (id 0.06 in and .125 in od; Dow Corning Corp. Michigan USA) in the portal vein was confirmed by palpation. Additional catheters (silastic) were placed into the iliac vein and mesenteric artery.

The catheters were flushed daily with heparinized sterile saline solution. Long acting terramycin and analgesic (Butorphanol, Ayerst, Winipeg, MB) were given i.v. at 0.1 mg/kg on the day of surgery and analgesic was given on the first day post-operation. The animals recovered quickly from surgery and were feeding within 3-4 h. The recovery was improved by 5 % dextrose (Baxter Corp. Toronto, ON) administered slowly intravenously. The sheep were allowed 10 d recovery period before any measurements were made. During this period the animals were accustomed to experimental manipulation and were trained to feed and feel comfortable in the metabolic hood. Treatments were randomly applied as shown under the experimental protocol. Twenty four hours before the start of an infusion experiment polyvinyl catheters (id 0.04 in and 0.07 in od; Norton Performance Plastic, Akron, Ohio) were inserted into both jugular veins of each animal. The blood flow measurements and sampling were made from a work station as described in Chapter 2.

3.2.3. Experimental Protocol

The infusions were randomly applied and designed such that each animal acted as it's own control. A two-day break was allowed between treatments. A 3 h control (vehicle;

sterile 0.9% saline + 0.1mg/100ml of ascorbic acid as an antioxidant) infusion preceded the test infusions viz; propranolol (Sigma Chemicals Co. St., Louis. Mo) a nonselective ß-blocker, metoprolol (Sigma Chemicals Co. St., Louis, Mo) a selective β_1-blocker and ICI 118551 (ICI Pharmaceuticals, Macclesfield, England) a selective β_2-blocker. The vehicle was made up into dark bottles and the ß-blockers were administered via the jugular vein such that a priming dose of propranolol (1 mg kg^{-1}), metoprolol and ICI 118551 (both at 100 µg kg^{-1}) was followed by a continuous infusion of 5 (Christopherson and Brockman 1989), 1.5 (Thorin et al. 1986) and 2.5 (Harmon 1992) µg kg^{-1} min^{-1} respectively. The infusions were initiated after a 1 hour pre-infusion sample was collected.

3.3. MEASUREMENTS

3.3.1. Oxygen Consumption

The oxygen consumption was determined by an open-circuit calorimetry apparatus (Young et al. 1975) connected to a ventilated hood to which the sheep were previously accustomed. Ventilation rate of the head hood was read from a flowmeter (Rotometer, Fisher and Porter, Warminster, PA) and the oxygen concentration difference between incoming and outgoing respired air, from a single channel oxygen analyzer (Servomix 540A Sussex, England). The data was acquired by a computerized program as described in Chapter 2 . The oxygen consumption was the product of ventilation rate (mL min^{-1}) at STP and oxygen decrement. The measuring system was calibrated with nitrogen as a zero gas and by the iron burn method of Young et al. (1984).

3.3.2. Blood flow

The Blood flow was measured with transit time ultrasonic blood flow probes connected to a blood flow meter (Transonic Systems, INC., Ithaca NY). The blood oxygen saturation and haemoglobin was determined in duplicate samples with a OSM 2 hemoximeter (Radiometer, Copenhagen, Denmark). Prior to infusion a pre-infusion zero time measurement was made and thereafter sampling was at scheduled intervals of 1 h. Organ (portal drained viscera and hindquarter) oxygen consumption was the product of blood flow and the A-V oxygen difference.

3.3.3. Amino Acid Analysis

The whole blood was deproteinized on ice with 3 % TCA solution. The whole blood amino acids were then derivatized using the O-phthaldialdehyde method (Jones and Gilligan 1983) and quantified using a Varian 5000 high perfomance liquid chromatograph and a Shimandzu RF-535 fluorescence detector (Shimadzu Scientific Instruments, Inc.). Samples were injected using a varian 9090 autosampler (Varian Associates, Inc. 1985). A supelcosil 3 micron LC-18 reverse phase column (4.6 x 150 mm; Oakville, On) equipped with a guard column (4.6 X 50 mm) packed with Supelco LC-18 reverse phase packing (20-40 micron) was used to separate the amino acids. The data was acquired and processed by the Shimadzu Ezchrom Chromatography Data System (Shimandzu Scientific Instruments, Inc.).

3.4. DATA ANALYSIS

The data for the cold experiment is presented as means plus standard error. The analysis for the repeated measures ANOVA was computed by the GLM procedure of SAS (SAS Inc., Gary, N.C.). The animal*treatment was the error term for the treatments. When ANOVA was significant, the treatment effects at each time were compared by the least significant difference. Data for the feeding experiment and absolute values for the blood parameters were analyzed as a randomized block design where animals were blocks. Computations were performed using the GLM procedure of SAS (SAS Inc., Gary, N.C.). Treatment was tested against animal * treatment and the means were compared by the SNK (Steel and Torrie 1980). For the amino acid data, the p values for treatment and treatment*time are shown.

3.5. RESULTS

Due to loss of catheter patency, data for portal drained viscera and hindquarter oxygen consumption was successfully collected from 4 and 3 animals, respectively.

3.5.1. Thermic Effects Of Acute Cold Exposure And ß-blockade

3.5.1.1. Whole body

Acute cold exposure (4°C) caused a 60% increase in oxygen consumption. Compared to the thermoneutral environment, acute cold resulted in a consistent and significant ($P<0.05$) elevation for the entire measurement period (Figure 3.1). All the blockers appeared to produce some suppression of the cold-induced whole body responses but

only the β_2-adrenoceptor blocker significantly reduced the cold-induced increase in oxygen consumption during the second hour of infusion.

3.5.1.2. Portal drained viscera

Acute cold exposure had no effect on the portal drained viscera oxygen consumption (Figure 3.2). Similarly, none of the β-blockers influenced the portal drained viscera oxygen consumption.

3.5.1.3. Hindquarters

The oxygen consumption by the hindquarters of the sheep was significantly increased by acute cold exposure (Figure 3.3) during the preinfusion period and during the first hour of infusion. The β-blockers did not produce any consistent change in the hindquarters metabolism during cold exposure although, propranolol and the selective β_2-adrenoceptor blocker ICI 118551 appeared to decrease hindquarter oxygen consumption very slightly.

3.5.2. Effects Of Acute Cold Exposure On Blood Parameters

Acute cold exposure and the β-blocking agents had no effect on the portal vein blood flow. However, cold-induced hemodynamic effects in the hindquarters were reduced ($P<0.05$) by propranolol and ICI 118551 by 31 and 43 % respectively. The reduction in response to metoprolol was 29% and not statitistically different from control, propranolol and ICI 118551 effects (Table 3.1).

There was a significant $P<0.05$ increase in haemoglobin concentration in response to acute cold exposure. This increase was not affected by the β-blockers (Table 3.2). The iliac vein oxygen saturation was slightly but not significantly lower during acute cold exposure compared to thermoneutral. However, iliac vein oxygen saturation values were significantly reduced by the β-blockers, resulting in a similar trend for the iliac vein oxygen content (Table 3.2). The hindquarters A-V oxygen difference increased in response to cold exposure and remained elevated even after the β-adrenoceptor blockade. The latter resulted in a small elevation in hindquarters oxygen extraction (%) for the sheep exposed to an acute cold environment, but, again the blockers had no significant effect on the increase (Table 3.2).

3.5.3. Thermic Effects of Feeding

3.5.3.1. Whole body

Results on the thermic effects of feeding are for the whole body and portal drained viscera of 3 sheep and are in Table 3.3. The hindquarter data was not successfully obtained from most of the animals due to loss of catheter patency. Feeding caused a 40% increase in whole body oxygen consumption. Although, the blockers appeared to induce a numerically reduced response, none of the β-adrenoceptor blockers caused a significant decrease in whole body oxygen consumption.

3.5.3.2. Portal drained viscera

There was a nonsignificant increase in oxygen consumption by the portal drained

viscera in response to feeding. Although, all the ß-blockers showed a trend towards a numerical decrease in portal drained viscera oxygen consumption, this effect was not statistically significant. However, the portal vein blood flow was significantly elevated in response to feeding. This increase was not responsive to β-adrenoceptor blockade. None of the blood parameters were affected by the treatment regime imposed.

3.5.3.3. Arterial whole blood amino acid concentration

The arterial whole blood amino acid concentrations were generally and numerically lower in cold exposed sheep compared to those in a thermoneutral environment. This trend was significant for citrulline (Tables 3.4) and the arterial total amino acid concentration approached significance (P≤0.1). Tryptophan showed a significant (P<0.05) treatment by time interaction while ornithine approached significance (P<0.1) which was difficult to interpret. The amino acid A-P difference and fluxes across the portal drained viscera were not affected by either the acute cold exposure or the ß-adrenergic blockers (Tables 3.4). The portal drained viscera A-V differences net fluxes associated with large variations. Therefore, meaningful conclusions could not be made from this data which is presented in Appendix 5 (Table A5.1-A5.3). The net fluxes were the product of A-V differences and blood flow (Appendix 6; Table 6.1b). In this appendix, the negative sign in front of net flux values denotes a release of the amino acid into the portal blood.

3.6. DISCUSSION

The significant increase in whole body oxygen consumption in response to cold is consistent with previous observations reported in the literature (Thompson et al.1975, Webster et al. 1969). These authors showed that cold exposure caused at least a two-fold increase in metabolic activity, a change comparable to the response observed in the present study. In the present study, propranolol slightly but not significantly blocked the cold-induced increase in oxygen consumption, whereas in Webster et al. (1969), propranolol caused a significant decrease in heat production of sheep exposed to a severe cold stress (-30°C). The lack of significant change in response to the nonselective blocker could be explained in part by the different experimental conditions used, i.e., severe cold (-30°C) vs mild (4°C) exposure as was found by Webster (1974). In addition, in laboratory animals, the significant inhibition of clenbuterol-induced anabolic responses by the β_2-blocker with only a trend for propranolol at lower doses have been attributed to the higher affinity of the former compared to the latter for the β_2-adrenoceptors (Choo et al. 1992). On the other hand, shivering or increased muscle tone were likely contributors towards whole body and hindquarter oxygen consumption. Shivering response mediated by somatic motor nerves may not be blocked by propranolol but may be facilitated by noradrenaline effects mediated via α-adrenoceptors (Zeisberger 1978). The latter author showed that intrahypothalamic infusion of noradrenaline resulted a shift of the shivering threshold to a higher temperature which was antagonized by phentolamine. However, propranolol may inhibit substrate mobilization that normally supports thermogenesis.

Therefore, in the present experiment, selective β-adrenoceptor blockers showed a similar trend to propranolol (a decrease) but, the β_2-adrenoceptor blocker (ICI 118551) reduced (P=0.02) whole body oxygen consumption by 24 % during the second hour of infusion. A whole body β_2-adrenoceptor predominant response could be associated with cold-induced metabolite/substrate mobilization from body organs including the skeletal muscles, liver and adipose tissue. The significance of the β_1-adrenoceptor-mediated effects may be limited since higher doses (2.5, 5.0 and 10 $\mu g.kg^{-1}.min^{-1}$) were ineffective in cold acclimated sheep (Harman 1990). Skeletal muscle β_2-adrenoceptor mediated metabolic responses have been reported (Meyer and Stull 1971). These thermogenic responses apparently could involve substrate cycles (Brooks et al. 1983) and the contribution of the Na^+-K^+ pumps in skeletal muscle which is predominantly under the influence of the β_2-adrenoceptor (Reid et al. 1986) could also be important. Reid et al. (1986) reported that ICI 118551 significantly affected the activity of the pump by causing a reduction in plasma K^+ concentration. Additionally, the activity of the ouabain-sensitive respiration (that associated with the Na^+-K^+ pump) is enhanced by cold exposure (Gregg and Milligan 1982).

The portal-drained-viscera oxygen consumption was not affected by the acute cold exposure confirming the data reported by Thompson et al. (1975) in which the liver but not the portal drained viscera was involved in the cold-induced thermogenesis. The nonselective and selective β-blockers had no apparent effects on portal drained viscera oxygen consumption. Our data differ somewhat from that of Thompson et al. (1975) in that we observed no increase in portal vein blood flow in

response to cold exposure. However, is consistent with their later study (Thompson et al. 1978) in which a small nonsignificant increase in the portal blood flow in response to an acute cold exposure was observed.

The hindquarter oxygen consumption was elevated in the cold during the preinfusion and 1st hour of infusion. This response was expected because the hind limb may contribute 15% of the increase in whole body oxygen consumption after a cold treatment (Bell et al. 1974). Since the predominant adrenoceptor population of skeletal muscle is the β_2-adrenoceptor, a role for this subtype of adrenergic receptors in modulating hindquarter oxygen consumption was expected. However, the nonselective and the β_2-adrenoceptor blocker induced only a small but statistically non-significant decrease in hindquarter oxygen consumption. This could mainly be due to a large between animal variability in oxygen consumption estimation by the blood flow and A-V oxygen difference method.

The increase in iliac artery blood flow was likely due to the dilatatory effects of the β_2-adrenoceptors on vascular smooth muscles. Hindquarters blood flow has been shown to increase in response to a β_2-agonist, clenbuterol (Eiseman et al. 1988). This data is consistent with this finding in that propranolol, a nonselective, and ICI 118551, a selective β_2-blocker but, not metoprolol the β_1-adrenoceptor blocker, effectively abolished the cold-induced hemodynamic effect.

The increase in haemoglobin concentration in response to acute cold exposure may reflect splenic contraction, normally ascribed to α-adrenergic mediation (Ignarro and Titus 1968). Therefore, the β-adrenoceptor blockade was expected to have no

effect on the increase in haemoglobin concentration. As the result of the increase in haemoglobin concentration, there was a subsequent increase in the arterial oxygen content. For the hindquarters, the mean A-V oxygen difference, oxygen venous saturation and the oxygen extraction coefficient changed predictably.

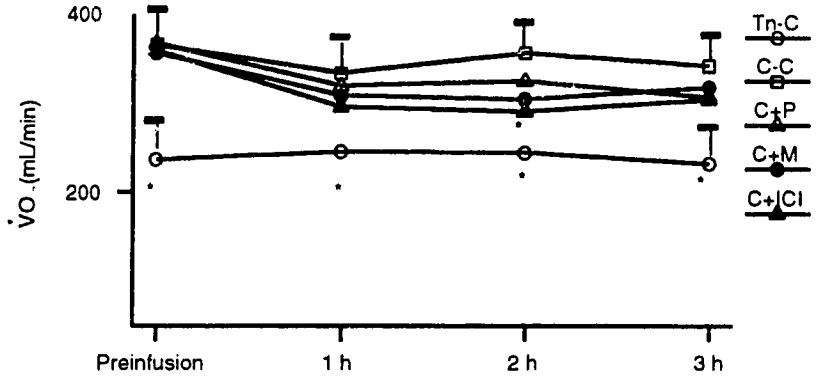
The increase in whole body oxygen consumption in response to feeding agrees favourably with published results (Webster and Hays 1968, Thompson et al. 1975, Christopherson and Brockman 1989). The lack of response to the β-blockers only partially agrees with the data of Christopherson and Brockman (1989) but is consistent with results reported by Webster and Hays (1968). In the latter study, propranolol did not affect the increase in energy expenditure of the sheep during eating. In the present study, the nonselective and the selective β-blockers showed a tendency to produce a numerical decrease in oxygen consumption which was not statistically significant. This trend was present for both whole body and the portal drained viscera oxygen consumption.

In response to feeding, portal vein blood flow was elevated. This was probably associated with nutrient uptake and transport to the liver for further processing. There was no evidence for the role of the β-adrenoceptor system in modulating the portal drained viscera hemodynamic effects in response to feeding.

The arterial whole blood amino acid concentrations for the thermoneutral control and cold controls compare favourably with published data (Thompson et al. 1978, Heitmann and Bergmann 1980a 1980b, Kelly 1987). The arterial whole blood amino acid concentrations were generally and numerically lower in cold exposed sheep

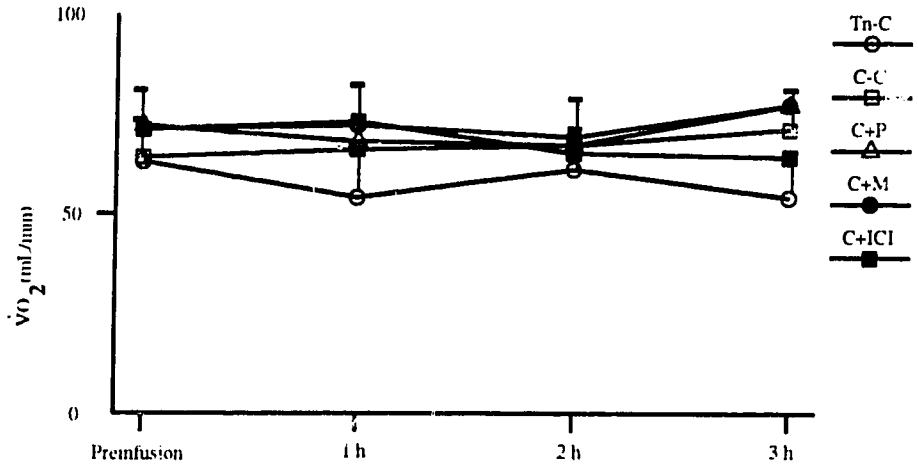
compared to those in a thermoneutral environment. The latter observation is in agreement with previous reports by Thompson et al. (1978). There were no significant treatment effects due to cold, with or without ß-adrenergic blockers, however, the changes in ornithine and citruline may be associated with the activity of the urea cycle. The data on amino acid changes show that acute cold exposure may exert relatively small effects on whole blood arterial amino acid concentrations. However, those changes that were significant or approached significance in response to acute cold exposure were always a reduction in plasma concentrations suggesting a generalized increase in uptake of amino acid by tissues.

In conclusion, this data suggest that during acute cold exposure, but not feeding, a portion of the whole body oxygen consumption of the sheep is mediated by β-adrenoceptor processes of which the β_2-adrenoceptor effects may predominate. This data also confirms previous conclusion by other workers that cold exposure had no effect on the oxygen consumption of the portal drained viscera of the sheep (Thompson et al. 1975). Evidently, the endogenous, unlike the exogenous adrenaline (Chapter 2) did not induce an increase in portal drained viscera metabolism in sheep during an acute cold exposure. One cannot, however, rule out a role for the adrenoceptor processes in the modulation of portal drained viscera metabolism in sheep exposed to a prolonged thermal exposure which is usually associated with increased gut mass, since data on the possible modulatory role of the adrenoceptor system in the gut of ruminants exposed to a prolonged cold exposure is lacking.



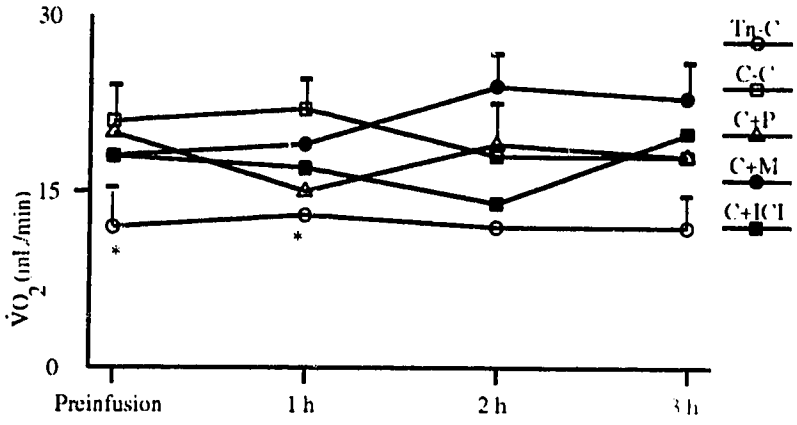
Tn-C = thermoneutral control; C-C = cold control; C+P = Cold+propranolol;
C+M = cold+metoprolol; C+ICI = cold+ICI 118551
*P<0.05 compared to C-C

Figure 3.1. The effect of acute cold exposure and beta-blockade on whole body oxygen consumption of the sheep



Tn-C = thermoneutral control; C-C = cold control; C+P = cold+propranolol;
C+M = cold+metoprolol; C+ICI = cold+ICI 118551

Figure 3.2. The effect of acute cold exposure and beta-blockade on portal darined viscera oxygen consumption of the sheep.



Tn-C = thermoneutral control; C-C = cold control; C+P = cold+propranolol; C+M = cold+metoprolol; C+ICI = cold+ICI 118551
*P<0.05 compared to C-C

Figure 3.3. The effect of acute cold exposure and beta-blockade on hindquarter oxygen consumption of the sheep.

Table 3.1. Effects of acute cold exposure (C) and β-blockers (P+propranolol a nonselective blocker, M=metoprolol a selective β_1-blocker and ICI=ICI 118551 a β_2-blocker) on portal vein and hindquarter blood flow of the sheep.

Item	Tn-C[1]	C-C	C+P	C+M	C+ICI	SE[3]
Blood flow (l/min)						
Portal vein	1.65[a]	1.80[a]	1.67[a]	1.72[a]	1.83[a]	0.08
Iliac artery	0.22[b]	0.35[a]	0.24[b]	0.25[ab]	0.20[b]	0.04

[1]Tn-C = Thermoneutral control (Vehicle)
[2]C-C = Cold plus control (vehicle)
[3]Pooled SE
[abc]Within rows values with different superscript are different (P<0.05).

Table 3.2. Effect of cold (C) exposure and beta-blockers (P=propranolol a nonselective blocker, M=metoprolol a β_1-blocker, ICI=ICI 118551 a β_2-blocker) on O_2 blood parameters of sheep

Item	Tn-C[1]	[2]C-C	C+P	C+M	C+ICI	SE[3]	Trt*T[6]
Haemoglobin							
g/dL	8.7[c]	10.0[ab]	9.9[ab]	10.2[a]	9.9[ab]	0.2	.002
Arterial O_2							
Saturation (%)	92.9	91.9	91.7	91.2	91.2	0.6	.969
portal vein O_2							
Saturation (%)	63.6	66.4	65.6	60.9	62.5	2.0	.028
Iliac vein O_2							
Saturation (%)	47.6[a]	37.9[ab]	27.8[b]	29.0[b]	29.5[b]	2.7	.826
Arterial O_2							
content(mL/dL)	11.0[b]	12.6[a]	12.5[a]	12.7[a]	12.4[a]	0.2	.002
portal vein O_2							
content(mL/dL)	7.2	8.7	8.7	8.3	8.6	0.4	.003
Iliac vein O_2							
content(mL/dL)	5.8[a]	5.2[ab]	3.8[c]	4.2[bc]	4.1[bc]	2.7	.856
PDV[4] O_2 A-P							
(mL/dL)	3.7	3.8	3.8	4.3	3.7	0.3	.551
HQ[5] O_2 A-V							
(mL/dL)	5.4[b]	7.6[a]	8.7[a]	8.3[a]	8.6[a]	0.5	.251
PDV O_2							
Extraction(%)	33.8	30.1	29.6	34.0	30.1	2.7	.11[illegible]
HQ O_2							
Extraction(%)	47.8[c]	50.8[bc]	57.1[ab]	55.8[ab]	67.4[a]	[illegible]	[illegible]

[1]Tn-C = Thermoneutral control (saline)
[2]C-C = Cold plus control (saline)
[3]Pooled standard error
[4]Portal drained viscera
[5]Hindquarter
[abc]Within rows values with different superscript are different (P<0.05)
[6]Means for significant treatment by time interaction (trt*t) are shown in Appendix 7.

Table 3.3. Effect of feeding (F) and beta-blockers (P=propranolol a nonselective blocker, M=metoprolol a β_1-blocker; ICI=ICI 118551 a β_2-blocker) on whole body (WB) and PDV[1] VO_2 and O_2 blood parameters of sheep.

Item	Tn-C[2]	F+C	F+P	F+M	F+ICI	SE[3]
WB VO_2(mL/min)	229[b]	322[a]	314[a]	308[a]	304[a]	11
PDV VO_2 (mL/min)	59	71	57	57	68	7
Portal blood flow(L/min)	1.58[b]	1.98[a]	1.88[a]	1.97[a]	2.04a	0.09
Haemoglobin g/dL	8.6	8.8	9.1	8.9	9.0	0.4
Arterial O_2 Saturation (%)	91.5	92.6	93.7	93.3	92.8	0.8
portal vein O_2 Saturation (%)	61.9	65.5	67.4	68.1	70.0	2.0
Arterial O_2 content(mL/dL)	10.9	11.3	11.6	11.4	11.5	0.5
portal vein O_2 content(mL/dL)	7.1	7.7	8.3	8.5	8.2	0.4
PDV A-p O_2 difference (mL/min)	3.9	3.6	3.2	2.9	3.4	0.3
PDV VO_2 Extraction(%)	35	32	28	26	29	2.4

[1]Portal drained viscera
[2]Tn-C = Thermoneutral control (saline)
[3]Pooled SE
[ab]Within rows values with different superscript are different (P<0.05)

Table 3.4. Effect of cold exposure(C) and ß-adrenoceptor blockade (P=propranolol a nonselective ß-blocker; metoprolol a selective $ß_1$-blocker; ICI 118551 a selective $ß_2$-blocker) on arterial whole blood amino acid ($\mu M.L^{-1}$) profile of sheep.

AMINO							P>F	
ACID	[1]Tn-C	C+C[2]	C+P	C+$ß_1$	C+$ß_2$	SE[3]	TRT	TRT*T[4]
ASP	26.2	21.4	27.6	30.2	19.1	1.2	.338	.286
GLU[5]	307.0	247.9	236.4	231.5	244.4	52.4	.192	.144
ASN	40.2	35.2	39.4	45.1	49.1	7.7	.259	.939
SER	49.5	24.5	31.1	36.6	42.4	17.5	.269	.504
HIS	89.0	71.4	74.5	79.6	80.4	6.8	.409	.488
GLY	720.1	346.4	437.5	446.8	471.8	203.3	.127	.792
THR	70.8	46.9	83.2	88.3	99.6	41.7	.285	.737
CIT	240.3	145.7	107.5	137.9	141.7	36.5	.021	.099
ARG	155.2	48.9	80.4	86.9	111.1	29.3	.173	.522
TAU	72.9	62.5	57.8	66.9	60.9	30.0	.262	.633
ALA	158.9	87.9	109.2	138.7	148.8	22.7	.307	.111
TYR	88.0	115.9	141.8	139.0	108.3	44.3	.501	.405
TRP	25.9	20.7	19.0	19.3	19.6	4.3	.129	.007
MET	12.8	9.9	11.4	11.4	19.1	3.0	.412	.395
VAL	203.2	189.9	211.9	211.4	192.3	11.5	.923	.511
PHE	49.5	45.9	51.1	52.6	50.6	6.1	.972	.218
ILE	86.0	79.5	87.9	87.0	89.7	6.1	.935	.116
LEU	130.2	130.1	140.6	147.4	133.5	8.9	.911	.138
ORN	158.1	132.6	158.7	139.4	148.6	35.7	.145	.053
LYS	157.6	122.7	145.9	144.2	138.2	18.7	.338	.369
TOTALS	2842.2	1985.8	2253.0	2340.4	2369.4	241.5	.097	.198
TOTAL[5] BCAA	402.1	399.6	440.4	445.8	415.5	25.2	.943	.477

[1]C = Thermoneutral control (saline)
[2]C = cold + control (saline)
[3]SE = pooled standard error
[4]means and SE not shown
[5]Include values for glutamine
[5]Total branched chain amino acids

3.8. REFERENCES

Bell A.W., Thompson G.E. and Findlay J.D. 1974. The contribution of the shivering leg to the metabolic response to cold of the young ox (Bos taurus). Pfugers Archiv 346:341-350.

Brooks B.J., Arch J.R.S. and Newsholme E.A. 1983. Effect of some hormones on the rate of the triacylglycerol/fatty-acid substrate cycle in adipose tissue of the mouse in vivo. Biosc. Reports 3: 263-267.

Choo J.J., Horan M.A., Horan R.A., Rothwell N.J. 1992. Anabolic effects of clenbuterol on skeletal muscle are mediated by $ß_2$-adrenoceptor stimulation. Am. J. Physiol. 263:E50-E56.

Christopherson R.J. and Brockman R.P. 1989. Effect of feeding and a beta-adrenergic blocking agent on oxygen consumption by the portal drained viscera, liver and hindquarter of sheep. EAAP 43:147-150.

Dauncy M.J. and Ingram D.L. 1979. Effect of dietary composition and cold exposure on non-shivering thermogenesis in young pigs and its alteration by the β-blocker propranolol. Br. J. Nutr. 41:361-370.

Eisemann J.H., Huntington G.B. and Ferrel C.L. 1988. Effects of dietary clenbuterol on metabolism of the hindquarters in steers. J. Anim. Sci. 66:342:353.

Eriksson L.S., Olsson M., Bjorkman O. 1988. Splanchnic metabolism of amino acids in healthy subjects: effects of 60 hours fasting. Metabolism 37(12): 1159-1162.

Graham A.D. and Christopherson R.J. 1981. Effects of adrenaline and noradrenaline on the heat production of warm- and cold-acclimated sheep. Can. J. Physiol. Pharmacol. 59:985-993.

Gregg V.A. and Milligan L.P. 1982. Role of Na^+K^+-ATPase in muscular energy expenditure of warm- and cold-exposed sheep. Can. J. Anim. Sci. 62:123-132.

Harmon M.C. 1992. The effects of beta-antagonist and epinephrine infusion on warm- and cold-acclimated sheep. M.Sc. Thesis, University of Alberta.

Heitmann R.N. and Bergman E.N. 1980a. Transport of amino acids in whole blood and plasma of sheep. Am. J. Physiol. 239:E242-E247.

Heitmann R.N. and Bergman E.N. 1980b. Integration of amino acid metabolism in sheep: effects of fasting and acidosis. Am. J. Physiol. 239:E248-E253.

Ignarro L.J. and Titus F. 1968. The presence of antagonistically acting alpha and beta adrenergic receptors in the mouse spleen. J. Pharmacol. exp. Ther. 160:172.

Jones B.N. and Gilligan J.P. 1983. o-Phthaldehyde precolum derivatization and reverse phase high perfomance liquid chromatography of polypeptide hydrosylates and physiological fluids. J. Chromatog. 226;471-482.

Jungas R.L., Halpern M.L. and Brosnan J.T. 1992. quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. Physiol. Rev. 72(2):419-449.

Kelly J.M. 1987. Digestibility of amino acids in sheep in cold . Msc. Thesis, University of Alberta.

MacRae J.C., Skine P.A., Connel A., Buchan V. and Lobley G.E. 1988. The action of ß-agonist clenbuterol on protein and energy metabolism in fattening wether lambs. Br. J. Nutr. 59:457-465.

Meyer S.E. and Stull J.T. 1971. Cyclic AMP in skeletal muscle. Ann. N.Y. Acad. Sci. 185:433.

Reeds P.J., Hay S.M., Dorward P.M. and Palmer R.M. 1988. The effect of ß-agonists and antagonists on muscle growth and body composition of young rats (Rattus sp). Comp. Biochem. Physiol. Vol. 89c(2):337-341.

Reid J.L., Whyte K.F. and Struthers A.D. 1986. Epinephrine-induced hypokalemia: the role of beta-adrenoceptors. Am. J. cardiol. 57:23F-27F.

Rothwell N.J., Saville M.E. and Stock M.J. 1980. Factors influencing the acute effect of food on oxygen consumption in the rat. Int. J. Obesity 6:53-59.

Shamoon H., Jacob R. and Sherwin R.S. 1980. Epinephrine-induced hypoaminocidemia in normal and diabetic man: effect of β-blockade. Diabetes 29:875-881.

Souba W.W. and Douglas W.D. 1983. Postoperative alteration of arteriovenous exchange of amino acids across the gastrointestinal tract. Surgery 94(2):342-350.

Steel R.G.D. and Torrie J.H. 1980. In Principles and Procedures of Statistics, McGraw-Hill Book Co., New York, N.Y.

Thompson G.E., Manson W., Clarke P.L. and Bell A.W. 1978. Acute cold and metabolism of glucose and some of it's precursors in the liver of the fed and fasted sheep. Quart. J. Expt. Physiol. 63:189-199.

Thompson G.E., Gardner J.W. and Bell A.W. 1975. The oxygen consumption, fatty acid and glycerol uptake of the liver on fed and fasted sheep during cold exposure. Quart. J. Expt. Physiol. 60:107-121.

Thorin D., Golay A., Simonsen D.C., Jequer E., Felber J.P. and DeFronzo R.A.1986. The effect of selective beta adrenoceptor blockade on glucose-induced thermogenesis in man. Metabolism 35(6):524-528.

Webster A.J.F. and Hays F.L. 1968. Effects of beta-adrenergic blockade on the heart rate and energy expenditure of sheep during feeding and during acute cold exposure. Can. J. Physiol. Pharmacol. 46:577-583.

Webster A.J.F., Heitman J.H., Hays F.L. and Olynyk G.P. 1969. Ca[illegible] and cold thermogenesis in sheep. Can. J. Physiol. Pharmacol. 47:719-72

Webster A.J.F. 1974. Physiological effects of cold exposure. In MTP International Review ed D. Robertshaw. Buthsworth and Co., Ltd., London, pp 33-69.

Young B.A., Kerrigan B. and Christopherson R.J. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55:17-22.

Young B.A., Fenton T.W. and McLean J.A. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol. 56(4):1120-1125.

Zeisberger E. 1978. Role of a [illegible]l catecholaminergic system in the thermoregulatory threshold de[illegible] H. Houdas and J.D. Guieu eds. New trends in Thermal Physiology. Massor[illegible] U.S.A pp 22-25.

4. Thermic Effects of Thermal Exposure and Feeding in Steers: The Role of α_2-Adrenoceptor Stimulation by Guanfacin

4.1. INTRODUCTION

Several studies have demonstrated an important role for the sympathoadrenal system in cold- and feed-induced thermogenesis (Graham and Christopherson 1981, Christopherson and Brockman 1989, Astrup et al. 1990). In most cases, data from these studies are based on the β-adrenoceptor mediated thermogenic processes. Much less is known about the thermogenic role of the α-adrenergic receptors. During cold pressor testing, guanfacin, an α_2-adrenoceptor agonist selectively reduced plasma noradrenaline concentration (an index of sympathetic outflow) while the α_1-adrenoceptor stimulation by bunazosin, a specific agonist had no effected (Koshiji et al. 1992). Noradrenaline has a greater affinity for and stimulatory effect on α_2-adrenoceptors, while adrenaline has a greater effect on the β-adrenoceptors (Ariens and Simonis 1983). Both the former and latter are elevated during cold exposure (Thompson et al. 1978). There is evidence that noradrenaline can have central, as well as peripheral, effects on metabolism mediated by α_2-adrenoceptors (Koshiji et al. 1992, Gazzola 1993). Relatively little information is available regarding the nature and subtypes of α-adrenoceptors that may be associated with influences on cold thermogenesis in domestic ruminants. Alpha-adrenoceptors are known to mediate peripheral vasoconstriction in cold-exposed ruminants and may play a role in energy conservation (Hidari et al. 1991). Guanfacin, an α_2-agonist, which is antithermogenic

in cattle (Hunter 1992) and mice (Sillence et al. 1992) may be used as a research tool to evaluate α_2-adrenoceptor mediated thermogenic effect of animals in different thermal environments. Although guanfacin has been proposed to be beneficial to animal production during dry season feeding in the tropics (Hunter 1992, Hunter et al. 1993), there is no data available on the effects of this drug on either heat- or cold-stressed animals. Further, the effects of α_2-adrenoceptor stimulation on feed intake is essential in evaluating the relationship between the energy sparing effects of guanfacin and animal productivity.

The major objective of this study was to examine some of the metabolic and thermoregulatory effects of guanfacin in steers fed a controlled diet and kept at different thermal environments with a view to assessing the role of α_2-adrenoceptors in improving energy conservation of animals in different environments. Additionally, the thermogenic role of α_2-adrenoceptor stimulation with guanfacin in warm and cold-acclimated steers fed ad libitum was examined.

4.2. MATERIALS AND METHODS

4.2.1. Animals and Experimental Procedure

In experiment one, 9 large-frame yearling Holstein steers with an average wieght of 343±1.7 kg were used. They were divided into three groups consisting of 3 animals per group and were housed in chambers maintained at temperatures of -9±1.2, 11±1.3 and 28±0.6 °C for the cold, warm and hot environments respectively. The relative humidity was not controlled, however, the wet and dry temperatures were 12±1.1 and

13±1.5 °C for the thermoneutral room and 19±1.9 and 27±0.6 °C in the hot room respectively. Daily feed intake was maintained at 25 g per kg body weight throughout the study (Table 4.1). Each steer received once daily (between 1500 and 1600 hours) a ration which consisted of 65 % grass hay, 25 % barley grain, 4 % canola, 5% soybean meal, 0.4 % fortified salts and 0.3 % calcium phosphate (DM basis) formulated to provide maintenance needs of steers in a thermoneutral environment (net energy content of feed = 1.41 Mcal/kg DM; NRC 1984). The determined DM (%), gross energy (Kcal/g DM) and crude protein (%) for the grain diet were 88, 4.5 and 21 respectively. On this diet, the steers with an initial average weight of 305 kg gained an average of 0.5 kg/d for the 79 d experimental period. The steers were exposed to the three environments in a crossover design for 21 d periods. The treatments of guanfacin (Sandoz Can. Inc. Dorval, Quebec) at 80 µg/kg body weight (the highest dose used by Hunter (1992)) and vehicle (physiological saline) were randomly administered intramuscularly to each animal within each thermal environment such that each steer acted as it's own control. The animals received each of the treatments only once after each of 21 d acclimation period. In experiment 1, guanfacin was injected at 1000 h following a pre-injection blood sample collection and oxygen consumption measurement. The 1100-1500 h data was used for the calculation of heat production. In the second experiment injection of guanfacin was given at time zero, however, only the data for the second hour was reported.

In the second experiment, eight steers used in the first experiment, weighing an average of 419±5 kg were divided into 2 groups of 4 animals and kept in warm and

cold environments, respectively. The temperatures for the warm and cold chambers were 22±2.4 and -19±3.3°C, respectively, and the wet-bulb and dry-bulb temperatures in the warm room were 19±2.1 and 19±2.5°C, respectively. The steers were fed to appetite a pelleted alfalfa ration (DM = 91, crude protein = 17% and gross energy = 4.4 Kcal/g DM). The treatments of guanfacin and saline control were applied to the steers in a nested experimental design. The groups were not crossed-over between the two periods. There were two 21 day periods in this experiment and the dosage of guanfacin was similar to experiment 1. In both experiments, water and salt was provided ad libitum and in the cold chamber water containers were fitted with heating elements to prevent the drinking water from freezing.

4.2.2. Heat Production

The steers were previously accustomed to the metabolic hood and measurement procedure. The oxygen consumption of the steers was determined by an open-circuit calorimetry apparatus (Young et al. 1975) connected to a ventilated hood for 6 h and 2 h following the 21 day acclimation period in experiment 1 and 2, respectively. Due to a limitation of metabolic rate measuring equipment, the oxygen consumption was measured in pairs of animals on separate days in experiment one and on day 21 in experiment two. Flow rate of the head hood was read from a flowmeter (Rotometer, Fisher and Porter, Warminster, PA) and the oxygen concentration difference between incoming and outgoing respired air, was determined using a dual channel oxygen analyzer (Taylor Servimix, Crowborough, Sussex UK). The data was acquired every

10 seconds and averaged over 5-minutes intervals by a computerized data acquisition program developed in our laboratory (Godby and Gregory 1992). The oxygen consumption was the product of the flow rate (mL min^{-1}) at STP and oxygen decrement. The measuring system was calibrated with nitrogen as a zero gas and by the nitrogen injection method as described by Young et al. (1984). The heat production was calculated from oxygen consumption using the equation of McLean (1972).

4.2.3. Other

Heart rate was recorded by auscultation with the aid of a stethoscope before and after (5 h) injection of guanfacin. The rectal temperature was measured with a rectal thermometer (Fisher Scientific Inc.) before and after (5 h) guanfacin injection. A single blood sample (30 mL) was collected before injection and at the end of the 5 h, after guanfacin injection using a heparinized venipuncture tube (vacutainer, Becton Dickinson, Missassauga, ON) for the determination of plasma glucose, glycerol, growth hormone, triiodothyronine and 3-methylhistidine. In experiment 1 all samples were collected at 1000 h and 1500 h. Feed intake was determined by measuring the orts prior to daily feeding.

4.3. LABORATORY ANALYSIS

4.3.1. Glucose

Plasma glucose concentration was determined enzymatically by a test kit (Sigma

Diagnostic., St Louis, Mo.) according to the method outlined by Trinder (1969). Briefly, glucose in the sample is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. The latter reacts with 4-aminoantipyrine and p-hydroxybenzyne sulfonate to form a dye (colour). The glucose concentration is proportional to the intensity of the colour produced as determined by a spectrophotometer (Milton Roy Spectronic 3000, Array, USA).

4.3.2. Glycerol

Glycerol concentration was determined using a test kit (Boehringer Mannheim, Laval, PQ) and a spectrophotometer (Milton Roy 3000, Array, USA). The samples were initially deproteinized with zinc sulfate and sodium hydroxide (Wieland 1984). The principle of this method involves the activity of glycerokinase and the conversion of NADH to NAD in the presence of pyruvate and lactate dehydrogenase , thus, pyruvate + NADH + H^+ = L-lactate + NAD^+. The amount of NADH oxidized is proportional to glycerol in the sample and is determined spectrophotometrically at 340 nm.

4.3.3. Growth Hormone and Triiodothyronine

Growth hormone was determined on single samples only. The concentration of the hormone was estimated from a double antibody radioimmunoassay as described by de Boer and Kennelly (1989). The total plasma triiodothyronine (T_3) concentration was determined using a radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles CA).

4.3.4. 3-Methylhistidine

Plasma 3-methylhistidine was determined by a modified method of Nakamura and Pisano (1976) and Wassner et al. (1980). Briefly, 200 µL plasma samples were mixed with 100 x 10^{-6} M histidinol (internal standard) and deproteinized with 0. [illegible] M perchloric acid. Derivatization was accomplished in screw [illegible]p vials [illegible] with 0.2 mL of the deproteinized sample, 0.125 mL of 1.5 M sod[illegible] hydroxide, [illegible].4 m[illegible] of 0.2 M sodium borate and while vortexing 0.250 mL of flourescamine (360 mg/[illegible] m[illegible] acetonitrile) was added. The samples wer[illegible] allowed to use up the excess fl[illegible] scamine and then 0.4 mL of 2 M HCL was added. The sampled were mixed and incubate[illegible] 45 minutes in a 90°C water bath. They were than extracted twice with 1.5 mL diethyl ether. The derivatized amino acid was quantified using a Varian 5500 high performance liquid chromatograph and a Shimadzu RF-535 fluorescence detector (Shimadzu Scientific Instruments, Inc.). Excitation 340 nm Emission 450 nm. The samples were injected with a varian autosamplar 9090 (Varian Associates Inc.). The separation of histidine, 3-methylhistidine and histidinol were accomplished using a gradient program. Solvent A was (2.5 mM acetyltrimethylammonium bromide, 0.1 M sodium acetate buffer; pH = 6.50) and solvent B was (10% 2.5 mM acetyltrimethylammonium bromide and 90 % acetonitrile; pH = 6.50). The gradient program was 25 % for 12. 25 % B to 80 % B over 2 minutes, 80 % B over 0.5 minute. Flow rate was 1.0 mL/min and total analysis time was 18 minutes. A supelcosil 3µ LC-18 reverse phase column (4.6x1.50 mm; Supelco, Oakville, ON) equipped with a guard column (4.6x150 mm) packed with a supelco LC-18 reverse

phase packing (20-40 μ) was used for the separation. The data was acquired and integrated by the Shimadzu Ezchrom Chromatography Data System (Shimadzu scientific Instruments, Inc.).

4.4. STATISTICAL ANALYSIS

The data of experiment 1 was analyzed as a cross-over design with the three treatments (Warm/Thermoneutral, Cold and Hot) utilizing the general linear model procedure of the Statistical Analysis System Institute (SAS Inc., Cary, NC.). The model included environment, drug, period(unit) and animal as the main effects. Comparisons between the guanfacin and vehicle within each environment (Warm, Cold and Hot) were made using least significance difference. The second experiment was analyzed as a nested design where steers were nested within treatments.

4.5. RESULTS

The main effects of acclimation temperature on metabolic parameters are presented for experiment 1 in table 4.1. Heat production of the warm-acclimated (11 °C) steers was significantly ($P<0.05$) lower compared to steers in the cold (-9°C) and hot (28°C) environments, resulting in a "U-shaped" relationship between heat production and temperature. The rectal temperature of the animals was significantly higher ($P<0.05$) in steers exposed to 28°C compared to those in warm (11 °C) or cold (-9°C) environments. Environment did not have a significant effect on the heart rate.

Plasma glucose concentration in steers was significantly ($P<0.05$) elevated in

the -9 compared to the 11 or 28°C environments, where values were similar. In the cold environment there was a slight increase in plasma glycerol concentration which was not statistically significant. The growth hormone levels were similar across temperatures. The plasma T_3 concentration was inversely related to environmental temperatures. At -9°C T_3 was significantly ($P<0.05$) higher compared to the 11°C environment whereas the T_3 values were reduced ($P<0.05$) in steers in the hot(28°C) environment. Plasma 3-methylhistidine concentrations were not influenced by the temperature treatments.

In table 4.2. the effects of guanfacin and vehicle within each acclimation temperature are shown. Compared to vehicle, guanfacin significantly ($P<0.05$) lowered heat production by 22 % in the cold(-9°C) and by 19 % in warm (11 °C) acclimated steers. At 28°C there was a small (8 %) reduction in heat production which only approached significance ($P=0.09$). The rectal temperature of steers was reduced at -9°C ($P<0.05$), remained unchanged at 11°C and was elevated ($P<0.05$) at 28°C in response to guanfacin. Guanfacin caused a significant ($P<0.05$) reduction in heart rate of the steers which was independent of the acclimation temperature.

Compared to vehicle, guanfacin induced a two-fold increase in plasma glucose concentration. The hyperglycaemia was not affected by the temperature treatments. Growth hormone showed a tendency to increase in response to guanfacin in steers at -9°C and 28°C. This tendency was significant at 11°C and, when the data was pooled across temperatures, guanfacin increased plasma growth hormone (33 vs 19 ng/mL for guanfacin vs vehicle; $P<0.05$, SE = 3). The plasma T_3 content was similar across

temperatures except at 11 °C where guanfacin lowered P<0.05 T_3. In response to guanfacin, plasma glycerol of the steers was significantly (P<0.05) lower at -9°C, unchanged at 11°C and significantly higher at 28°C. For 3-methylhistidine, guanfacin, caused an increase (P<0.05) which was independent of temperature.

The body weights of the steers in the second experiment were similar at 415 vs 418±13 kg for warm- and cold-acclimated steers respectively. Table 4.3 summarizes the data from the second experiment which shows the effects of guanfacin and environmental temperature on feed intake, heat production and rectal temperature. Compared to vehicle, guanfacin induced a 12 % reduction (P=0.02) in heat production when the data was pooled across temperatures and the feed intake was reduced by 14% (P<0.1). The rectal temperature was not affected by either environmental temperature or guanfacin in the ad libitum fed steers.

4.6. DISCUSSION

In experiment 1, the constant intake feeding strategy was planned to avoid possible confounding effects of differences in feeding level with temperature, since temperature is known to affect intake and intake can influence heat production (Forbes 1986). The "U-shaped" relationship between temperature and heat production and the increase in heat production of steers kept in the hot (28°C) environment is consistent with previous reports (Blaxter and Wainman 1961). The likely explanation for the similarity in heat production of steers in the cold (-9°C) and hot (28°C) environments, is that ---- 9°C is a relatively mild cold exposure which may have been only slightly below the

lower critical temperature for these yearling steers, while, on the other hand, the 28°C temperature was likely above the upper critical temperature resulting in mild heat stress (Christopherson and Young 1986). This is confirmed by the elevated rectal temperature of the steers kept at 28°C, a response similar to that reported by Blaxter and Wainman (1961) for steers exposed to an ambient temperature of 35°C. The increase in heat production, plasma glucose and triiodothyronine concentration in the cold (-9°C) exposed animals is evidence for the existence of mild stress in these steers. The thyroid responses observed are in agreement with published data (Ole Miaron and Christopherson 1992).

In the present study, guanfacin, an α_2-adrenoceptor agonist was anticalorigenic in steers kept in the -9°C and 11°C thermal environments. The results for guanfacin effects in steers exposed to a temperature of 11°C are in general agreement with previously published data for cattle in a thermoneutral environment (Hunter 1992). At the temperature of 28°C, the guanfacin-induced decrease in heat production of the steers was only 8 % ($P<0.1$). This is the first study designed to examine the interaction between guanfacin and the thermal environment. The rectal temperature responses to guanfacin in the different environments appeared to reflect the changes in heat production. Therefore, guanfacin lowered the rectal temperature in steers exposed to an ambient temperature of -9°C, produced no change at 11°C and caused an elevation of rectal temperature at 28°C. It is possible that the guanfacin-induced increase in rectal temperature prevented a reduction in heat production in response to the drug when the steers were exposed to 28°C. In addition, the sympathetic nervous system

activity in thermogenic tissues might have been decreased to minimal by heat exposure ruling out the possibility of a further reduction by guanfacin. The high rectal temperature at 28 ° C in response to guanfacin could have resulted from reduced heat dissipation from the core to skin surface. The latter is normally associated with the α-adrenoceptor-mediated vasoconstrictor effects. This suggestion is supported by results presented in Chapter 5, which show that the skin-temperature is lower in guanfacin-treated sheep compared to controls.

The lower heat production by the steers could be partly explained by the lower rectal temperature at -9°C but not at 11 °C. The mechanism by which guanfacin reduced heat production is not known, but might involve reduced sympathetic outflow mediated by central α_2-adrenoceptors. Peripheral noradrenaline (mediator of sympathetic outflow) release during cold pressor testing has been shown to be inhibited by guanfacin in humans (Koshiji et al. 1992).

The possible physiological mechanisms that could be associated with changes in heat production may include cardiovascular responses such as heart rate and peripheral vascular resistance, substrate mobilization such as glucose, glycerol and protein turnover as well as metabolic hormones which could be represented by growth hormone and triiodothyronine. The lower heart rate of the steers is consistent with reduced metabolic rate and the antihypertensive nature of guanfacin observed in animals (Scholtysik 1986).

In the present study, guanfacin influenced glucose metabolism. This effect was manifested by a marked hyperglycaemia which was independent of the acclimation

temperature. High blood glucose concentration, in itself, cannot be a cause of reduced heat production, but may be an indication of reduced glucose utilization. Elevated blood glucose concentration may result from enhanced glycogenolysis, gluconeogensis and decreased glucose utilization and or combination of all these factors. The responses reported here (hyperglycaemia) agree favourably with published data in which other α_2 -adrenoceptor agonists were shown to cause an increase in plasma glucose concentration (Nakadate et al. 1980; DiTullo et al. 1984). In a recent study, α_2 -adrenoceptor stimulation with guanfacin resulted in increased glucosuria in mice (Sillence et al. 1992). The increase in glucose concentration could therefore, be mediated via α_2-adrenoceptor processes involving gluconeogenesis and reduced peripheral glucose utilization (DiTullo et al. 1984). Tissue protein breakdown could be a source of gluconeogenic amino acid precursors. In this experiment, plasma concentrations of 3-methylhistidine, a specific index of myofibrillar protein breakdown, was elevated by guanfacin. Plasma 3-methylhistidine is a useful indicator of protein breakdown in goats subjected to a starvation stress (Nagasawa et al. 1993) and was deliberately used here because plasma levels of 3-methylhistidine were expected to reflect acute changes in protein degradation. The plasma 3-methylhistidine values in this study compare favourably to those reported for dairy cattle by Nagasawa et al. (1991). Glucose synthesis from amino acids could be substantial (Jungas et al. 1992).

The hyperglycaemia observed in this study is also consistent with the increased growth hormone concentration. Growth hormone release is episodic in cattle, with

baseline and peak levels which vary with time of day (Wheaton et al. 1986). However, in this experiment, the variability in plasma growth hormone concentrations was low within treatment, probably, because, each steer acted as it own control and that α_2-adrenoceptor stimulation by guanfacin could have influenced the sporadic nature of growth hormone surges. It is known, at least in the rat, that α_2-adrenoceptor effects are responsible for the episodicity of growth hormone release (Kaesoh et al. 1983). Guanfacin is an inducer of growth hormone synthesis in humans (Balldin et al. 1993) and the latter stimulates gluconeogenesis and decreases peripheral glucose utilization (Rizza et al. 1982).

As an indicator of lipolysis, glycerol rather than free fatty acids was measured in the present experiment. Interpretation of the guanfacin-mediated in vivo lipolytic responses and hyperglycaemia in cold exposed animals, should be done with caution because they could be complicated by beta-adrenoceptor responses and the α_1-adrenoceptor mediated hepatic glycogenolysis (Kunos 1984). In the cold (-9°C), the reduced glycerol level in response to guanfacin is, in part, consistent with increased hepatic gluconeogenesis utilizing glycerol as a substrate observed in young steers (Bell et al. 1975). The lack of change in glycerol content in the steers at 11° C, suggests that these animals were not stressed and gluconeogenesis from glycerol was not likely important. It is difficult to isolate the specific effects of α_2-adrenoceptor stimulation, which is known to inhibit lipolysis in sheep (Watt et al. 1991) but not pig (Mersmann 1984) adipocytes. The apparent lipolytic responses in the cold (-9°C) during control (vehicle) injection could be a result of changes in the β-adrenoceptor

system (increased adrenaline effects in lieu of the noradrenaline decrease) an effect which may be suppressed by guanfacin acting on α_2-adrenoceptors in adipose tissue. The general response in fat mobilization (increase glycerol concentration) in the hot environment seems contradictory. However, it is consistent with previous reports which show that in the hot environment, the adipose tissue in mammals may be a preferred source of energy (Yates 1993). In the hot (28° C) environment, the increased lipolysis is not consistent with the expected inhibitory effect of the α_2 -adrenergic receptors on lipolysis reported by Watt et al. (1991). On the other hand, the responses observed in the hot (28°C) thermal environment support the data of Sillence et al. (1992) in which, guanfacin was postulated to promote a reduction in body energy and fat in mice. It may be postulated that, the increased lipolysis at 28°C with guanfacin may be an adjustment to guanfacin impairment of glucose utilization induced by growth hormone.

The lack of significant changes in plasma triiodothyronine concentrations in steers in the cold (-9°C) and hot (28°C) environment agree favourably with the work of Hunter (1992) and may suggest that the guanfacin-induced metabolic responses were not a result of changes in the peripheral thyroid activity. While the latter is in full agreement with work of Hunter (1992), the guanfacin-induced reduction in T_3 in the 11°C environment is consistent with the inhibitory effect of α_2-agonist on the thyroid secretory activity reported by Yamashita et al. (1980) and at this temperature thyroid hormone changes could explain the reduced metabolic rate.

In the second experiment, cold exposure had no significant effect on heat

production of steers fed ad libitum, even though the steers ate slightly more feed in the cold environment. However, from this data , the steers ate only 9% more feed in the cold compared to warm environment. The latter, may suggest that, other factors accounted for their comfort, for example, large body size and the insulation cover provided by the hair coat. Further, the apparent low metabolic rate of the steers in the second experiment could be associated with the older age (Sillence et al. 1992) and also the nature of feed. The steers in the second experiment received a pelleted diet which is associated with a lower diet-induced thermogenesis. The 12 % decrease in heat production in response to guanfacin in the steers fed ad libitum was consistent with but, slightly smaller than, that of steers fed a fixed diet. There was also a tendency to reduce feed intake in guanfacin treated animals. The latter partially agrees with data reported by Hunter (1992) and Sillence et al. (1992).

In conclusion, α_2-adrenoceptor stimulation by guanfacin resulted in a marked reduction in heat production of restricted fed steers fasted for 18 h and exposed to an ambient temperature of -9 and 11 but not 28°C. The rectal temperature response to α_2-adrenoceptor stimulation reflected the heat production effects with a reduced rectal temperature in steers at -9, unchanged at 11 and an increase at 28°C. The anticalorigenic effects of guanfacin could not be attributed to any of the individual blood parameters affected by guanfacin, however, the reduced heart rate, low glycerol levels in the cold and the increased plasma glucose concentration (implying either an enhanced gluconeogenesis from protein breakdown precursors ,i.e., increased plasma 3-methylhistidine and/or blunted peripheral glucose utilization) all are consistent with

the reduced thermogenesis. Additionally, guanfacin was antithermogenic in steers fed ad libitum. Finally, although Hunter et al. (1993) have suggested that decreased maintenance requirements of cattle during dry season feeding might be a beneficial effect of guanfacin, the results from the present study indicates that environmental temperature will markedly modify the effectiveness of this approach.

Table 4.1. The effects of acclimation temperature on heat production (HP), rectal temperature (Tr), heart rate (HR) and plasma glucose (GLU), growth hormone (GH), triiodothyronine (T_3) , glycerol (GLY) and 3-methylhistidine (3M-H) concentration of steers kept at different thermal environments (cold = -9°C; thermoneutral = 11°C and hot = 28°C).

Trait	-9°C	11°C	28°C	S.E[1]
Weights(kg)	345[a]	342[a]	342[a]	3.8
FI[2] (kg/d)	8.8[a]	8.7[a]	8.9[a]	0.85
HP (watts/kg)	1.87[a]	1.56[b]	1.86[a]	0.05
Tr (°C)	38.4[b]	38.5[b]	38.8[a]	0.08
HR (beats/min)	78.1	74.6	76.7	1.70
GLU (mmol/L)	5.6[a]	4.5[b]	4.3[b]	0.27
GLY (μM)	146.1[a]	123.8[a]	137.0[a]	14.5
GH (ng/mL)	16.6[a]	19.7[a]	20.3[a]	5.3
T_3 (ng/mL)	1.73[a]	1.32[b]	0.99[c]	0.07
3M-H(nmol/mL)	3.05[a]	2.81[a]	3.32[a]	0.19

[1]S.E. = Standard error of mean and N (number of animals) = 9.

[2]FI = feed intake (ration of alfalfa hay + grains given at ratio 3:1).

[ab]Within rows lsmeans with different superscript differ P<0.05.

Table 4.2. The effects of α_2-adrenoceptor stimulation by guanfacin on heat production (HP), rectal temperature (Tr), heart rate (HR) and plasma glucose (GLU), growth hormone (GH), triiodothyronine (T^3) , glycerol (GLY) and 3-methylhistidine (3M-H) concentrations of steers kept at different thermal environments (cold = -9°C; thermoneutral= 11°C and hot = 28°C).

	COLD(-9°C)				THERMONEUTRAL(11°C)				HOT(28°C)			
Trait	G[1]	V[2]	SE[3]	P[4]	G[1]	V[2]	SE[3]	P[4]	G[1]	V[2]	SE	P[4]
HP (W/kg)	1.41	1.82	0.06	*	1.23	1.53	0.06	*	1.65	1.80	0.06	+
Tr (°C)	38.1	38.9	0.09	*	38.9	38.8	0.09	ns	39.6	39.1	0.09	*
HR (beats/min)	63	75	2.4	*	62	71	2.1	*	62	71	2.1	*
GLU (mmol/L)	13.2	5.2	0.7	*	13.5	4.6	0.7	*	12.9	4.9	0.7	*
GH (ng/mL)	27.9	16.6	5.3	ns	43.7	19.7	5.3	*	27.5	20.3	5.3	ns
T_3 (ng/mL)	1.63	1.73	0.07	ns	1.11	1.32	0.07	*	1.02	0.99	0.07	ns
GLY (µM)	96	146	15	*	109	124	15	ns	177	137	13	*
3M-H(nmol/mL	3.64	3.05	0.16	*	3.79	2.81	0.19	*	3.89	3.32	0.19	*

[1]G = guanfacine at 80 µg/kg
[2]V = vehicle (physiological saline)
[3]S.E. = Standard error of the mean and N (number of animals) = 9
[4]P = test of significant level *= P<0.05; + = P<0.1; ns = not significant).

Table 4.3. The effects of α_2-adrenoceptor stimulation by guanfacin on heat production (HP), feed intake (FI) and rectal temperature (Tr) of steers kept at two different thermal environments and fed ad libitum.

	WARM (22°C)				COLD (-19°C)				POOLED			
Trait	G[1]	V[2]	S.E[3]	P[4]	G[1]	V[2]	S.E.[3]	P[4]	G[1]	V[2]	S.E.[3]	P[4]
HP (watts/kg)	1.49	1.76	0.09	+	1.48	1.62	0.09	ns	1.48	1.68	0.05	*
FI (kg/d)	11.85	12.51	0.89	ns	11.09	13.58	0.89	+	11.47	13.05	0.62	+
Tr (°C)	38.75	38.90	0.20	ns	39.35	38.85	0.20	ns	39.1	38.9	0.11	ns

[1]G = guanfacine.
[2]V = vehicle (physiological saline).
[3]S.E. = standard error of the means N (number of animals) = 4.
[4]P = test of significant level * = P<0.05; + = P<0.1; ns = not significant

4.7. REFERENCES

Ariens E.J. and Simonis A.M. 1983. Physiological and pharmacological aspects of adrenergic receptor classification. Biochem. Pharmacol. 32 (10):1539-1545).

Astrup A.V. Christensen N.J., Simonsen L. and Bulow J. 1990. Effect of nutrient intake on sympathoadrenal activity and thermogenic mechanism. J. Neurosc. Methods 34:187-192.

Balldin J., Berggren U., Eriksson E., Lindstedt G. and Sundkler A. 1993. Guanfacine as an alpha-2-agonist inducer of growth hormone secretion- a comparison with clonidine. Psychoneuroendocrinology 18(1):45-55.

Bell A.W., Gardner J.W., Manson W. and Thompson G.E. 1975. Acute cold exposure and metabolism of blood glucose, lactate and pyruvate and plasma amino acids in the hind leg of the fed and fasted young ox. Br. J. Nutr. 33:207-217.

Blaxter K.L. and Wainman F.W. 1961. Environmental temperature and the energy metabolism and heat emission of steers. J. Agric. Sci. 56:81-90.

Christopherson R.J. and Young B.A. 1986. Effects of cold environments on domestic animals. In Grazing Research at Northern Latitudes. Ed. O. Gudmundsson, Plenum Publishing Corporation pp 247-257.

Christopherson R.J. and Brockman R.P. 1989. Effect of feeding and a beta-adrenergic blocking agent on oxygen consumption by the portal drained viscera, liver and hindquarter of sheep. EAAP 43:147-150.

de Boer G. and Kennelly J.J. 1989. Effect of somatotropin injection and dietary protein concentration on milk yield, and kinetics of hormones in dairy cows. J. Dairy Sci. 72:419-428.

DiTullo N.W., Cieslinski L., Matthews W.D., and Storer B.1984. Mechanisms involved in the hyperglycaemic response induced by clonidine and other α_2-adrenoceptor agonists. J. Pharmacol. Expt. Ther. 228 (1):168-173.

Forbes J.M. 1986. (Ed). The Voluntary Food Intake of Farm Animals pp114-128. Butterworths, London.

Gazzola C. 1993. α_2-Adrenoceptor-mediated effects on resting energy expenditure. Int. J. Obesity. 17:637-641.

Godby G. and Gregory P. 1992. Datagrabber. Department of Animal Science, University of Alberta.

Graham A.D. and Christopherson R.J. 1981. Effects of adrenaline and noradrenaline on the heat production of warm- and cold-acclimated sheep. Can. J. Physiol. Pharmacol. 59:985-993.

Hidari H., Christopherson R.J. and Ole Miaron J.O. 1991. Effects of a cold environment and alpha and beta adrenergic blocking agents on metabolic rate in steers and beta blockade on oxygen consumption in sheep. EAAP 58:76-79.

Hunter R.A. 1992. The effect of α_2-adrenergic agonist, guanfacine, on the energy metabolism of steers fed on low quality roughage diets. Br. J. Nutr. 67:337-343.

Hunter R.A., Sillence M.N., Gazzola C. and Spiers W.G. 1993. Increasing animal growth rate of cattle by reducing maintenance energy requirements. Aust. J. Agric. Res. 44;579-595.

Jungas R.L., Halpern M.L. and Brosnan J.T. 1992. Quantitative analysis of amino acid oxidation and related gluconeogensis in humans. Physiol. Rev. 72(2):419-449.

Koshiji M., Ito H., Minatoguchi S., Watanabe H., Imai Y., Kakami M and Hirakawa S. 1992. A comparison of guanfacine, bunazosin, atenolol and nadolol on blood pressure and plasma noradrenaline responses to cold pressor testing. Clinical. Exp.Pharmacol. and Physiol. 19:481-488.

Kunos G. 1984. The hepatic α_1-adrenoceptor. TIPs. 380-384.

Kocsoh B., Opp J.S., Crowley W.R. and Grosvenor C.E. 1993. Interaction between the α_2-adrenergic system and nursing in the regulation of growth hormone secretion in neonatal rat. Acta Endocrinologica 128:184-191.

McLean J.A. 1972. On the calculation of heat production from open-circuit calorimetric measurement. Br. J. Nutr. 27:597-600.

Mersmann H.J. 1984. Absence of α-adrenergic inhibition of lipolysis in swine adipose tissue. Comp. Biochem. Physiol. 79c(1):165-170.

Nagasawa T, Hashiguchi N. and Onodera R. 1993. Effect of starvation on plasma 3-methylhistidine concentration in goats. Biosc. Biochem 57(3):517.

Nagasawa T, SaKai T. and Onodera R. 1991. Simple and sensitive determination of plasma N-tau-methylhistidine by high-performance liquid chromatography using pre-column derivative formation with 0-phthaldehyde-2-mercaptoethanol. J. Chromat. 566(10):223-227.

Nakadate T., Nakaki T., Muraki T. and kato R. 1980. Adrenergic regulation of

blood glucose levels: possible involvement of postsynaptic alpha-2 type adrenergic receptor regulating insulin release. J. Pharmacol. Expt. Ther. 215(1):226-230.

Nakamura H. and Pisano J.J. 1976. Fluorescamine derivatives of histidine, histamine, and certain related imidazoles: Unique flourescence after heating in acid. Arch. Biochem. Biophy. 177:334-335.

National Research Council 1984. Nutrient requirements for beef cattle. NAS-NRC, Washington. DC.

Ole Miaron J.O. and Christopherson R.J. 1992. Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and -particulate passage rate constants and apparent digestibility in steers. Can. J. Anim. Sci. 72:809-819.

Rizza R.A., Mandarino L.S. and Gerich J.E. 1982. Effects of growth hormone on insulin action in man: mechanisms of insulin resistance, impaired suppression of glucose production and impaired stimulation utilization. Diabetes 31: 663-669.

Scholtysik G. 1986. Animal pharmacology of guanfacine. Am. J. Cardiol. 57:13E-17E.

Sillence M.N., Tudor G.D., Mathews M.L. and Lindsay D.B. 1992. Effects of the α_2-adrenoceptor agonist guanfacine on growth and thermogenesis in mice. J. Anim. Sci. 70:3429-3434.

Trinder P. 1969. Determination of glucose in blood using glucose oxidase with and alternative oxygen acceptor. Ann. Clin. biochem. 6:24-27.

Thompson J.R., Christopherson R.J., Hammond V.A. and Hills G.A. 1978. Effects of acute cold exposure on plasma concentration of noradrenaline and adrenaline in sheep. Can. J. Anim. Sci. 58:23-28.

Watt P.W., Finley E., Cork S., Clegg R.A. and Vernon R.G. 1991. Chronic control of the β- and α-adrenergic system of sheep adipose tissue by growth hormone and insulin. Biochem. J. 273:39-42.

Wassner S.J., Schlitzer and Li J.B 1980. A rapid, sensitive method for the determination of 3-methylhistidine levels in urine and plasma using high-pressure liquid chromatography. Anal. Biochem. 104;284-289.

Wheaton J.E., Al-Raheem S.N, Massri Y.G. and Marcek J.M. 1986. Twenty-four hour growth hormone profiles in Angus steers. J. Anim. Sci. 62:1267-1272.

Wieland O.H. 1984. Glycerol UV-method. In Methods of Enzymatic Analysis. Eds

H.R. Bergmeyer, J.Bergmeyer and M Grabi, Vol VI pp 504-510

Yamashita K., Yamashita S., and Aiyoshi Y. 1980. Effect of α_2-adrenergic action on cyclic AMP levels in canine thyroid slices. Life Sci. 27:1127-1130.

Yates A.A. 1993. Food intake, appetite and work in hot environments. In B.M. Morriott ed. Nutritional Needs in Hot Environments. pp 297-304.

Young B.A., Kerrigan B. and Christopherson R.J. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55:17-22.

Young B.A., Fenton T.W. and McLean J.A. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol. 56(4):1120-1125.

5. The Thermogenic Role Of α_2-Adrenoceptor Stimulation in Fasted and Fed Sheep.

5.1. INTRODUCTION

Studies on research techniques based on physiological principles that may allow for the manipulation of energy balance through reduced maintenance costs in the beef industry have been initiated by other workers (Gazzola 1993, Hunter 1992 and Sillence et al. 1992 and were also the focus of Chapter 4). These studies have focused on compounds that could reduce metabolic rate (an index of energy biotransformation) in ruminants. One such chemical compound is guanfacin, an α_2-adrenoceptor agonist used therapeutically as an anti-hypertensive drug with both central and peripheral effects in humans (Van Zweiten 1980, 1986) and animals (Scholtysik 1986). Guanfacin is anticalorigenic in fasted steers (Hunter 1992) and, in laboratory animals, α_2-adrenoceptor stimulation by guanfacin resulted in the reduction of the resting energy expenditure. The latter was thought to be mediated by changes in noradrenaline release (Gazzola 1993). Heat production of 18 hour fasted and ad libitum fed steers in thermoneutral and cold environments is reduced by guanfacin (Chapter 4). Therefore, the regulation of thermogenesis at rest could involve α_2-adrenoceptor responses but it is not clear whether the heat increment of feeding is affected by α_2-adrenoceptor stimulation.

There is some experimental evidence to link the thermic effect of feeding (TEF) to the activity of the sympathoadrenal system (Christopherson and Brockman

1989, Astrup et al. 1990). The TEF is a result of the oxidation of nutrients (LeBlanc 1985) in association with ingestion, digestion and metabolism of dietary nutrients. The oxidative process involving substrate mobilization and utilization is divided into two phases, viz; the cephalic phase controlled by the palatability of food and activity of eating and the digestive/absorptive phase. Both appear to be under the influence of the sympathetic system via noradrenaline release (LeBlanc 1985, LeBlanc and Blondel 1985) as well as a delayed adrenaline response (Astrup 1990). In humans, α_2-adrenoceptor stimulation by clonidine, an α_2 -agonist, resulted in a reduction in TEF which was paralleled by a reduction in plasma noradrenaline appearance rate but not adrenaline (Schwartz et al. 1988). It has been speculated that an increase in TEF is associated with a reduced feed efficiency (LeBlanc 1985). Antithermogenic agents like guanfacin, in addition to conserving energy, might improve digestive efficiency of beef steers (Hunter 1992). This could be particularly important during the dry-season in the tropics when feed availability is limited.

In ruminants, β-adrenoceptor involvement in the TEF have been reported in sheep (Christopherson and Brockman 1989). However, much less is known about the involvement of α-adrenoceptors in the sympathetically-mediated thermogenesis in sheep either at rest or during feeding. This experiment was designed to investigate the effects of α_2-adrenoceptor stimulation by guanfacin in fasted and fed sheep on thermogenesis and other thermoregulatory responses, and to establish a dose metabolic response relationship for guanfacin in sheep. A third objective was to determine the effect of guanfacin on thermic responses to feeding.

5.2. MATERIALS AND METHODS

5.2.1. Animals and Experimental Procedure

Eight whether sheep with an average final body weight of 99±15 SD kg were used. They were studied in two groups (fasted and fed) consisting of 4 animals per group and were housed in individual pens in a thermoneutral environment. The animals were accustomed to being fed individually at 0900 hours a diet consisting of 1 kg pelleted alfalfa (dry matter % = 92; crude protein 19 %; gross energy = 4.6 Kcal/g DM) plus 0.5 kg grass cut hay (dry matter 91 % and crude protein 11 %) per day for 3 weeks prior to the experiment. On the day of the experiment following a 1 hour pre-treatment sampling period, food, but not water, was withheld from the fasted group and the fed group received a ration of 1 kg pelleted diet at time zero. Measurements of oxygen consumption were made for 1 h before and over a 5 hour period following time zero. The normal daily feed allotment was provided to the fasted group immediately after each 6-hour metabolic measurement session.

5.2.2. Treatments

The treatments consisted of a control physiological saline and the guanfacin (Sandoz Canada Inc. Dorvel, Quebec) doses were 0.8, 1.6 and 2.4 mg/sheep for low, medium and high doses respectively. The doses were randomly administered intravenously as a single bolus at time zero via a jugular catheter. Only one animal was measured on any day. There were eight days in one period. Once an animal had received a given drug treatment there were seven days before that animal was measured again, allowing a

minimum of one week for metabolic clearance between drugs. The animals were measured in the same order for each of the four periods.

5.3. MEASUREMENTS

5.3.1. Heat Production

The post-treatment oxygen consumption was determined for 5 hours by an open-circuit calorimetry apparatus (Young et al. 1975) connected to a ventilated hood to which the sheep were previously accustomed. Ventilation rate of the head hood was read from a flowmeter (Rotameter, Fisher and Porter, Warminster, Pa) and the oxygen concentration difference between incoming and outgoing respired air, was determined using a single-channel oxygen analyzer (Servomix 540A, Sussex, England). The data was acquired (every 5 seconds and averaged over 2 minutes) by a computerized data acquisition program developed in our laboratory (Godby and Gregory 1992). The oxygen consumption was the product of ventilation rate of the hood (mL min^{-1}) at STP and oxygen decrement. The measuring system was calibrated with nitrogen as a zero gas and by the iron burn method of Young et al. (1984). The heat production was calculated from the equation of McLean (1972).

5.3.2. Other

Heart rate was recorded by an a multichannel chart recorder model 69-7488

electrocardiograph (ECG; Carolina Biological Supply Co. Burlington, NC). The ECG peaks were counted at hourly intervals over 5 minutes and expressed as beats per minute. The rectal temperature was measured with a Fisher Digital Thermometer (Fisher Scientific Inc., ON). The skin temperature was measured from one point on the intrascapular region by a thermometer (Fisher Scientific Inc.). Blood (1 mL) was collected hourly into heparinized tuberculin syringes for haemoglobin and haematocrit determinations. Haemoglobin was determined in duplicate samples with an OSM 2 hemoximeter (Radiometer, Copenhagen, Denmark). The haematocrit was determined by an MB microcentrifuge (International Equipment, Niidhen Heights, MS).

5.4. STATISTICAL ANALYSIS

The experiment was conducted and analyzed as a split-plot with two levels of feed (fasted and fed) and four animals nested within each feed. Within a feed the four levels of drug (saline, guanfacin = low, guanfacin = medium, and guanfacin = high) were applied as a four-factor change-over design, balanced for first-order residual effects, with animals (a=4), period(p=4) and drugs (d=4). Comparisons between the four levels of the drug were made using least significant difference. Computations were performed using the general linear model procedure of the Statistical Analysis System Institute (SAS 1985).

5.5. RESULTS

The effect of feeding is shown in table 5.1. Heat production was higher in fed sheep

compared to fasted ($P<0.05$). The heart rate, haemoglobin concentration and haematocrit were significantly elevated in the fed sheep. The rectal temperature was not altered by feeding regime.

The effects of guanfacin are shown in Table 5.2. In the fasted sheep, the heat production was significantly ($P<0.05$) lower in the guanfacin vs vehicle treated animals. All the doses used lowered ($P<0.05$) the resting heat production of the fasted sheep. The heart rate and the rectal temperature of fasted sheep was not affected by guanfacin. However, all doses of guanfacin caused a significant ($P<0.05$) reduction in the skin temperature of fasted sheep. The low and the medium dose of guanfacin had no effect on the blood haemoglobin content but the highest dose significantly lowered the haemoglobin content and the haematocrit mirrored the haemoglobin response.

In the fed sheep, the low level of guanfacin had no effect on heat production. There was a small but significant ($P<0.05$) reduction in heat production at the medium and high dose of guanfacin when the values were compared to the vehicle. The heart rate of the fed sheep was not affected by any level of the guanfacin used in this study. The rectal temperature of the fed sheep was significantly increased ($P<0.05$) by 2.4 mg compared to vehicle, low or the medium doses of guanfacin. The skin temperature of the fed sheep was significantly ($P<0.05$) lower at the 2.4 mg dose compared to vehicle and the other two doses of guanfacin. The blood haemoglobin content was significantly ($P<0.05$) reduced at the highest dose compared to vehicle, and low and the medium doses of guanfacin. Although the haematocrit appeared to be reduced at the highest dose used in this experiment, the effect was not statistically significant.

Finally, the increment in heat production between the fed and the fasted sheep was not affected by the guanfacin treatments.

5.6. DISCUSSION

The increase in heat production in response to feeding of the present study is consistent with previously published data for sheep (Christopherson and Brockman 1989, Thompson et al. 1975, Webster and Hays 1968). The elevated heart rate in response to feeding is in agreement with results of other workers (Webster and Hays 1968). However, the absolute heart rate values are somewhat lower compared to those reported by Webster and Hays (1968). This discrepancy may be attributed to the older age and heavier weight (99 kg) of the sheep used in this experiment. The increase in haemoglobin and haematocrit in response to feeding would to help meet the increased demand for oxygen in the fed state.

The decrease in heat production of fasted sheep in response to the α_2-adrenoceptor stimulation by guanfacin is in full agreement with previous observations with cattle (Hunter 1992), mice (Sillence et a. 1992) and rats (Gazzola 1993). The actual physiological mechanism involved have yet to be fully elucidated. However, the study by Gazzola (1993) has shown that, the centrally-located neural α_2-adrenergic receptors may be involved and supports the hypothesis that peripheral noradrenaline release is suppressed in response to α_2-adrenoceptor stimulation by guanfacin. A recent study on cold pressor testing has shown that plasma noradrenaline release is indeed inhibited in response to α_2 -adrenoceptor stimulation by guanfacin (Koshiji et a. 1992).

On the other hand, the reduced skin temperature in fasted sheep in this study is evidence for a peripheral vasoconstrictor effect of guanfacin acting on α-adrenoceptors. The reduced haemoglobin and haematocrit content in response to α_2 -adrenoceptor stimulation is consistent with the reduced heat production and metabolic oxidative demands of the fasted sheep. The lack of increase in haemoglobin content in response to α-adrenoceptor stimulation is, perhaps surprising, since, α-adrenoceptor - mediated splenic contraction has been shown to lead to an increase in haemoglobin content (Ignarro and Titus 1968). Whether the effects noted in the present study were centrally or peripherally mediated has yet to be determined.

The small but significant decrease in heat production of the fed sheep in response to the medium and high dose of guanfacin is not entirely consistent with the results obtained by Schwartz et a. (1988) in man. The latter authors showed that TEF was completely inhibited by α_2-adrenoceptor stimulation by clonidine. The present study, on the other hand, suggests that α_2 -adrenergic activity does not affect the change in heat production resulting from feeding. Although, Christopherson and Brockman (1989) reported that, in sheep, the sympathoadrenal system partially modulates TEF, this effect was related to peripheral β-adrenoceptor effects. The possibility that α_2-adrenoceptor stimulation with guanfacin might act centrally to suppress sympathetic activity cannot be ruled out. Measurements of plasma noradrenaline might help to clarify this suggestion since, in other species, the reduced TEF in response to α_2-adrenoceptor stimulation was associated with a decrease in plasma noradrenaline but not adrenaline appearance rates (Schwartz et a. 1988). In

humans and laboratory animals it is known that the TEF is accompanied by a biphasic response in which noradrenaline levels remain high (LeBlanc 1985). In the present experiment even after a very short feeding period (animals consumed the feed in about 10 minutes), the heat production peaked at 20-40 minutes after feeding and then slowly declined over the remainder of the 5 h period to a level that was above the prefeeding average heat production; see Appendix 3 (Figure A3.1). One may therefore speculate that, in sheep the TEF may be under some neuroendocrine control and a suitable candidate for this regulation is noradrenaline. Surprisingly, the change in heat production between the fed and the fasted sheep was not influenced by guanfacin. LeBlanc (1985) suggested that, in man, only the cephalic phase of TEF is under a direct sympathetic tone. This meant that only a portion of the 'heat increment of food' in his experiment was influenced by the sympathetic system. This contradicts the finding of Schwartz et a. (1988) in which the total TEF was completely inhibited by an α_2-agonist.

In conclusion, guanfacin in doses ranging from 0.8 - 2.4 mg/sheep were anticalorigenic in fasted sheep, but in the fed sheep only the medium and high doses were effective in reducing the total post-feeding heat production of the sheep and guanfacin had no effect on the change in heat production associated with feeding. Secondly, the changes in skin temperature and rectal temperature were indicative of increased heat and energy conservation in response to guanfacin treatment in sheep.

Table 5.1. The effects of feeding on heat production (HP), heart rate (HR), rectal temperature (Tr), skin temperature (Ts), haemoglobin (HB) and haematocrit (HCT) of sheep.

	FASTED	FED	S.E.[1]
HP (Watts/kg)	1.21[b]	1.64[a]	0.04
HR (beats/min)	48.7[b]	56.6[a]	1.94
Tr (°C)	39.3[a]	39.1[a]	0.15
Ts (°C)	31.8[a]	31.7[a]	0.34
Hb (g/dl)	10.25[b]	11.44[a]	0.23
HCT (%)	29.55[b]	33.00[a]	0.69

[1]S.E. = standard error of the mean and N (number of animals) = 4.

[ab]means within rows with different superscripts differ P<0.05.

Table 5.2. The effects of α_2-adrenoceptor stimulation by guanfacin (vehicle = 0 mg; low = 0.8 mg; medium = 1.6 mg; high = 2.4 mg in vehicle= physiological saline) on heat production (HP), heart rate (HR), rectal temperature (Tr), skin temperature (Ts), haemoglobin (HB) and haematocrit (HCT) of fasted and fed sheep. Values are means of times 1-5 h).

	Trait	Vehicle	Low	Medium	High	S.E.[1]
FASTED						
	HP (W/kg)	1.21[a]	0.99[b]	1.09[ab]	0.98[b]	0.04
	HR (beats/min)	48.7[a]	43.5[a]	45.3[a]	46.3[a]	1.94
	Tr (°C)	39.3[a]	39.3[a]	39.4[a]	39.3[a]	0.15
	Ts (°C)	31.8[a]	30.7[b]	30.7[b]	30.6[b]	0.35
	Hb (g/dl)	10.25[a]	10.04[a]	9.95[a]	9.54[b]	0.23
	HCT (%)	29.55[a]	28.70[a]	28.20[a]	27.15[b]	0.69
FED						
	HP (W/kg)	1.64[a]	1.59[a]	1.43[b]	1.55[ab]	0.04
	HR (beats/min)	56.6[a]	54.9[a]	56.5[a]	51.4[a]	1.94
	Tr (°C)	39.1[b]	39.4[b]	39.5[b]	39.9[a]	0.15
	Ts (°C)	31.7[a]	31.4[a]	31.3[a]	30.3[b]	0.35
	Hb (g/dl)	11.44[a]	11.67[a]	11.36[a]	10.55[b]	0.23
	HCT (%)	33.00[a]	32.70[a]	31.85[a]	31.50[a]	0.69
ΔHP(fed-fasted) (W/kg)		0.43[a]	0.61[a]	0.34[a]	0.57[a]	0.17

[1]S.E. = standard error of the mean and N (number of animals) = 4.
[ab]Lsmeans within rows with different superscripts differ P<0.05.

5.7. REFERENCES

Astrup A.V. Christensen N.J., Simonsen L. and Bulow J. 1990. Effect of nutrient intake on sympathoadrenal activity and thermogenic mechanism. J. Neurosc. Methods 34:187-192.

Christopherson R.J. and Brockman R.P. 1989. Effect of feeding and a beta-adrenergic blocking agent on oxygen consumption by the portal drained viscera, liver and hindquarter of sheep. EAAP 43:147-150.

Gazzola C. 1993. α_2-Adrenoceptor-mediated effects on resting energy expenditure. Int. J. Obesity 17:637-641.

Godby G. and Gregory P. 1992. Datagrabber. Department of Animal Science, University of Alberta

Hunter R.A. 1992. The effect of α_2-adrenergic agonist, guanfacine, on the energy metabolism of steers fed on low quality roughage diets. Br. J. Nutr. 67:337-343.

Hunter R.A., Sillence M.N. Gazzola C. and Spiers W.G. 1993. Increasing animal growth rate of cattle by reducing maintenance energy requirements. Aust. J. Agric. Res. 44;579-595.

Ignarro L.J. and Titus F. 1968. The presence of antagonistically acting alpha and beta adrenergic receptors in the mouse spleen. J. Pharmacol. Expt. Ther. 160:172-

Koshiji M., Ito H., Minatoguchi S., Watanabe H., Imai Y., Kakami M and Hirakawa S. 1992. A comparison of guanfacine, bunazosin, atenolol and nadolol on blood pressure and plasma noradrenaline responses to cold pressor testing. Clinical Exp. Pharmacol. and Physiol. 19:481-488.

LeBlanc J. 1985. Thermogensis in relation to feeding and exercise training. Int. J. Obesity 9 (supl.2):75-79.

LeBlanc J. and Blondel L. 1985. Role of palatability on meal-induced thermogenesis in human subjects. Am. J. Physiol. 248:E333-336.

McLean J.A. 1972. On the calculation of heat production from open-circuit calorimetric measurement. Br. J. Nutr. 27:597-600.

Schwartz R.S., Jaeger L.F., and Veith R.C. 1988. Effect of clonidine on the thermic effect of feeding in humans. Am. J. Physiol. 254:R90-R94.

Scholtysik G. 1986. Animal pharmacology of guanfacine. Am. J. Cardiol. 57:13E-17E.

Sillence M.N., Tudor G.D., Mathews M.L. and Lindsay D.B. 1992. Effects of the α_2-adrenoceptor agonist guanfacine on growth and thermogenesis in mice. J. Anim. Sci. 70:3429-3434.

Thompson G.E., Gardner J.W. and Bell A.W. 1975. The oxygen consumption, fatty acid and glycerol uptake of the liver on fed and fasted sheep during cold exposure. Quart. J. Expt. Physiol. 60:107-121.

Van Zweiten P.A. 1980. Pharmacology of centrally acting hypotensive drugs. Br. J. Pharmacol. (Supl. 1):135-205.

Van Zweiten P.A. 1986. Overview of alpha$_2$-adrenoceptor agonist with central action. Am. J. Cardiol 57:3E-5E.

Webster A.J.F. and Hays F.L. 1968. Effects of beta-adrenergic blockade on the heart rate and energy expenditure of sheep during feeding and during acute cold exposure. Can. J. Physiol. Pharmacol. 46: 577-583.

Young B.A., Kerrigan B. and Christopherson R.J. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55:17-22.

Young B.A., Fenton T.W. and McLean J.A. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol. 56(4):1120-1125.

6. GENERAL DISCUSSION AND CONCLUSION

The major conclusions from this thesis have been summerized under the specific chapters. In this chapter, the various conclusions are discussed in relation to each other under the following subheadings:

a. Physiological mechanisms in adrenergic regulation

b. Adrenoceptors, thermogenesis and energy conservation

c. Limitations and future directions

d. Application in animal production

6.1. Physiological Mechanisms in Adrenergic Regulation of Thermogenesis

6.1.1. ß-Adrenoceptors

A series of experiments was conducted to assess the adrenoceptor regulation of thermogenesis in sheep and cattle. In the initial set of experiments, selective ß-blocking agents were used to characterize ß-adrenoceptor responses and assess their role in thermogenic and metabolic responses in sheep. Upon intravenous infusion, adrenaline induces a larger metabolic response than noradrenaline in sheep (McDowell and Annison 1991, Graham and Christopherson 1981). This may be due, in part, to it's role as a circulating hormone. Consequently adrenaline was used as an agonist to study the thermic effects of the peripheral β-adrenoceptor on whole body and specific organs of sheep.

In the first experiment, the hypothesis that $ß_1$- and $ß_2$-adrenoceptors differentially modulate whole body and organ thermic effects of adrenaline infusion was addressed. In this experiment, the whole body and organ (portal drained viscera) thermic effect of adrenaline infusion was differentially influenced by the selective β_1- and β_2-adrenoceptor antagonists. The portal drained viscera thermogenic response is consistent with the activity and presence of the β-adrenoceptors in the gastrointestinal tract as reported previously in humans (McIntrye et al. 1992) and in sheep (Brikas et al. 1989). By stimulating the portal drained viscera β-adrenoceptors with exogenous naturally-occurring adrenaline rather than a synthetic agonist, it was possible to assess responses which could reflect the physiological conditions in the gut. This study partially confirms the hypothesis that β-adrenoceptors could mediate a portion of the portal drained viscera metabolic rate. Portal drained viscera oxygen consumption of the sheep is elevated in response to feeding (Christopherson and Brockman 1989, Kelly et al. 1993). The latter authors showed that the increased thermic effect of feeding in the gut was associated with the increased activity of Na^+/K^+ -ATPase. The mechanism involved in the thermic effect of adrenaline infusion may also be linked to increase Na^+/K^+-ATPase activity (Kelly et al. 1993) and triacylglycerol/fatty acid substrate cycle. In the rat, the latter response to feeding was abolished by β-adrenoceptor blockade (Brooks et al. 1983). ICI 118551, the selective β_2-adrenoceptor antagonist has been shown to inhibits lipolysis (Ole Hansen et al. 1990) and it abolishes the adrenaline-induced hypokalaemia in the present experiment with sheep and in man (Struthers and Reid 1984). The adrenaline infusion in the presence and absence of

non-selective ß-blockers clearly and definitively establishes that whole body, portal drained viscera and hindquarters metabolic rate are modified by the adrenergic system. Finally, for the first time this study provided a definitive role for the ß-adrenoceptor modulation of the portal drained viscera and hindquarter metabolic rate during adrenaline infusion in sheep.

Following the adrenaline infusion experiments the next step was to assess the organ metabolic responses in sheep to other natural stimuli that may influence metabolism, i.e., cold and feeding. The hypothesis that whole body, hindquarter and portal drained viscera thermogenesis is elevated in response to cold exposure and a portion of the increase is mediated by the β-adrenoceptor system was examined. Cold exposure is known to cause an increase in endogenous plasma catecholamines in sheep (Christopherson et al. 1978) which could play a role in the increase in cold-induced thermogenesis. The whole body metabolic response is partly inhibited by the nonselective β-adrenoceptor blocker, propranolol (Webster et al. 1969). The results from the acute cold experiments may point to a more important and differential role for the β_2-adrenoceptor mediated whole body cold thermogenesis. The latter is the first report on ruminants (sheep). Cold exposure, however, had no effect on the portal drained viscera oxygen consumption of the sheep and, therefore, there appears to be no role for the β-adrenoceptor system in this tissue relative to cold thermogenesis. The hindquarter oxygen consumption was higher in the cold-exposed animals compared to the controls in a thermoneutral environment. There were large variations in the hindquarter thermic responses to adrenaline infusion and thermic effects of cold

exposure as well as in the responses to the nonselective and the selective β-adrenoceptor antagonists. This large between-animal variability plus the fact that measurements were completed in only three sheep resulted in an inability to detect statistically significant differences in the hindquarter response to cold exposure that could be attributed to ß-adrenoceptors. Therefore, the β-adrenoceptors modulate whole body and organ thermic effects of adrenaline infusion and a minor but significant adrenergic regulation of the whole body, but not portal drained viscera, cold-induced thermogenesis is reported.

In a separate experiment, the hypothesis that a portion of the whole body and portal drained viscera thermic responses to feeding is modulated by the β-adrenoceptor system was examined in the same sheep. Feeding, predictably increased the oxygen consumption of the sheep. This effect was not altered by either the nonselective or the selective β-adrenoceptor blockade. The portal drained viscera oxygen consumption was slightly, but not significantly, higher in response to feeding and none of the ß-blockers had any effect on the portal drained viscera oxygen consumption. The limited role for β-adrenoceptor mediation in the metabolic responses to feeding in sheep observed in the present study is consistent with that of Webster and Hays (1968) who specifically studied the energy cost of eating. This finding is not universal because, depending on the diet offered, the amount of feed eaten and the timing of the measurements, the modulatory role of β-adrenoceptor in the thermic effect of feeding has been demonstrated in man (LeBlanc 1985A, strup et al. 1990), the pig (Dauncy and Ingram 1979) and the sheep (Christopherson and Brockman 1989).

6.1.2. α_2-Adrenoceptors

In the last set of experiments, the role of the α_2-adrenoceptors in the regulation of thermogenesis was studied in cattle and sheep. The sympathoadrenal system consists of two components, namely, the adrenal medulla and the sympathetic nervous system (Astrup et al. 1990). Noradrenaline tissue turnover is associated with sympathetic nervous system mediated metabolic effects. However, under certain conditions these effects could be confounded with noradrenaline secreted by the adrenal medulla (Young et al. 1984). Alpha-2-adrenergic agonists that selectively influence noradrenaline metabolic effects (Koshiji et al. 1992, Schwartz 1988) could be used to estimate the sympathetic component of the sympathoadrenal system during thermal exposure and feeding. One such chemical agent used as a research tool in this overall study is guanfacin. Guanfacin is both a centrally and peripheral acting α_2-adrenoceptor agonist used therapeutically as an antihypertensive drug in humans (Van Zweiten 1980, 1986) and has been shown to be antihypertensive in animals (Scholtysik 1986). In the experiment with steers, the hypothesis that guanfacin, an α_2-adrenergic agonist, is anticalorigenic in steers kept in different thermal environments was tested. I conclude that in steers (18 hours post feeding) α_2-adrenoceptor stimulation resulted in a reduction of heat production in the cold as well as in the thermoneutral environment. The decrease in a hot environment only approached significance. The latter is the first information on the interaction between guanfacin, ambient temperature and heat production (index of energy biotransformation) in ruminants. These changes might have been due to a reduced sympathetic outflow, because during cold pressor testing

in humans, a reduction in plasma noradrenaline (marker of sympathetic stimulation) was observed with guanfacin (Koshiji et al. 1992) a response that suggests a decrease sympathetic activity.

Suppression of sympathetic activity during fasting, with a consequent decrease in metabolic rate, would contribute to the conservation of energy during a time of energy scarcity. The reduction of heat production in the fed state could be economically important if it is associated with reduced maintenance costs. Research techniques based on physiological principles that could lead to a manipulation of energy maintenance costs that favour improved beef cattle production efficiency (Hunter et al. 1993, Hunter 1992) could have a major effect in the beef industry especially in the tropics. For the last few decades, genetically-selected ruminants (based on ability to resist excessive heat load) reared in this regions are generally less productive and at times their economic value is minimal. In the past, several techniques to improve feed efficiency in the tropics have been tried, including infrequent provision of water (Jewell and Nicholson 1989) which may result in increased dry matter digestibility. Unfortunately, the apparent increase in digestibility will not override the negative effects of water scarcity and heat load. The second and much more promising technique is sheltering of animals from the harmful effects of solar radiation typical of arid and tropical Africa. However, this technique is expensive and impractical for the nomads who roam the vast region of the tropics especially in Africa. In view of this and the population explosion with the ever decreasing food base in Africa, the need to look for better techniques that may lead to improved

animal productivity is justified. The possibility of activation of α_2-adrenoceptor mechanisms with the use of agonists, such as guanfacin hold promise and should be investigated thoroughly.

The α_2-adrenoceptor-induced changes in heat production in a simulated tropical environment were minimal, possibly because the breed of the steers (Holstein) used in the present experiment was not a heat tolerant breed. Further research to assess guanfacin responses in a more heat resistant breed of cattle is needed. The α_2-adrenoceptor-mediated increase in plasma glucose concentration is consistent with the reduced peripheral glucose utilization and increased gluconeogenesis as reported by other workers (DiTullo et al. 1984). The latter could be associated with energy conservation by means of a reduced substrate utilization. To appreciate the significance of the reduced glucose utilization, the long-term effects of hyperglycaemia need to be addressed.

There appear to be conflicting reports on physiological mechanism underlying α_2-adrenoceptor mediated thermogenic responses. Hunter et al. (1993) suggested that, in cattle, these responses are not mediated centrally but rather peripherally in a thermoneutral environment. These authors based their reasoning on the facts that intracerebral administration of guanfacin had no effect on heat production of steers but an intramuscular administration of a dose as low as 5 mg had a significant effect on heat production. A peripheral effect is possible because postsynaptic α_2-adrenoceptors, in addition to being present in the brain stem and cerebral cortex are also found in the kidneys, spleen, skeletal muscle and lungs (Flordelis et al. 1990) and ovine adipocytes.

The α_{2A}-adrenoceptor subspecies found in the rat kidney has a higher affinity for guanfacin compared to other tissues (Uhlen and Wikberg 1991). Furthermore, reduced skin temperature in sheep treated with guanfacin is consistent with a peripheral vasoconstrictor effect of α_2-adrenoceptor stimulation. On the other hand, there is no agreement on the effects of guanfacin on plasma catecholamines. Hunter et al. (1993) reported that noradrenaline, a possible indicator of peripheral sympathetic tone, was not affected by guanfacin given to steers in a thermoneutral environment. However, reports on other species show that a prolonged use of guanfacin results in low levels of plasma noradrenaline and even adrenaline (Knypi et al. 1989). In addition, plasma noradrenaline levels were reduced by guanfacin during a cold pressor testing in man (Koshiji et al. 1992). Nevertheless, the responses to heat production observed in this experiment and that of Hunter (1992) could be mediated by other unknown mechanisms. It has been reported that guanfacin inhibits excitatory amino acid release from the rat cerebral cortex via a new non-α_2/α_1 -adrenoceptor mechanism (Uhlen et al. 1989). It remains to be determined whether guanfacin influences thermogenesis in cattle and sheep by some non-specific effects not involving α_2-adrenoceptors.

I tested the hypothesis that guanfacin is antithermogenic in ad libitum fed warm- and cold-acclimated steers. It was concluded that, the guanfacin induced a 12% reduction in heat production when the data was pooled across acclimation temperatures and, in the cold environment, was associated with a small reduction in feed intake. A lack of significant change in feed intake in response to guanfacin reported by Hunter (1992) was associated with an increase in fluid retention time in the rumen. The latter

may increase digestibility and fermentation which, in turn, would result in increased volatile fatty acids in the rumen. Increase in propionate metabolism could involve gluconeogensis. Therefore, the hyperglycaemia observed in the present study could also result from glucose precursors such as propionate.

In a final experiment, I tested the hypothesis that guanfacin was antithermogenic in fasted and fed sheep and that guanfacin could suppress the increased heat production associated with feeding. All doses reduced heat production of sheep at rest and the medium and high dose lowered the heat production of fed sheep but had no effect on the heat increment of feeding. This is the first information on α_2-adrenoceptor influence on heat increment of feeding in sheep.

Taken together, the use of β-adrenoceptor antagonists in elucidating the differential effects for the β_1- and β_2-adrenoceptor mediated metabolic responses are now well documented in most species including ruminants (with a substantial contribution from this study). Further, ß-adrenoceptor antagonists coupled with the α_2-agonist, guanfacin, are useful research tools for assessing the significance of the sympathoadrenal system (the adrenal gland and the sympathetic nervous system) in modulating metabolic responses to adrenaline infusion, thermal exposure and feeding in sheep and cattle. This study supports the hypothesis that β_1- and β_2-adrenoceptors, differentially modulates the whole body and organ (portal drained viscera) oxygen consumption of the sheep during adrenaline infusion. During acute cold exposure the whole body but not the portal drained viscera oxygen consumption is influenced by ß-adrenoceptor process with a major role for the β_2-adrenoceptors. In response to

feeding, the thermogenic effects were not affected by β-adrenoceptor blockade. The α_2-, β_1- and β_2-adrenoceptors when stimulated with specific agonist and antagonists are involved in energy conservation in sheep and cattle by means of reduced heat production. The α_2-adrenergic responses could be associated with reduced substrate utilization. The increased glucose and glycerol concentrations in this study may be evidence for the latter. Guanfacin due to its hyperglycaemic effect, may be useful in veterinary medicine in the prophylaxis and the treatment of bovine ketosis, ovine pregnancy toxaemia and neonatal hypoglycaemia in piglets. The treatment of these conditions may be particularly feasible if the potential for gluconeogenesis is still present in the affected animals.

6.2. Adrenoceptors, Thermogenesis and Energy Conservation

Energy conservation strategies in animals may involve the adrenoceptor system. In the temperate regions animals adopt strategies which allow them to optimize the build up of their energy reserves in anticipation of stressful and unfavourable weather conditions. Body fat is the preferred energy storing tissue in the body. The ß-adrenoceptors are instrumental in fat mobilization and their interaction with insulin could be important in lipogenesis (Selberg et al. 1991). During lipogenesis these receptors could be suppressed and could be activated during lipolysis induced by seasonal changes in climate e.g. winter or dry seasons when food is scarce. The physiological mechanisms responsible for the suppression of ß-adrenoceptors in vivo are unclear, however, it may involve downregulation of the adrenergic receptors

resulting in reduced sensitivity to circulating catecholamines which result from the interaction with other hormones e.g. insulin. In the study of Selberg et al. (1991), hyperinsulinemia evoked a reduction in adrenaline-induced thermogenesis in man. The only evidence for the suppression of the adrenergic receptors in the latter study was a reduction in lipolysis in the hyperinsulinemic individuals treated with adrenaline. In addition, the lipid environment of the receptors could modify the adrenoceptor-agonist interaction, since dietary lipid supplementation has been implicated in the decreased coupling between the ß-adrenoceptors and the Gs protein (Nijkamp et al. 1992). Other intracellular events mediated by phosphodiesterase that lead to a decrease in cAMP and hence in substrate mobilization (Jourdan et al. 1984) and molecular modulation of the expression of the ß-adrenoceptors (Hadcock and Malbon 1988) may be instrumental in the suppression of the ß-adrenoceptors in vivo. Irrespective of the mechanism involved, the suppression of the ß-adrenoceptors may be associated with energy conservation. This viewpoint is supported by the present study. On the other hand, in the arid and dry regions, the α_2-adrenoceptors (Hunter et al. 1993) may be more important in energy conservation. New data from the present study on the interaction between the ambient temperature and the effects of α_2-adrenoceptor stimulation by guanfacin on heat production of restricted and ad libitum fed steers, indicate that the α_2-adrenoceptor activation could be an important energy conservation strategy employed by domestic ruminants. In conclusion cattle and sheep have the potential to improve energy conservation by either activation of α_2-adrenergic mediated processes or by suppression the $ß_1$- and $ß_2$-adrenoceptor pathways. However,

it remains to be determined whether the adrenoceptor system is particularly important in physiological states of wild and domestic ruminants associated with reduced metabolic such as dehydration, feed scarcity, seasonal energy biotransformation and topor.

6.3. Limitations and Future Directions

Meaningful physiological changes associated with thermal and diet-induced thermogenesis are studied on the whole animal level. Heat production was calculated from the decrement in oxygen concentration and ventilation rate as described by MacLean (1972). In ruminants, the use of this equation in the calculation of heat production is accurate within ±2%. However, erratic feeding regimens may influence results. Therefore, the feeding regimen was taken into account in all of the experiments reported in this thesis. The errors which could arise in an open circuit system were controlled and accounted for by nitrogen injection or iron burn calibrations as described by Young et al. (1984).

The blood flow probes were bench tested before and after implantation and the signal received was within the 13% range recommended by the manufacturer and in addition, zero flow reference was confirmed at the time of autopsy. Blood flow measurement by spot reading is traditionally associated with a 10-20% error. The inherent error (of up to 15% on individual readings as specified by the manufacturer) associated with all blood flow probes could potentially mask some physiologically important but small metabolic differences. This error was minimized and compensated

for, in the present study, however, by the continuous measurement and the averaging of frequent repeated measurements over 1 hour interval. The blood flow transit-time technology allowed for accurate determination of blood flow and detection of major treatment differences in this study.

The use of ß-adrenoceptor blockers such as propranolol, metoprolol and ICI 118551 as research tools to study whole body and organ adrenoceptor-mediated thermogenesis and guanfacin for α_2-adrenoceptor whole animal antithermogenic effect, provide physiologically important information on the adrenoceptor regulation of thermogenesis. However, this approach will likely reflect the summation of presynaptic and postsynaptic effects on a variety of interconnecting adrenoceptor systems in various organs, or tissues and cells within a specific organ, i.e., the portal drained viscera or the hind limb of the sheep. For future studies in animals, there is a need to identify techniques for the direct study of adrenoceptors in vivo. One such technique is the radionuclide scan of adrenoceptors or receptor scans mentioned by Motulsky and Insel (1987). With the use of the latter method, it would be feasible to monitor the distribution of the adrenoceptors in various tissues or organs in vivo. Another futuristic approach is the use of adrenoceptor antibodies. The latter may broaden our knowledge on the adrenoceptors and may provide a potential means for manipulation of adrenoceptors for direct application in animal production.

6.4. Application in Animal Production

The ß-adrenoceptor antagonist have not been used as dietary supplements. If these

compounds are given to well fed animals, they are likely to increase fat deposition. The latter is an undesirable effect in the beef industry unless it could specifically improve marbling by favouring intramuscular deposition of fat. The latter possibility needs to be explored. However, in cattle reared in dry tropical regions where feed availability is scarce and for efficient management of dams prior to pregnancy it might be desirable to increase their fat depots by use of ß-adrenoceptor blockers as feed supplements especially during the rainy seasons. In the tropics, the camel is known to accumulate fat during the rainy seasons when nutrients are abundant and it is possible that these fat stores allow it to better cope with the harsher drier seasons when forage availability is low. The mechanism of the rapid fat deposition could involve the ß-adrenoceptor processes. Preliminary research on cattle should be designed to ascertain whether the ß-adrenoceptor antagonist supplementation during the rainy season (plentiful food supply) could improve productivity during the prolonged dry seasons. This application might be difficult to employ in extensive pastoral animal production unless a practical system of delivery of the agents can be developed.

In young animals, which have the potential to grow rapidly when feed is abundant, it may be most appropriate for the animal to increase energy expenditure as part of a natural investment which favours lean tissue growth at the expense of fat deposition. Activation of ß-adrenoceptors (as shown by use of ß-agonists) would tend to favour protein accretion at a particular metabolic energy cost which requires diversion of food energy away from fat depots. Hence, depending upon the stage of the life cycle and season of the year, the conservation of energy may not always be in

the long term interest of the species. Achieving significant animal growth among the offspring is an important energy demanding process that needs to be supported. Beta adrenoceptor activation may be important in these circumstances.

The α_2-agonists have not been successfully used as food supplements in animal production (see Chapter 1). However, the reduced metabolic rate in response to guanfacin, an α_2-agonist, could be a potential strategy for improving energy balance. Knowledge on the means by which the saving in energy is achieved could be important in animal production efficiency. Nevertheless, the combination of the ß-antagonists and α_2-adrenoceptor agonists as feed supplements may be of some economic importance in animals that experience drastic variations in seasonal food availability.

6.5. REFERENCES

Astrup A.V. Christensen N.J., Simonsen L. and Bulow J. 1990. Effect of nutrient intake on sympathoadrenal activity and thermogenic mechanism. J. Neurosc. Methods 34:187-192.

Brikas P., Bueno L., and Fioromonti J. 1989. Central and peripheral β-adrenergic influences on reticulo-rumen and upper-gut myoelectrical activity in sheep. J. Vet. Pharmacol. Therap. 12:430-437.

Brooks B.J., Arch J.R.S. and Newsholme E.A. 1983. Effect of some hormones on the rate of the triacylglycerol/fatty acid substrate cycle in adipose tissue of the mouse in vivo. Biosc. Reports 3:263-267.

Christopherson R.J. and Brockman R.P. 1989. Effect of feeding and a ß-adrenergic blocking agent on oxygen consumption by the portal drained viscera, liver and hindquarters of the sheep. EAAP 43:147-150.

Christopherson R.J. , Thompson J.R., Hammond V.A. and Hills G.A. 1978. Effects of thyroid status on plasma adrenaline and noradrenaline concentration in sheep and during acute and chronic cold exposure. Can. J. Physiol. Pharmacol. 56:490-496.

Dauncy M.J. and Ingram D.L. 1979. Effect of dietary composition and cold exposure on non-shivering thermogenesis in young pigs and its alteration by the β-blocker propranolol. Br. J. Nutr. 41:361-370.

DiTullo N.W., Cieslinski L., Matthews W.D., and Storer B.1984. Mechanisms involved in the hyperglycaemic response induced by clonidine and other α_2-adrenoceptor agonists. J. Pharmacol. Expt. Ther. 228 (1):168-173.

Flordellis C.S., Castellano M. and Franco R. 1990. Expression of multiple α_2-adrenergic receptor messenger RNA species in rat tissues. Hypertension 15:881-887.

Graham A.D. and Christopherson R.J. 1981. Effects of adrenaline and noradrenaline on the heat production of warm and cold acclimated sheep. Can. J. Physiol. Pharmacol. 59:985-993.

Hadcock J.R. and Malbon C.C. 1988. Down-regulation of ß-adrenergic receptors: Agonist-induced reduction in receptor mRNA levels. Proc. Natl. Sci. USA. 85:5021-5025.

Hahn L.G. 1981. Housing and management to reduce climatic impacts on livestock. J.

Anim. Sci. 52:175-185.

Hunter R.A. 1992. The effect of α_2-adrenergic agonist, guanfacin, on the energy metabolism of steers fed on low quality roughage diets. Br. J. Nur. 67:337-343.

Hunter R.A., Sillence M.N., Gazzola C. and Spiers W.G. 1993. Increasing animal growth rates of cattle by reducing maintenance energy requirements. Aust. J. Agric. Res. 44:578-595.

Jewell P.A. and Nicholson M.J. 1989. Strategies for water economy amongst cattle pastoralists and in wild ruminants pp73-85. In P.A. Jewell and G.M.O. Maloiy (Eds.). The Biology of Large African Mammals in Their Environments. Zool. Soc. Lond. Clarendon Press. Oxford.

Jourdan M.L., Wang L.C.H. and Christopherson R.J. 1984. Effects of fasting and aminophylline on norepinephrine-stimulated non-shivering thermogenesis. Life Sciences 34:1101-1109.

Kelly J.M., Southorn B.G., Kelly C.E., Milligan L.P. and McBride B.W. 1993. Quantification of in vitro and in vivo energy metabolism of the gastrointestinal tract of fed and fasted sheep. Can. J. Anim. Sci. 73:855-868.

Koshiji M., Ito H., Watanabe H., Imai Y., Kakami M . and Hirakawa S. 1992. A comparison of guanfacine, bunazosin, atenolol and nadolol on blood pressure and plasma noradrenaline responses to cold pressor testing. Clin. Expt. Pharmacol. Physiol. 19:481-488.

Knypi K., Waclaw B., Chodakowska J., Brym E., Czerniewska -E., Waclawek M.J., Kucynska K., Berent H. and Januszewski W. 1989. Effect of long-term treatment with guanfacine on selected humoral metabolic indices in patients with primary hypertension. Pol. Tyg. Lek. 44(12-13):293-296.

LeBlanc J. 1985. Thermogenesis in relation to feeding and exercise training. Int. J. Obesity 9(suppl.2):75-79.

McLean J.A. 1972. On the calculation of heat production from open-circuit calorimetric measurement. Br. J. Nutr. 27:597-600.

McDowell G.H. and Annison E.F. 1991. Hormonal control of energy and protein metabolism. In T. Tsuda, Y. Sasaki and R. Kawashina eds. Physiological Aspects of Digestion and Metabolism in Ruminants. Proc. Intern. Symp. on Ruminant Physiology. Academic Press, Inc. pp231-253.

McIntyre A.S., Thompson D.G., Days,. Durnham W.R. and Walker E.R. 1992.

Modulation of human upper intestinal nutrient transit by beta adrenoceptor mediated pathway. Gut 33:1062-1070.

Nijkamp F.P., Engels F., Hendricks P.A.J. and Van Oosterhout A.J.M. 1992. Mechanisms of ß-adrenergic receptor regulation in lungs and its implications for physiological responses. Physiol. Rev. 72(2):323-362.

Ole Hansen, Johansson B.W. and Nielsson-Ehle P. 1990. Metabolic, electrocardiographic, and hemodynamic responses to increase circulating adrenaline: effects of selective and nonselective beta adrenoceptor blockade. Angiology 41 (3):175-188.

Scholtysik G. 1986. Animal pharmacology of guanfacine. Am. J. Cardiol. 57:13E-17E.

Schwartz R.S., Jaeger L.F., and Veith R.C. 1988. Effect of clonidine on the thermic effect of feeding in humans. Am. J. Physiol. 254:R90-R94.

Selberg O., Schlaak S., Balks H., Muhlen A. and Muller M.J. 1991. Thermogenic effect of adrenaline: interaction with insulin. Eur. J. Appl. Physiol. 63:417-423.

Sillence M.N., Tudor g.D., Mathews M.L. and Lindsay D.B. 1992. Effect of α_2-adrenoceptor agonist guanfacine on growth and thermogenesis in mice. J. Anim. Sci. 58:23-28.

Struthers A.D. and Reid J. 1984. Adrenaline causes hypokalemia in man by β_2-adrenoceptor stimulation. Clin. Endocrinology. 20:409-414.

Uhlen S. and Wikberg J.E.S. 1991. Delineation of rat kidney α_{2A}- and α_{2B}-adrenoceptors with [^{3}H]RX821002 radioligand binding: computer modelling reveals that guanfacine is an α_{2A}-selective compound. Eur. J. Pharmacol. 202:235-243.

Uhlen S., Nasstrom J., Post C. and Wikberg J.E. 1989. Guanfacine inhibits excitatory amino acids release from rat cerebral cortex through a non-adrenergic mechanism. Pharmacol. Toxicol. 65(5):357-361.

Van Zweiten P.A. 1980. Pharmacology of centrally acting hypotensive drugs. B.J. Pharmacol. (suppl.1):135-205.

Van Zweiten P.A. 1986. Overview of alpha$_2$-adrenoceptor agonist with central action. Am. J. Cardiol. 57:3E-5E.

Webster A.J.F., Heitman J.H., Hays F.L. and Olynyk G.P. 1969. Catecholamines and cold thermogenesis in sheep. Can. J. Physiol. Pharmacol. 47:719-724.

Webster A.J.F. and Hays F.L. 1968. Effects of beta-adrenergic blockade on the heart rate and energy expenditure of sheep during feeding and during acute cold exposure. Can. J. Physiol. Pharmacol. 46;477-583.

Young J.B., Rosa R.M. and Landsberg L. 1984. Dissociation of sympathetic nervous system and adrenal medullary responses. Am. J. Physiol. 247:E35-E40.

APPENDIX

Appendix 1

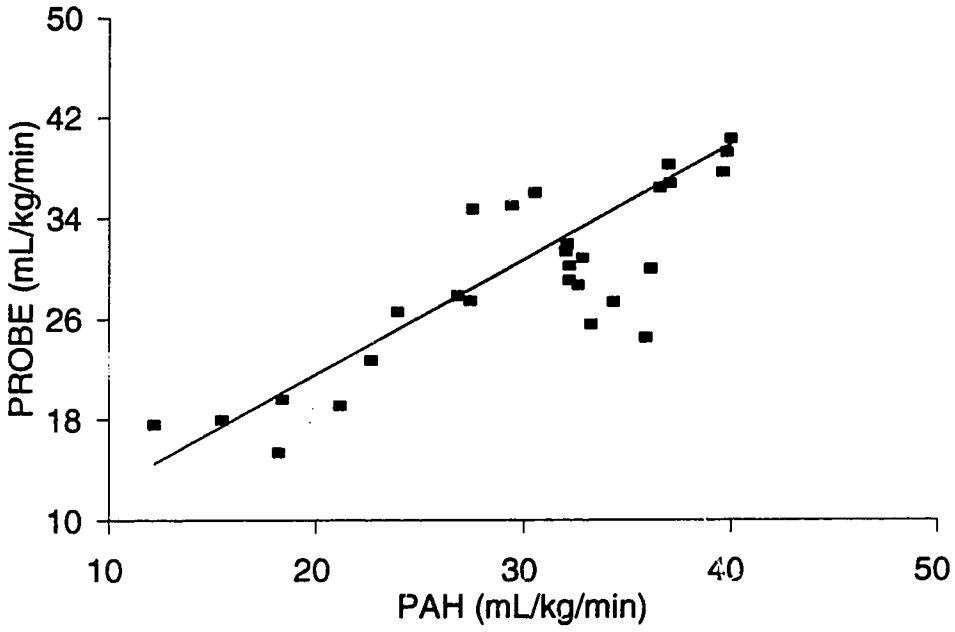
Table A1.1 Portal blood flow in sheep as determined by p-Aminohippuric acid (PAH) dilution and other techniques.

Body weight (kg)	Technique	Portal blood flow (mL/kg/min	Source[3]
63	PAH	29±5.1	present study
	Transit-time	30±3.5	present study
39	Doppler shift	26	Prewitt et al. 1975
56	PAH dilution	28	Naylor et al. 1985
40	PAH	33-50	Kelly et al. 1993
61	PAH dilution	38	Katz wt al. 1969

[3]Are in Chapter2 reference list

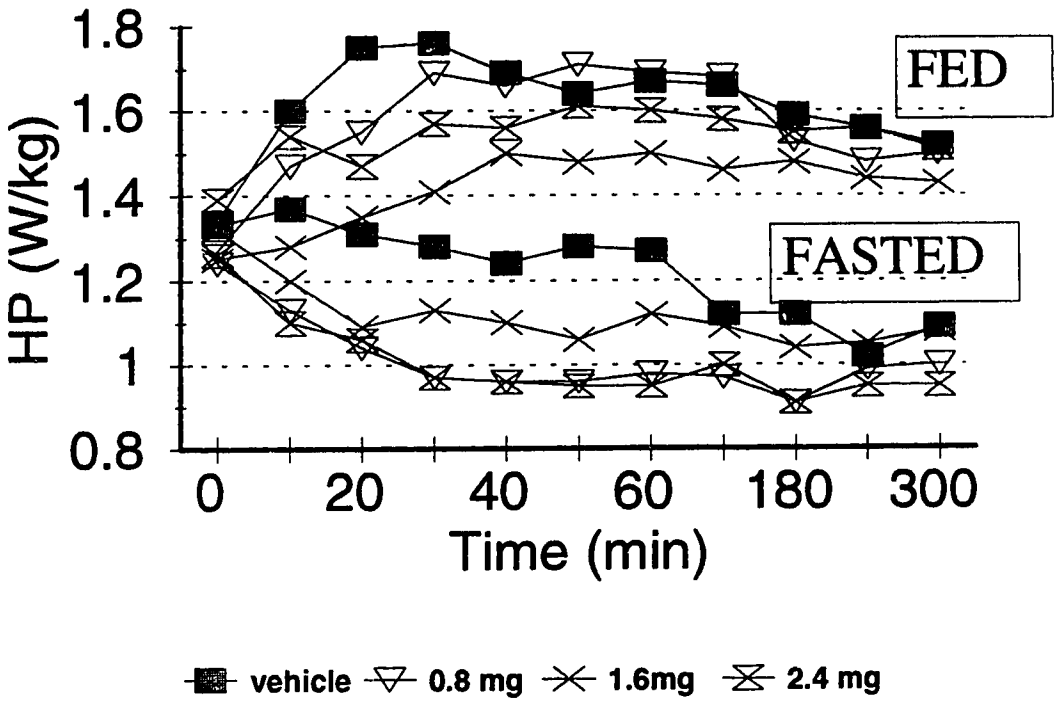
Appendix 2

Figure A2.1. Comparison of portal blood flow in sheep as determined by p-Aminohippuric acid (PAH) dilution and transit-time techniques.


50
42
34
26
18
10
PROBE (mL/kg/min)
10
20
30
40
50
PAH (mL/kg/min)
y=.908X+3.41 R²=.71

Appendix 3

Figure A3.1. Effect of guanfacin on heat production of fasted and fed sheep.


1.8
1.6
1.4
1.2
1
0.8
HP (W/kg)
FED
FASTED
0
20
40
60
180
300
Time (min)
vehicle
0.8 mg
1.6mg
2.4 mg

Appendix 4. The effect of adrenaline with or without ß-adrenergic blockers on portal, iliac venous whole blood amino acid profiles, A-V differences and net fluxes across these vascular beds.

Table A4.1. Effect of adrenaline(A) infusion and ß-adrenoceptor blockade (P=propranolol, a selective ß-blocker, metoprolol; a selective $ß_1$-blocker, ICI 118551; a selective $ß_2$-blocker) on portal vein whole blood amino acid ($\mu M.L^{-1}$) profile of sheep.

AMINO							P>F	
ACID	[1]C	A	A+P	A+$ß_1$	A+$ß_2$	SE[2]	TRT	TRT*T[3]
ASP	25.2	23.8	19.1	24.4	25.6	6.7	.685	.499
GLU[4]	340.4	265.9	217.4	283.9	286.5	101.0	.254	.138
ASN	37.8	30.1	27.6	27.3	30.6	4.4	.323	.137
SER	47.4	23.8	14.4	20.1	24.8	14.4	.097	.275
HIS	86.2	55.1	47.4	63.8	85.3	22.6	.146	.585
GLY	714.2	512.4	442.4	506.9	429.6	270.2	.244	.293
THR	70.0	44.1	49.6	69.3	70.1	13.4	.482	.904
CIT	244.4	181.1	130.4	146.8	142.9	50.3	.017	.316
ARG	162.6	146.3	80.4	124.8	111.3	69.7	.153	.279
TAU	79.4	86.9	102.3	84.2	87.6	81.6	.723	.694
ALA	148.4	181.3	153.9	199.9	150.0	68.2	.230	.289
TYR	78.0	67.7	51.2	58.1	63.9	23.0	.669	.441
TRP	23.6	12.4	17.2	16.7	21.3	3.9	.138	.746
MET	12.2	8.9	6.6	12.1	12.7	2.8	.326	.712
VAL	194.8	174.3	128.6	157.7	160.5	17.8	.193	.342
PHE	47.1	46.4	34.8	47.1	51.7	4.9	.315	.048
ILE	80.4	79.0	56.1	65.7	66.8	3.2	.341	.066
LEU	123.4	119.3	83.4	126.3	124.0	16.3	.051	.188
ORN	143.2	145.8	125.0	170.9	137.3	34.78	.724	.532
LYS	149.1	131.0	95.5	162.8	125.9	25.6	.385	.085
TOTALS	2807.7	2338.9	1892.8	2368.7	2208.0	442.2	.172	.389
TOTAL[4]								
BCAA	398.6	372.6	268.4	349.7	350.3	30.3	.168	.139

[1]C = control (saline)
[2]SE = pooled standard error
[3]means and SE not shown
[4]Include values for glutamine
[4]Total branch chain amino acids

Table A4.2. Effect of adrenaline(A) infusion and ß-adrenoceptor blockade (P=propranolol; nonselective ß-blocker. Metoprolol; selective $ß_1$-blocker. and ICI 118551; selective $ß_2$) on hindquarters venous whole blood amino acid ($\mu M.L^{-1}$) profile of sheep.

AMINO							P>F	
ACID	[1]C	A	A+P	A+$ß_1$	A+$ß_2$	SE[2]	TRT	TRT*T[3]
ASP	25.5	25.4	19.5	19.0	18.3	6.4	.402	.650
GLU[4]	330.9	242.5	208.0	283.9	298.5	76.9	.143	.541
ASN	36.1	30.5	27.9	19.4	27.2	3.0	.283	.390
SER	52.9	22.2	18.1	14.3	16.8	13.5	.056	.554
HIS	72.4	56.6	101.3	57.5	74.1	38.3	.691	.727
GLY	744.3	551.6	443.4	481.1	474.1	302.8	.148	.159
THR	76.6	44.5	37.6	67.1	67.2	9.3	.047	.879
CIT	231.6	189.7	152.3	146.3	125.5	24.3	.018	.196
ARG	159.0	95.8	63.7	98.1	84.2	46.0	.340	.335
TAU	84.6	63.3	70.4	78.2	75.6	56.9	.590	.400
ALA	168.2	206.9	147.1	202.5	138.9	84.8	.157	.352
TYR	88.3	72.8	58.3	59.1	64.2	24.9	.733	.743
TRP	21.7	14.0	16.3	17.0	22.3	2.2	.199	.183
MET	25.1	8.9	7.5	9.6	12.3	7.3	.148	.474
VAL	185.1	176.9	145.8	130.1	141.4	3.2	.225	.780
PHE	45.2	43.3	37.6	43.5	48.9	6.8	.308	.270
ILE	77.3	74.7	67.0	62.5	57.8	9.2	.334	.308
LEU	115.5	18.9	92.0	117.5	106.7	13.7	.142	.226
ORN	142.3	124.7	127.4	151.1	115.1	36.5	.621	.021
LYS	134.9	115.2	105.5	129.8	112.6	25.9	.775	.023
TOTALS	2818.5	2278.4	1946.7	2187.5	2081.3	507.9	.047	.587
TOTAL[4]								
BCAA	377.9	370.6	304.9	310.1	305.8	22.0	.234	.528

[1]C = control (saline)
[2]SE = pooled standard error
[3]means and SE not shown
[4]Include values for glutamine
[4]Total branch chain amino acid

Table A4.3. Effect of adrenaline infusion and ß-adrenoceptor blockade (P=propranolol; a nonselective ß-blocker, metoprolol; a selective $ß_1$-blocker, ICI 118551; a selective $ß_2$-blocker) on whole blood amino acid A-V difference ($\mu M.l.^{-1}$) across the portal drained viscera of sheep.

AMINO							P>F	
ACID	[1]Tn-C	A	A+P	A+$ß_1$	A+$ß_2$	SE[2]	TRT	TRT*T[3]
ASP	1.06	-0.22	-0.16	-0.83	-4.20	3.43	.847	.588
GLU[4]	-33.42	15.70	-10.04	-6.40	21.89	14.93	.282	.556
ASN	2.39	1.59	2.32	2.032	1.56	3.52	.999	.356
SER	2.12	-0.61	-0.53	4.34	-7.28	5.81	.694	.482
HIS	2.60	6.19	3.82	3.99	1.77	7.56	.995	.233
GLY	5.99	21.06	0.75	36.86	-41.42	21.89	.223	.611
THR	0.78	6.26	-3.88	-15.97	12.52	15.64	.753	.908
CIT	-4.03	7.21	11.31	7.14	-13.61	8.93	.343	.453
ARG	-7.43	9.66	16.74	3.03	-27.36	17.28	.466	.276
TAU	-6.47	1.65	-1.89	1.41	3.51	5.271	.701	.891
ALA	10.36	9.91	3.23	15.46	2.56	11.27	.909	.658
TYR	10.00	7.66	-6.81	-0.39	18.98	9.31	.408	.192
TRP	2.27	1.34	0.63	2.08	3.13	2.19	.938	.073
MET	0.51	-0.84	0.33	0.68	1.13	1.47	.900	.199
VAL	9.16	7.87	8.39	-2.24	-5.64	17.75	.957	.721
PHE	2.38	-3.76	-1.26	-2.00	1.82	4.16	.809	.101
ILE	5.61	0.33	0.36	-2.64	1.58	5.94	.956	.019
LEU	6.81	6.50	-14.59	1.12	1.63	14.32	.945	.248
ORN	14.90	-5.50	-4.60	-11.12	-10.42	14.74	.726	.879
LYS	8.52	-10.36	6.08	-14.37	3.23	15.66	.782	.664
TOTALS	34.27	82.36	41.82	28.76	-42.40	153.08	.984	.365
TOTAL[5]								
BCAA	21.57	14.70	22.62	-0.72	-2.43	36.41	.976	.266

[1]C = control (saline)
[2]SE = pooled standard error
[3]means and SE not shown
[4]Include values for glutamine
[5]Total branch chain amino acids

Table A4.4. Effect of adrenaline infusion and ß-adrenoceptor blockade (P=propranolol, a nonselective ß-blocker, metoprolol; a selective $ß_1$-blocker, ICI 118551; a selective $ß_2$-blocker) on whole blood amino acid A-V difference ($\mu M.L^{-1}$) across the hindquarter of sheep.

AMINO							P>F	
ACID	[1]C	A	A+P	A+$ß_1$	A+$ß_2$	SE[2]	TRT	TRT*T[3]
ASP	0.64	-1.79	-0.67	4.57	3.16	3.14	.675	.594
GLU[4]	-23.98	32.76	-1.56	-0.63	23.97	25.69	.574	.296
ASN	4.04	1.56	2.02	10.02	4.78	5.42	.797	.583
SER	-3.47	1.00	-4.23	10.20	0.72	4.87	.319	.799
HIS	16.64	7.69	-50.03	10.22	13.04	24.16	.337	.807
GLY	-24.12	-17.57	-0.633	62.57	-85.89	32.86	.115	.142
THR	-5.78	5.81	8.18	-14.06	16.06	11.88	.458	.978
CIT	8.79	-1.37	-10.66	7.72	3.70	15.54	.895	.155
ARG	-3.84	60.11	33.37	29.74	-0.24	31.18	.604	.639
TAU	-11.60	25.31	30.04	7.48	15.49	14.01	.323	.490
ALA	-9.40	-15.71	20.00	12.84	13.40	13.27	.315	.435
TYR	-0.27	2.57	-13.94	-1.40	18.69	8.45	.202	.281
TRP	4.20	-0.34	1.51	1.83	2.13	1.25	.724	.016
MET	-12.32	-0.82	-0.56	3.16	1.46	5.80	.418	.449
VAL	18.84	5.31	-8.97	25.36	15.44	20.09	.779	.685
PHE	3.32	-0.66	-3.86	1.53	4.68	5.28	.783	.305
ILE	8.76	4.57	-11.29	5.85	10.73	5.29	.106	.242
LEU	14.64	6.82	6.34	7.63	17.94	12.61	.945	.179
ORN	15.71	15.62	-6.97	8.64	11.80	20.03	.920	.045
LYS	22.63	-5.50	-3.87	18.63	16.67	18.80	.848	.005
TOTALS	23.64	142.92	-12.11	209.97	84.28	162.46	.868	.188
TOTAL[5]								
BCAA	42.24	16.70	-13.89	38.84	42.12	36.69	.778	.346

[1]C = control (saline)
[2]SE = pooled standard error
[3]means and SE not shown
[4]Include values for glutamine
[5]Total branch chain amino acid

Table A4.5. Effect of adrenaline infusion and ß-adrenoceptor blockade (P=propranolol a nonselective ß-blocker; metoprolol a selective $ß_1$-blocker; ICI 118551 a selective $ß_2$-blocker) on whole blood amino acid exchange $\mu M.kg^{-1}.Min^{-1}$) across the portal drained viscera of sheep.

AMINO							P>F	
ACID	[1]C	A	A+P	A+$ß_1$	A+$ß_2$	SE[2]	TRT	TRT*T[3]
ASP	0.028	-0.008	-0.003	-0.025	-0.097	0.090	.893	.543
GLU[4]	-0.883	0.549	-0.149	-0.082	0.332	0.449	.289	.736
ASN	0.063	0.054	0.049	0.059	0.036	0.085	.999	.196
SER	0.056	-0.021	-0.011	0.128	-0.167	0.159	.754	.461
HIS	0.069	0.208	0.082	0.118	0.041	0.211	.981	.165
GLY	0.158	0.709	0.016	1.087	-0.953	0.602	.251	.165
THR	0.021	0.211	-0.083	-0.471	0.288	0.397	.696	.909
CIT	-0.107	0.243	0.243	0.211	-0.313	0.254	.466	.491
ARG	-0.197	0.325	0.360	0.089	-0.629	0.439	.517	.185
TAU	-0.171	0.056	-0.041	0.042	0.081	0.118	.584	.831
ALA	0.274	0.333	0.069	0.456	0.052	0.289	.829	.698
TYR	0.265	0.258	-0.146	-0.012	0.436	0.232	.452	.213
TRP	0.060	0.045	0.014	0.062	0.072	0.060	.962	.089
MET	0.013	-0.028	0.007	0.019	0.026	0.041	.892	.147
VAL	0.243	0.265	0.180	-0.066	-0.129	0.464	.955	.539
PHE	0.063	-0.127	-0.027	-0.059	0.042	0.121	.801	.067
ILE	0.149	0.011	-0.008	0.078	0.036	0.157	.953	.010
LEU	0.181	0.219	0.314	-0.033	0.038	0.378	.964	.094
ORN	0.395	-0.185	-0.099	-0.328	-0.239	0.388	.713	.925
LYS	0.226	-0.349	0.131	-0.423	0.075	0.452	.784	.556
TOTALS	0.908	2.772	0.899	0.849	-0.975	3.989	.975	.306
TOTAL[5]								
BCAA	0.572	0.495	0.486	-0.021	-0.056	0.961	.979	.108

[1]C = control (saline)
[2]SE = pooled standard error
[3]means and SE not shown
[4]Include values for glutamine
[5]Total branch chain amino acids

Table A4.6.Effect of adrenaline infusion and ß-adrenoceptor blockade (P=propranolol a nonselective ß-blocker; metoprolol a selective $ß_1$-blocker; ICI 118551 a selective $ß_2$-blocker) on whole blood amino acid flux ($\mu M.kg^{-1}.min^{-1}$) across the hindquarter of sheep.

AMINO							P>F	
ACID	[1]C	A	A+P	A+$ß_1$	A+$ß_2$	SE[2]	TRT	TRT*T[3]
ASP	0.003	-0.017	-0.002	0.029	0.012	0.020	.592	.395
GLU[4]	-0.091	0.371	-0.007	-0.017	0.009	0.164	.377	.320
ASN	0.016	0.016	0.011	0.007	0.176	0.029	.630	.448
SER	-0.013	0.009	-0.015	0.065	0.003	0.028	.302	.921
HIS	0.064	0.072	-0.175	0.066	0.048	0.092	.329	.679
GLY	-0.092	-0.164	-0.002	0.407	-0.315	0.172	.126	.719
THR	-0.022	0.054	0.029	-0.091	0.059	0.066	.494	.872
CIT	0.034	-0.013	-0.037	0.050	0.014	0.097	.967	.321
ARG	-0.015	0.561	0.117	0.193	-0.001	0.273	.594	.961
TAU	-0.045	0.236	0.105	0.049	0.057	0.106	.492	.323
ALA	-0.036	-0.147	0.070	0.084	0.049	0.072	.234	.445
TYR	-0.001	0.024	-0.049	-0.009	0.069	0.047	.536	.229
TRP	0.016	-0.003	0.005	0.012	0.008	0.015	.905	.002
MET	-0.047	-0.008	-0.002	0.021	0.005	0.025	.446	.396
VAL	0.072	0.049	-0.031	0.165	0.049	0.126	.864	.503
PHE	0.013	-0.006	-0.013	0.009	0.017	0.030	.936	.137
ILE	0.036	0.043	-0.039	0.038	0.039	0.031	.349	.155
LEU	0.056	0.064	0.022	0.049	0.066	0.075	.993	.025
ORN	0.060	0.146	-0.024	0.056	0.043	0.108	.860	.112
LYS	0.087	-0.051	-0.135	0.121	0.061	0.111	.931	.001
TOTALS	0.061	1.333	-0.042	1.365	0.309	0.936	.714	.236
TOTAL[4]								
BCAA	0.162	0.156	-0.049	0.252	0.155	0.225	.902	.117

[1]C = control (saline)
[2]SE = pooled standard error
[3]means and SE not shown
[4]Include values for glutamine
[4]Total branch chain amino acid

Appendix 5. The effect of acute cold exposure with or without the ß-adrenergic blockers on portal whole blood amino acid profiles, A-V differences and net fluxes across the portal drained viscera.

Table A5.1 Effect of cold exposure(C) and ß-adrenoceptor blockade (P=propranolol a nonselective ß-blocker; metoprolol a selective $ß_1$-blocker; ICI 118551 a selective $ß_2$-blocker) on portal vein whole blood amino acid ($\mu M.L^{-1}$) profile of sheep.

AMINO							P>F	
ACID	[1]Tn-C	C+C[2]	C+P	C+$ß_1$	C+$ß_2$	SE[3]	TRT	TRT*T[4]
ASP	25.1	24.8	36.3	33.9	19.3	4.4	.402	.972
GLU[6]	340.4	275.5	240.4	249.9	248.3	75.9	.051	.072
ASN	37.8	44.5	33.4	50.8	52.0	16.5	.174	.733
SER	47.4	27.8	30.3	53.0	51.5	25.9	.269	.504
HIS	86.4	71.5	79.7	95.2	95.8	20.3	.213	.126
GLY	714.2	373.6	442.9	496.3	484.0	165.7	.216	.992
THR	70.0	47.9	72.2	123.8	106.0	52.6	.306	.509
CIT	244.4	148.2	125.1	145.9	152.7	40.2	.016	.764
ARG	162.6	56.1	77.1	74.4	97.5	41.2	.197	.517
TAU	79.4	61.2	62.7	67.1	63.6	29.5	.455	.899
ALA	148.4	135.5	116.6	150.7	152.2	9.5	584	.346
TYR	78.0	118.3	141.7	154.1	112.3	63.9	.429	.325
TRP	23.6	19.7	19.3	23.5	22.6	5.7	.293	.568
MET	12.28	11.2	12.7	13.6	15.2	2.9	.363	.324
VAL	194.2	189.1	209.9	220.2	21[	31.9	.787	.820
PHE	47.1	50.4	52.8	54.5	57.5	1[illegible]	.8[illegible]2	.879
ILE	80.4	81.5	88.3	98.6	96.1	18.3	.284	.754
LEU	123.4	135.7	139.4	154.3	139.5	23.0	.494	.797
ORN	143.2	135.9	149.1	143.9	160.7	22.8	.537	.021
LYS	149.1	128.8	143.8	149.4	148.1	11.4	.579	.126
TOTALS	2807.7[6]	2137.9	2272.9	2553.1	2492.8	114.9	.320	.312
TOTAL[5]								
BCAA	398.6	406.3	436.6	473.14	453.6	71.9	.619	.897

[1]Tn-C = Thermoneutral control (saline)
[2]C+C=cold + control (saline)
[3]SE = pooled standard error
[4]means and SE not shown
[6]Include values for glutamine
[5]Total branch chain amino acids

Table A5.2 Effect of cold(C) exposure and ß-adrenoceptor blockade (P=propranolol a nonselective ß-blocker; metoprolol a selective $ß_1$-blocker; ICI 118551 a selective $ß_2$-blocker) on whole blood amino acid A-V difference ($\mu M.l.^{-1}$) across the portal drained viscera of sheep.

AMINO							P>F	
ACID	[1]Tn-C	C+C[2]	C+P	C+$ß_1$	C+$ß_2$	SE[3]	TRT	TRT*T[4]
ASP	1.06	-3.41	-8.72	-3.69	-0.211	4.07	.522	.305
GLU[*]	-33.32	-27.57	-3.93	-18.40	-3.86	13.56	.468	.107
ASN	2.39	-9.37	5.88	-5.60	-2.92	5.25	.329	.988
SER	2.12	-3.27	0.87	-16.38	-9.08	6.25	.290	.552
HIS	2.60	-0.11	-6.96	-6.11	-9.37	8.81	.495	.142
GLY	5.99	-27.26	-5.43	-49.48	-12.19	28.79	.701	.977
THR	0.78	-1.00	11.00	-35.58	-6.40	17.17	.441	.712
CIT	-4.03	-2.51	-17.59	-7.91	-10.98	12.12	.904	.515
ARG	-7.43	-7.14	3.28	12.50	13.63	17.08	.833	.799
TAU	-6.47	0.72	-4.90	-0.12	-2.72	4.36	.754	.861
ALA	10.36	-47.68	-7.37	-12.02	-3.34	19.96	.392	.646
TYR	10.00	-2.46	0.12	-15.02	-4.06	13.64	.782	.652
TRP	2.27	0.84	-0.31	-4.18	-2.27	2.52	.412	.474
MET	0.51	-1.32	-1.22	-2.23	3.89	2.67	.539	.451
VAL	9.16	0.87	2.97	-8.77	-25.56	7.42	.134	.916
PHE	2.38	-4.47	-1.66	-2.01	-6.78	3.19	.401	.963
ILE	5.61	-1.98	-0.37	-11.61	-6.38	5.98	.379	.977
LEU	6.81	-5.60	-1.19	-6.93	-5.98	7.11	.615	.953
ORN	14.90	-3.34	9.56	-4.54	-12.13	7.41	.160	.681
LYS	8.52	-6.08	2.04	-5.19	-9.82	8.72	.628	.915
TOTALS	34.27	-152.12	-19.84	-212.76	-123.30	107.01	.518	.917
TOTAL[5]								
BCAA	21.57	-6.71	3.80	-27.31	-37.92	19.39	.286	.949

[1]Tn-C = Thermoneutral control (vehicle)
[2]C+C=cold + control (saline)
[3]SE = pooled standard error
[4]means and SE not shown
[*]Include values for glutamine
[5]Total branch chain amino acids

Table A5.3. Effect of cold(C) exposure and ß-adrenoceptor blockade (P=propranolol a nonselective ß-blocker; metoprolol a selective $ß_1$-blocker; ICI 118551 a selective $ß_2$-blocker) on whole blood amino acid exchange ($\mu M.kg^{-1}.min^{-1}$) across the portal drained viscera of sheep.

AMINO							P>F	
ACID	[1]Tn-C	C+C[2]	C+P	C+$ß_1$	C+$ß_2$	SE[3]	TRT	TRT*T[4]
ASP	0.028	-0.101	-0.238	-0.105	-0.006	0.116	.546	.305
GLU[5]	-0.883	-0.818	-0.108	-0.524	-0.114	0.378	.476	.102
ASN	0.063	-0.278	0.161	-0.159	-0.086	0.145	.307	.986
SER	0.056	-0.097	0.024	-0.467	-0.268	0.175	.275	.551
HIS	0.069	-0.003	-0.144	-0.445	-0.455	0.245	.463	.119
GLY	0.159	-0.809	-0.149	-1.410	-0.359	0.794	.677	.973
THR	0.021	-0.029	0.301	-1.014	-0.189	0.486	.441	.706
CIT	-0.107	-0.075	-0.481	-0.225	-0.324	0.335	.904	.568
ARG	-0.197	-0.212	0.089	0.356	0.402	0.490	.833	.803
TAU	-0.171	0.021	-0.134	-0.003	-0.080	0.124	.778	.870
ALA	0.274	-1.414	-0.202	-0.432	-0.098	0.569	.368	.649
TYR	0.265	-0.073	0.003	-0.428	-0.119	0.383	.788	.633
TRP	0.060	0.025	-0.009	-0.119	-0.088	0.069	.376	.538
MET	0.013	-0.039	-0.033	-0.063	0.115	0.077	.535	.453
VAL	0.243	0.026	0.081	-0.249	-0.754	0.215	.126	.910
PHE	0.063	-0.133	-0.045	-0.057	-0.199	0.093	.403	.966
ILE	0.149	-0.059	-0.010	-0.330	-0.189	0.172	.413	.982
LEU	0.181	-0.166	-0.032	-0.198	-0.176	0.208	.650	.959
ORN	0.395	-0.099	0.261	-0.129	-0.358	0.214	.177	.685
LYS	0.226	-0.180	0.056	-0.148	-0.289	0.257	.645	.918
TOTALS	0.908	-4.513	-0.542	-6.064	-3.637	2.960	.486	.915
TOTAL[5]								
BCAA	0.572	-0.199	0.104	-0.778	-1.119	0.563	.304	.953

[1]Tn-C = Thermoneutral control (vehicle)
[2]C+C=cold + control (saline)
[3]SE = pooled standard error
[4]means and SE not shown
[5]Include values for glutamine
[5]Total branch chain amino acids

Appendix 6. The effect of adrenaline infusion and acute cold exposure with or without the ß-adrenergic blockers on organ blood flow in sheep.

Table A6.1a. The effect of adrenaline (A) infusion and beta-blockade (P=propranolol a nonselective, M=metoprolol a $ß_1$-adrenoceptor blocker and ICI=ICI118551 a $ß_2$-adrenoceptor blocker) on portal vein (PBF) and hindquarters blood flow (L/min; HBF) of the sheep.

	S[1]	A	A+P	A+M	A+ICI	S.E
PBF	1.59^{b}	2.02^{a}	1.29^{b}	1.77^{ab}	1.38^{b}	0.079
HBF	0.23^{c}	0.56^{a}	0.21^{c}	0.39^{b}	0.22^{c}	0.041

S[1] = Saline (control) infusion.
[abc]Within rows means with different superscripts are different ($P<0.05$)

Table A6.1b. The effect of acute cold exposure (C) and beta-blockade (P=propranolol a nonselective, M=metoprolol a $ß_1$-adrenoceptor blocker and ICI=ICI118551 a $ß_2$-adrenoceptor blocker) on portal vein blood flow (L/min; PBF) of the sheep

	S[1]	C	C+P	C+M	C+ICI	S.E
PBF	1.59	1.78	1.64	1.71	1.77	0.077

S[1] = Saline (control) infusion

Appendix 7

Appendix 7. The effect of acute cold exposure (C) and beta-blockade (P=propranolol a nonselective, M=metoprolol a $ß_1$-adrenoceptor blocker and ICI=ICI118551 a $ß_2$-adrenoceptor blocker) on blood haemoglobin, arterial oxygen content, portal vein oxygen saturation and portal vein oxygen content of the sheep. Comparisons were made between warm thermoneutral control (Tn-C), cold control (C-C), cold exposure and propranolol (C+P), cold exposure and metoprolol (C+M) and cold exposure and ICI 118551 (C+ICI).

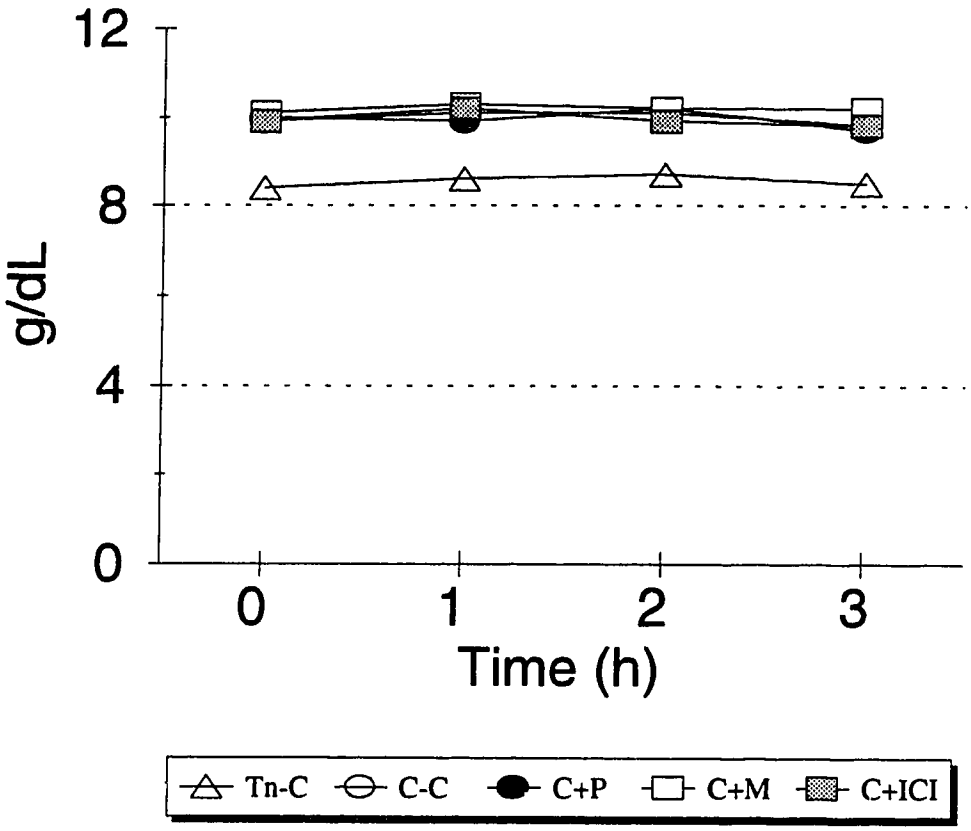


Figure A7.1. Blood haemoglobin

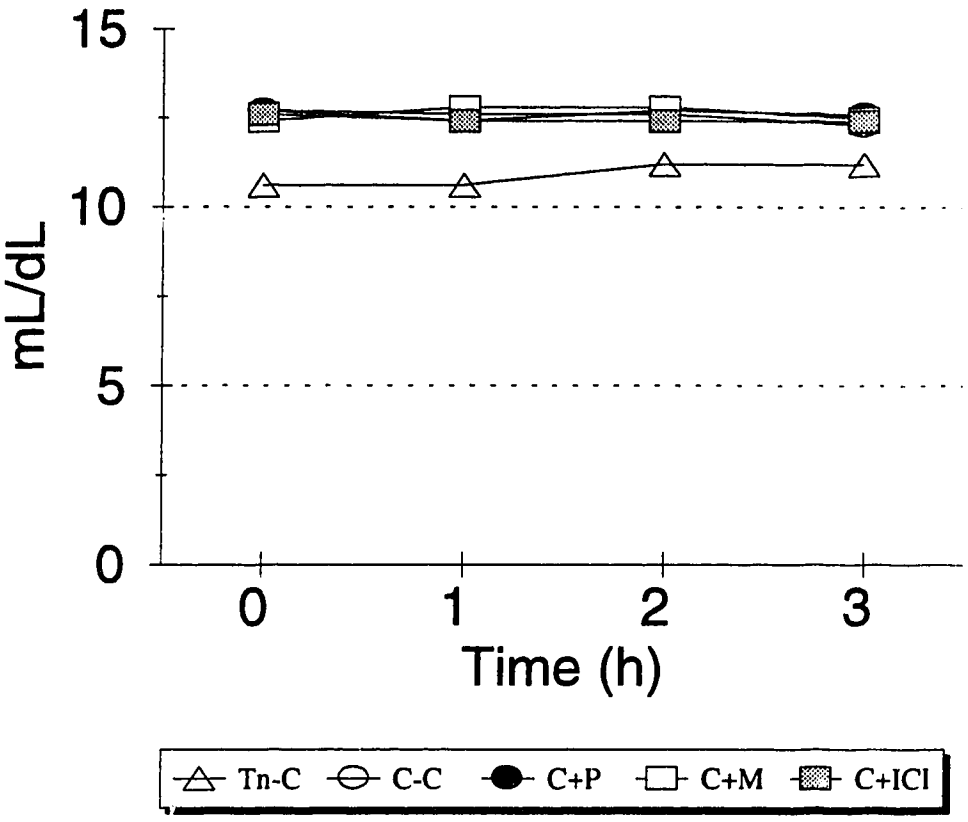


Figure A7.2. Arterial oxygen content

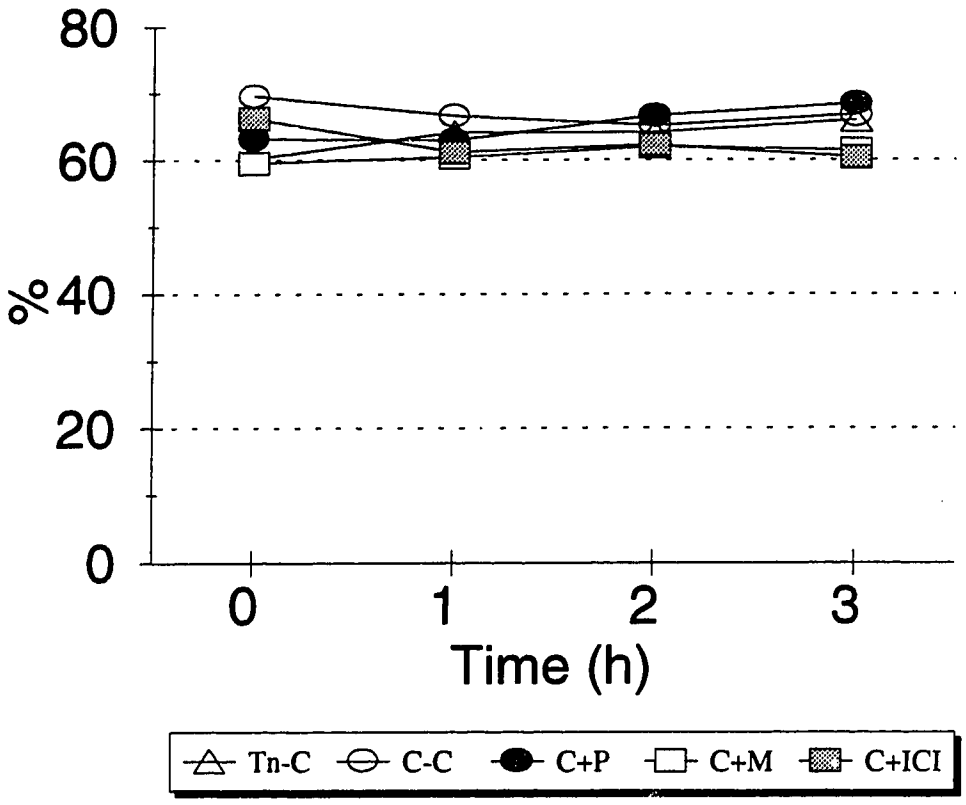


Figure A7.3. Portal oxygen saturation

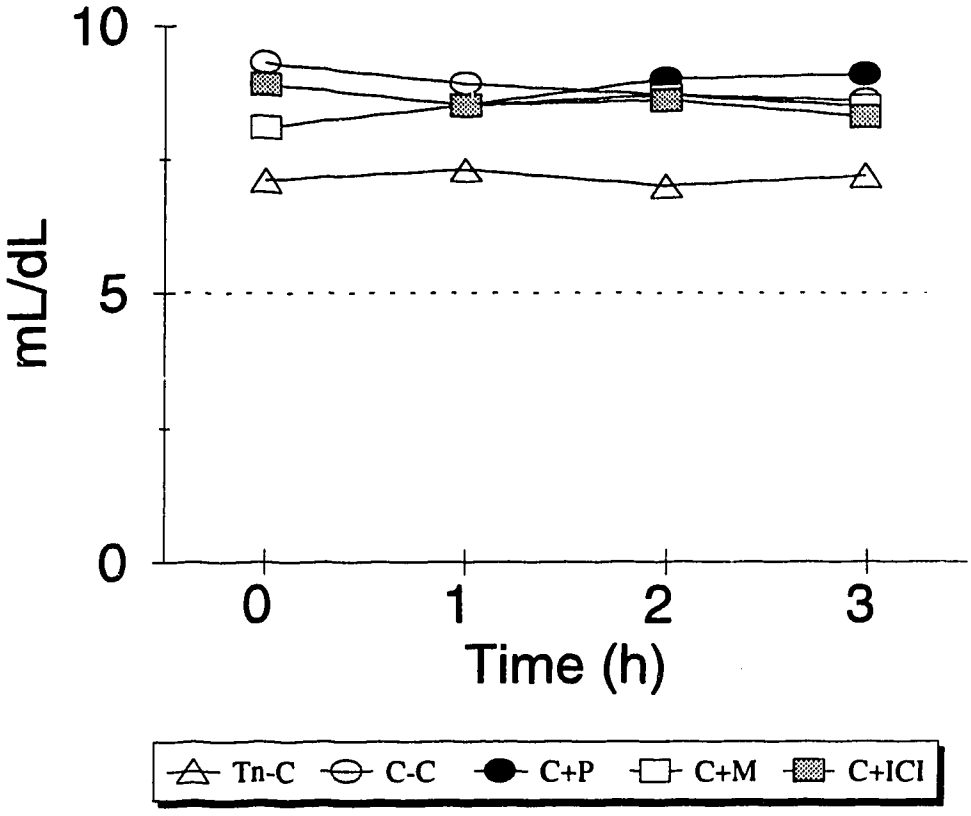


Figure A7.4. Portal oxygen content