University of Alberta

Vasopressin and the Asphyxiated Neonate

by

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This work is dedicated to my wonderful spouse for her endless patience

and to my daughter, for reminding me of the good things in life.

ABSTRACT

Cardiovascular dysfunction in asphyxiated neonates leads to significant morbidity and mortality and the hemodynamic support is limited to the use of catecholamines. In a pilot study, we showed that low-dose vasopressin improved myocardial tissue lactate levels, suggesting decreased anaerobic metabolism. We postulated that starting this infusion earlier would benefit systemic hemodynamics. The aim was to compare the systemic and regional hemodynamic effects of vasopressin with dobutamine, a synthetic beta-adrenoreceptor agonist commonly used clinically. This study is the first to demonstrate that a low-dose vasopressin infusion used in the setting of a neonatal swine model of hypoxia-reoxygenation improves cardiac output and mesenteric perfusion, with results comparable to those of dobutamine. Improved myocardial biomarkers and evidence of decreased mesenteric oxidative stress confirmed our results. We did not demonstrate a benefit to the combined use of vasopressin and dobutamine. We conclude that low-dose vasopressin may be useful in the treatment of perinatal asphyxia.

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LIST OF ABBREVIATIONS

AC	Adenylate Cyclase
ADP	Adenosine Diphosphate
AHA	American Heart Association
ANOVA	Analysis of Variance
ANP	Atrial Natriuretic Peptide
AMP	Adenosine Monophosphate
AQ2	Aquaporin-2 Channel
ATP	Adenosine Triphosphate
β-AR	β-Adrenoreceptor
CAFI	Carotid Artery Flow Index
CaM	Calmodulin
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
CI	Cardiac Index
CT	Computed Tomography
CVP	Central Venous Pressure
DAG	Diacylglycerol
dDAVP	Desmopressin
DI	Diabetes Insipidus
DNA	Deoxyribonucleic acid
ECG	Electrocardiography
ER	Endoplasmic Reticulum
FiO_2	Fraction of Inspired Oxygen
GC	Guanylate Cyclase
GSH	Glutathione
GSSH	Glutathione Disulphide
GTP	Guanosine Triphosphate

HCO_3	Bicarbonate
HIE	Hypoxic-Ischemic Encephalopathy
H-R	Hypoxia-Reoxygenation
I-FABP	Intestinal Fatty Acid Binding Protein
IP_3	Inositol 1,4,5-trisphosphate
I-R	Ischemia-Reperfusion
K-ATP channel	Adenosine Triphosphate-dependent Potassium Channel
LPO	Lipid Hydroperoxyde
MAP	Mean Arterial Pressure
MLCK	Myosin Light Chain Kinase
MRI	Magnetic Resonance Imaging
NEC	Necrotizing Enterocolitis
NMDA	N-methyl-D-Aspartate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OTR	Oxytocin Receptor
$PaCO_2$	Partial Pressure of Arterial Carbon Dioxide
PaO_2	Partial Pressure of Arterial Oxygen
PAP	Pulmonary Arterial Pressure
pCO_2	Partial Pressure of Carbon Dioxide
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PKA	Protein Kinase A
PKC	Protein Kinase C
PLC	Phospholipase C
pO_2	Partial Pressure of Oxygen
PPH	Persistent Pulmonary Hypertension
RAFI	Renal Artery Flow Index
ROCC	Receptor Operated Cation Channel

RONS	Reactive Oxygen-Nitrogen Species
ROS	Reactive Oxygen Species
$S_{CV}O_2$	Central Venous Oxygen Saturation
SMAFI	Superior Mesenteric Artery Flow Index
SOCC	Store Operated Calcium Channel
SpO_2	Pulse Oximeter Oxygen Saturation
TnC	Troponin C
V1R	V_1 Vasopressin Receptor
V2R	V_2 Vasopressin Receptor
VGCC	Voltage Gated Calcium Channel
VWF	Von Willebrand Factor

Chapter 1: Perinatal Asphyxia

1. INTRODUCTION

The neonatal period is defined as the first four weeks of life. This represents a particularly vulnerable age, as up to 40% of deaths occurring in children under five years old occurs within this time period, and the proportion is increasing.[1] Perinatal asphyxia, caused by insufficient oxygen delivery to the fetus or neonate, contributes significantly to the morbidity and mortality in this age group, and will be discussed in depth.

2. EPIDEMIOLOGY

Of all neonatal deaths, it is estimated that nearly a quarter can be attributed to perinatal asphyxia. This thus accounts for 0.7 to 1.6 million neonatal fatalities annually – comparable to the proportion of children who die of malaria.[1,2] Along with preterm complications, it is one of the two most common causes of neonatal mortality,[3,4] while being reported as the most common in recent large studies.[5,6] It has also been shown that the absolute incidence of perinatal asphyxia and its incidence in full term infants are increasing in certain populations.[7] Nearly half of these children die within the first 24 hours of life and three quarters of deaths will occur within the first week.[8] In addition to the post-natal mortality, another 1.02 million annual stillbirths can also be attributed to perinatal asphyxia.[1]

This information is limited by the fact that only 3% of deaths in the neonatal age group occur in countries where detailed information is available, suggesting that these statistics may in fact represent an underestimation. Also, the lack of a standard definition makes accurate data collection difficult.[2] Additionally, the term “birth asphyxia” may also be avoided in litigious societies, adding another potential source of bias.[1] Although most of the cases of this diagnosis occur in the developing world, it is estimated that it has an incidence of 2-4 per 1000 live births in the industrialized world,[9] and it has been reported in up to 2.5% of deliveries in a study based out of Finland.[10]

Along with the significant mortality, chronic complications develop in at least as many, including epilepsy, cerebral palsy and developmental delay. [2,11] In fact, hypoxic-ischemic injury in the perinatal period is the most recognized cause of cerebral palsy.[12] Additionally, mild-moderate injury has been associated with behavioral and cognitive complications, including autism, hyperactivity and attention disorders, decreased intelligence quotients, schizophrenia as well as other psychotic disorders.[13] Perinatal asphyxia is thus responsible for a significant worldwide health burden.

3. DEFINITION

The lack of a standard definition of perinatal asphyxia has complicated attempted epidemiological studies of the disease.[2] The World Federation of Neurology Group task force has generally defined asphyxia as a condition of impaired gas exchange leading to progressive hypoxemia and hypercapnia. This definition is insufficient however as this may occur transiently without significant detrimental effect. [14,15] In reality, due to uterine contractions, all newborns experience a certain level of asphyxia.[16]

Clinical parameters have to be used to confirm the presence of asphyxia and to classify its severity. Historically, neonatal asphyxia was classified as either *asphyxia livida,* when cyanosis was present with a strong heartbeat and reflexes, or *asphyxia pallida*, when the child was pale with a weak pulse and absent reflexes.[2] These definitions were then replaced by the more objective Apgar score (Table 1.1), first proposed by Virginia Apgar in 1952.[17] With this tool, the birth attendant gives a score to the infant based on physiological adaptation at one minute and five minutes following birth. It gained acceptance after it was shown that a low score could predict survival and is strongly correlated with the presence of multi-organ dysfunction.[18] Furthermore, a score of <7 at 5 minutes has been linked with decreased academic performance later in life.[19]

This score has come under scrutiny however, as while it has some predictive ability on mortality, it is not useful in determining neurological outcomes in those who survive.[16] Also, it has been suggested that it is somewhat subjective and thus has a poor inter-observer variability.[20]

Table 1.1: Apgar Score[17]

Sign	**0**	**1**	**2**
Heart rate	Absent	Below 100	Above 100
Respiratory effort	Absent	Slow, irregular	Good, crying
Muscle tone	Flaccid	Some flexion of extremities	Active motion
Reflex irritability	No response	Grimace	Vigorous cry
Color	Pale	Cyanotic	Completely pink

Another method suggested in the diagnosis of perinatal asphyxia is the use of umbilical blood gases. In most centers, a pH ≥ 7.2 from the umbilical artery is considered normal and a pH of 7.0-7.2 mild to moderate acidemia. Severe acidosis is present if the pH is <7 and the base deficit >12. Used in isolation, metabolic acidosis is a poor predictor of asphyxial injury, with 21% sensitivity for adverse outcomes. Interestingly, it has even been suggested that metabolic acidosis may be protective, with hypercarbia resulting in cerebral vasodilatation (and increased blood flow) and acidosis decreasing cerebral oxygen demand. Also, acidemia

promotes the unloading of oxygen from fetal hemoglobin by shifting the oxygen dissociation curve.[16]

Table 1.2: Normal Umbilical Cord Blood Gases[16]

	pH	PaO2 (mm Hg)	PaCO2 (mm Hg)	HCO3 (mEq/L)
Umbilical artery	7.27 ± 0.08	25±19	45±10	22±3.7
Umbilical vein	7.34 ± 0.07	36±10	40±6	23±2.2

4. DIAGNOSIS

There isn't a gold standard test for neonatal asphyxia, and the predictive value of umbilical artery pH, base deficit, Apgar scores, fetal heart rate patterns and the need for resuscitation have come into question, especially if considered in isolation.[8,15,21] As such, the diagnosis of perinatal asphyxia has progressed from individual parameters (i.e. pH or Apgar score) to the use of multiple factors, as numerous studies have reported an increase in the predictive value when doing so.[22-24] Position statements by both the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics have therefore defined perinatal asphyxia as including the following conditions:[16]

1. Umbilical cord arterial pH <7
2. Apgar score of <4 for >5 minutes

3. Neonatal neurological manifestations (i.e. seizures, coma, hypotonia)
4. Multisystem organ dysfunction

Also, the need to exclude other causes of neurologic damage has been suggested[2] as well as the fact that the diagnosis should not be made without evidence of an interruption in fetal oxygen delivery. This can be maternal (hypotension, toxemia, tetany, uterine rupture), placental, from the umbilical cord (abruption, infection/inflammation, cord compression) or from the fetus (central nervous system depression, anomalies, infection).[16]

5. PROGNOSTIC STUDIES

In an effort to predict the short and long term outcomes of perinatal asphyxia, a number of tools are currently being utilized. The importance of these tools cannot be understated, as they can help predict who would benefit form the various treatment strategies, which will be outlined in the following chapter. Additionally, most therapeutic strategies for this condition are time-sensitive, underlining the need for early recognition and prognostication.[21] Sarnat and Sarnat developed a system employing clinical and electroencephalographic findings in an effort to predict outcomes of hypoxic-ischemic encephalopathy (HIE),[25] the predominant neurological injury associated with perinatal asphyxia.(Table 1.3)

Table 1.3: Sarnat And Sarnat HIE System[25]

	Mild HIE	Moderate HIE	Severe HIE
Level of consciousness	Hyperalert	Lethargic or obtunded	Stuporous
Neuromuscular control:			
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive	Decreased/ absent
Segmental Myoclonus	Present	Present	Absent
Complex reflexes:			
Suck	Weak	Weak/absent	Absent
Moro	Strong	Weak	Absent
Occulovestibular	Normal	Overactive	Weak/absent
Tonic neck	Slight	Strong	Absent
Autonomic function	Sympathetic	Parasympathetic	Depressed
Pupils	Mydriasis	Myosis	Variable/ depressed
Heart rate	Tachycardia	Bradycardia	Variable
Secretions	Sparce	Profuse	Variable
GI motility	Normal/ decreased	Increased	Variable
Seizures	None	Common: focal/ multifocal	Uncommon
EEG findings	Normal	Early: low-voltage, continuous delta and theta waves Late: Periotic pattern Seizures: focal 1-1.5 Hz spike and wave	Early: Periotic pattern with isopotential phases Late: Isopotential
Duration	<24 hours	2-14 days	Hours to weeks

Although useful, the Sarnat and Sarnat system is limited by its subjectivity and the variability of its criteria over time,[26] although clinically evident neurological behavior after birth is still the best predictor of morbidity and mortality.[21]

Tests evaluating for neurological outcomes include neonatal clinical examination and clinical course, monitoring general movements, early electrophysiology testing, cranial ultrasound imaging, visual evoked potentials, sensory evoked potentials, Doppler blood flow velocity measurements, magnetic resonance imaging (MRI), MR spectroscopy and MR microscopy. [13,27] There is some evidence that early MRI imaging may be the best prognostic test available, and is preferable to computed tomography, as the injury can be identified within 24 hours.[21] Cerebral injury has a different appearance in the neonate as opposed to adults, as myelination is occurring and the water content is much higher. As such, lack of myelination of the posterior limb of the internal capsule and subtle alterations in brain water are early signs of injury that can be detected using MRI technology.[28] The basal ganglia and thalami, being extremely sensitive to hypoxia-reoxygenation injury due to their high metabolic rates, are the most commonly injured structures following acute perinatal asphyxia. Early MRI imaging of these structures was studied by Martinez-Biage et al, and they found that a well timed study provided useful

prognostic information concerning mortality, cerebral palsy, feeding problems, communication problems, hearing and visual impairment, cognition and seizure activity.[29] Other potential findings on MRI include white matter infarction, which is thought to be related to chronic or repetitive insults, or perinatal stroke.[30]

A recent systematic review and meta-analysis has been performed, comparing all prognostic tools for perinatal asphyxia. They found that amplitude-integrated electroencephalography, electroencephalography and visual evoked potential had the greatest predictive ability for outcomes when performed within the first week of life, and that early MRI examination had the greatest specificity. The authors conclude that a combination of these tests would be wise and would likely improve the overall prognostic ability.[27]

Being that neuroimaging is contraindicated in unstable patients, other technologies have been developed. These plasma, cerebral spinal fluid and urinary markers include S-100B, neuron specific enolase, umbilical interleukin-6, creatinine kinase-BB, glial fibrillary acidic protein, myelin basic protein, ubiquitin carboxyl-terminal hydrolase L1, phosphorylated neurofilament H,[26] malondialdehyde and nitrates/nitrites.[31] These markers

are released with neuronal cell death and represent a future direction in the diagnosis and prognostication of perinatal asphyxia.

6. ETIOLOGY

Perinatal asphyxia can occur due to a variety of etiologic factors and under different circumstances.[32] The insults can be acute or chronic and can be classified as ante-partal, intra-partal or post-partal, which have been estimated to be responsible for 50%, 40% and 10% of cases respectively. This varies depending on the country of origin, with intra-partum causes being more common in developing countries and less so in developed countries.[2] Due to this variety of circumstances surrounding the insult, the clinical presentations will differ. While ante-natal causes have traditionally thought to have been the most important in the development of perinatal asphyxia,[33] this has been disputed. Cowan et al. studied MRI images of infants with either established perinatal asphyxia or early-onset seizures and found that the images suggested an acute insult in 80% and 69% of infants respectively. They concluded that the immediate perinatal period was the most important in neonatal brain injury.[34]

Risk factors for perinatal asphyxia have been reported in multiple studies,[10,35-37] and can be separated in maternal, antepartal, and delivery or infant-related.(Table 1.4)

Table 1.4: Risk Factors For Perinatal Asphyxia[10,35-37]

Maternal	Ante-partal	Delivery/Infant
Single civil status	Prenatal viral infections	Preterm birth
Unemployment	Severe pre-eclampsia	Intrauterine meconium release
Age >40	Maternal bleeding	Oxytocin augmentation
High or low BMI	Placental abnormalities	Breech delivery
Infertility treatment	Intrauterine growth restrictions	Cord complications
Thyroid disease	Uterine rupture	Nighttime delivery
Primiparity		Small for gestational age
Prior cesarean section		Dystocia
Alcohol use		General Anesthesia
Short stature		Multiple Fetuses
Previous neonatal death		
Smoking		

In a recent study by Martinez-Biarge, certain perinatal events were found to be at very high risk for the development of perinatal asphyxia. These sentinel events included uterine rupture, placental abruption, cord prolapse and amniotic fluid embolism. The infants subjected to one of these sentinel events were at a 2-6 time greater risk of perinatal asphyxia and thus identifies a group of infants who may require early resuscitative efforts.[29]

7. PATHOPHYSIOLOGY

The underlying pathogenesis of perinatal asphyxia is associated with energy failure. Due to a lack of oxygen, less efficient anaerobic metabolism is utilized, with its inherent consequences, which include: decreased adenosine triphosphate (ATP) creation, lactate formation, decreasing pH, decreased phosphorylation and the subsequent production of large amounts of reactive oxygen-nitrogen species (RONS)[38] once reoxygenation occurs.[13]

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and are generated in physiologic conditions, in which case the body is protected by endogenous anti-oxidant mechanisms. They are in fact useful in certain situations as they are utilized by our immune system for a potent and rapid attack of pathogens. However, during hypoxia-reoxygenation, the excessive production of these molecules overwhelms the endogenous anti-oxidant systems and their high reactivity results in damage to cellular lipids, proteins and deoxyribonucleic acids (DNA). They originate from a number of sources, including mitochondria, xanthine oxidase and activated neutrophils.[39]

ROS are intermediates of cellular metabolism, and include the superoxide anion ($O_2^{\cdot -}$) as well as other compounds. Superoxide anion is of particular

importance as it is considered the “primary” ROS, and is the first step in the generation of all other ROS outlined in table 1.5,[39] the “secondary” ROS. While they are also produced by the endoplasmic reticulum and the nuclear membrane, the single most important source is likely the mitochondria.[39,40] The mitochondria’s main role in the cell is that of cellular respiration and the production of ATP. In order to accomplish these functions, it is required that oxygen is reduced to form water via the electron transport chain. It is estimated however, that 1-3% of all electrons spill out during this process and combine with oxygen to form the superoxide anion.[39] Considering that 90% of inhaled oxygen is consumed in this fashion by mitochondria, this is a significant source of ROS under physiologic conditions.[41] During hypoxia-reoxygenation, ROS production by the mitochondria is further increased[41] and could overwhelm endogenous antioxidant defenses.

Table 1.5: Reactive Oxygen-Nitrogen Species[39]

Reactive Oxygen Species	Reactive Nitrogen Species
Superoxide Anion ($O_2^{\cdot -}$)	Nitric oxide ($NO^{\cdot}$)
Hydroxyl Radical ($OH^{\cdot}$)	Peroxynitrite ($ONOO^{-}$)
Peroxyl Radical ($HOO^{\cdot}$)	
Hydrogen Peroxide (H_2O_2)	

During hypoxia, ROS production also takes place in the peripheral tissues. During ischemia, ATP is consumed which leads to the production and

accumulation of hypoxanthine. Upon reoxygenation, the enzyme xanthine oxidase uses the cellular oxygen to convert the hypoxanthine to xanthine, a process that also releases superoxide radicals and uric acid.[42] Hypoxia/reoxygenation is thus a cause of superoxide generation in the tissues.

As previously stated, our immune system uses these chemically reactive molecules to aid in the destruction of pathogens. Activated neutrophils produce large quantities of ROS and also have the ability to excrete myeloperoxidase. This enzyme, when released into the extracellular space, is able to produce the potent oxidant hypochlorous acid using hydrogen peroxide and a chloride ion.[43]

Nitric oxide is a labile gas produced by the vascular endothelium and serves important physiological functions. Large quantities are produced during reperfusion,[38] along with ROS including the superoxide anion. Nitric oxide and superoxide anion quickly react and generate the oxidizing and nitrating agent peroxynitrite ($ONOO^-$).[44] This molecule damages a multitude of cellular components, including lipids, proteins, carbohydrates and nucleic acids.[45,46]

The newborn has inherent protective mechanisms to combat RONS. (Table 1.6) These include enzymes such as catalase, glutathione peroxidase and super-oxide dismutase, with the latter likely being the most important.[39,47] Non-enzymatic antioxidants, which include glutathione, ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), carotenoids (Vitamin A), flavonoids and uric acid, also serve an important part of these defenses via free radical scavenging. Metal-binding proteins also play an important role in our defenses as free metals such as copper and iron are involved in the production of "secondary" RONS. Injury may occur however when these mechanisms are overwhelmed, such as when significant hypoxia is followed by reoxygenation.[47]

Table 1.6: Adaptive Responses To Reactive Species (Antioxidants)[47]

Enzymatic	Non-enzymatic	Metal binding proteins
Catalase	Glutathione	Transferrin
Superoxide dismutase	Ascorbic acid (Vitamin C)	Ferritin
Glutathione peroxidase	α – tocopherol (Vitamin E)	Lactoferrin
Ceruloplasmin	Carotenoids (Vitamin A)	
	Flavonoids	
	Ubiquinol (Co-enzyme Q_{10})	
	Urate	

8. EFFECTS OF NEONATAL ASPHYXIA

8.1 Systemic effects

Asphyxia leads to hypoxemia, hypercapnia and metabolic acidosis, and it has been shown that 2% of all births have a base deficit >12 and that 0.5% have a base deficit >16.[14] Hypoxemia causes centralization in blood flow, also known as the diving seal reflex[48] and decreased oxygen consumption. This decrease in oxygen consumption occurs most drastically in the peripheral organs in favor of the central, more essential organs. Oxygen consumption only decreases when oxygen extraction is maximal, and this occurs in different organs at different times, which is modulated by a chemoreceptor in the carotid artery. With this, the fetus can increase the amount of oxygen extracted at the level of the placenta as well as in each individual organ.[49] In sheep during normal fetal circulation under normoxic conditions, a relatively small amount of the blood flow is directed to the brain (3%), the heart (2.6%), the mesentery (2.6%), the kidneys (2.3%) and the adrenals (0.006%). Under hypoxic conditions however, these hemodynamic values change dramatically, with some variability based on etiology.[50]

During maternal hypoxia, fetal bradycardia and hypertension is induced, but the cardiac output is maintained as long as the pH is normal. Circulatory distribution changes with a concentration of blood flow to vital

organs, namely the brain, heart and adrenals. Also, the amount of blood that passes through the ductus venosus increases and a larger proportion of blood is shunted through the foramen ovale. Additionally, blood flow is increased to the umbilical arteries, thus recirculating a larger proportion of oxygenated blood through the umbilical vein.[49]

If there is a reduction in umbilical blood flow, the umbilical partial pressure of carbon dioxide (pCO_2) is fairly resistant, as it still flows freely across the placenta to the maternal circulation in spite of a decreased flow of up to 50%. It does however cause bradycardia, hypertension and a decrease in the cardiac output. Blood flow is different than in the previously described situation, as only the flow to the lungs decrease while the blood flow to the brain, heart, adrenals, kidneys, gastro-intestinal tract and spleen all increase. The amount of blood shunted through the ductus venosus increases. [49,50]

The effects of a reduction in uterine blood flow vary according to severity. A graded reduction causes mild bradycardia and increased blood pressure without a change to the cardiac output. The flow preferentially goes to the brain, heart and adrenals, while it decreases to the skin and skeletal tissue. The flow through the ductus venosus, foramen ovale and the umbilical arteries increases.[49] A prolonged arrest in uterine flow for longer

than 4 min causes severe asphyxia associated with a decreased heart rate and cardiac output and an increased blood pressure at this stage. The blood flow is redistributed to essential organs (brain, heart, adrenals) and the blood flow to the cerebrum falls in favor of an increase to the brainstem. This circulatory redistribution cannot be maintained, and decentralization of blood flow occurs with a decrease in vascular resistance in the periphery and an increase in the vascular resistance to vital organs. Also, flow to the placenta decreases due to increased resistance. This, and the associated metabolic derangements make fetal demise imminent, unless resuscitation occurs rapidly.[49]

8.2 Multi-Organ Dysfunction

As stated above, multi-system organ dysfunction has been listed as one of the criteria for the diagnosis of perinatal asphyxia. In a study of 72 full-term asphyxiated newborns, 56% of them had multi-organ dysfunction, with the central nervous system being the most common organ system involved.[18] The incidence may be even higher depending on your inclusion criteria for asphyxia. In a Canadian retrospective study of asphyxiated newborns, it was found that every patient who had hypoxic-ischemic encephalopathy also had at least one other organ affected, thus meeting the criteria for multi-system organ dysfunction.[51]

8.3 Brain:

Hypoxia-reoxygenation is the single most important cause of neonatal brain injury[52] and the brain is the most common organ affected in fetal asphyxia.[18] The neurological injury manifests itself as HIE, a relatively common form of neonatal encephalopathy, and its presence is a strong prognostic factor, as the mortality of infants found to have severe HIE ranges from twenty to fifty percent.[26] The degree of brain injury seen in severe asphyxia is correlated with the degree of injury to other organ systems, as proven in an animal model on fetal lambs.[53] Long-term neurologic disabilities, such as cerebral palsy and developmental delay are at high risk to occur in survivors.

Cerebral hemodynamics undergoes a compensatory mechanism in response to hypoxia. Initially, there is an increase in blood flow to the brain associated with a blunted response to the partial pressure of arterial carbon dioxide ($PaCO_2$), which has the net result of an increased cerebral blood volume. The delivery to the brainstem is increased as compared to the cerebrum.[49] Possible explanations for these findings include altered prostanoid metabolism and increased nitric oxide production. The injury does not end with re-establishment of normoxia, with severity of cerebral edema peaking at 36-48 hours post-injury, which suggests an evolving process.[2] On the other hand, with complete arrest of uterine blood flow,

flow to the brain does not increase, likely due to vasoconstriction increasing the cerebral vascular resistance through the steep rise in catecholamines. Thus, with complete uterine blood flow arrest, oxygen delivery to the brain decreases; but with moderate hypoxemia, the delivery increases.[49]

The cerebral injury can be divided into the acute injury and the secondary injury. In the acute phase, there is initial compensatory maintenance of cerebral blood flow, as described above. This is limited however as due to vasoconstriction or cardiac dysfunction, a decrease in cerebral blood flow ultimately results[54] and may be the pathogenic mechanism in HIE. This leads to the onset of excitotoxicity,[54] the utilization of energy reserves, the shift to anaerobic metabolism and the accumulation of lactic acid. The ability to maintain cellular functions is compromised, and a lack of energy to perform active ion pumping leads to an electrolyte imbalance in both intra and extracellular spaces. Specifically, the inability to activate the ATP-dependent Na^+-K^+ transmembrane channel causes cellular edema and subsequent necrosis. Excitatory neurotransmitters released through cell lysis overwhelm the neuronal reuptake capabilities, increase cellular energy utilization and activate apoptotic pathways. Oxygen free radicals accumulate and disrupt cellular components with the end result being cell death. [12,13]

In the second phase of injury, perfusion and oxygenation are restored, usually in the post-partal period, and the intra-cellular pH returns to normal. However, a second episode of cerebral injury occurs, usually within 6-48 hours, secondary to a second episode of energy failure. Mechanisms of this energy failure include: an accumulation of cytosolic calcium, excitatory neurotransmitter release, the formation of oxygen free radicals and inflammatory mediators.[12]

Liu et al. found that cerebral flow velocities were significantly reduced for up to three days after birth. They also found that the severity of encephalopathy was correlated with severity of cardiac dysfunction.[11] This is further supported by the fact that serum troponin concentrations have been found to correlate strongly with the severity of encephalopathy.[55] This thus leads us to believe that the treatment of cardiac dysfunction would potentially aid in the treatment of HIE.

8.4 Heart:

Cardiovascular dysfunction occurs in 50-80% of asphyxiated neonates[56] with an increased proportion in pre-term infants.[57] During normoxia, tachycardia causes only a small rise in cardiac output (10-15%), and cardiac output is very sensitive to changes in afterload.[58] These findings suggest that the fetal heart operates at the upper limit of its functional

curve during physiological conditions, making it all the more difficult for the neonate to adapt to a hypoxic environment.[49] Although initially in the course of a hypoxic insult, an increased sympathetic response actually improves cardiac contractility,[59] hypoxemia eventually causes a drop in the cardiac output and the heart rate with an increase in the systemic vascular resistance. This is associated with a persistent increase in the pulmonary vascular resistance and an increase in coronary blood flow.[49,59]

Perinatal asphyxia has significant effects on the myocardial tissue. Following the initial insult of in-utero hypoxemia, immediate resuscitation at birth, which may include the use of oxygen therapy, reestablishes tissue oxygenation and/or perfusion and could lead to undesirable consequences. Oxidative stress during reoxygenation contributes significantly to the extent of myocardial injury and dysfunction. In animal models, it has been noted that superoxide anion and the hydroxyl radical have been increased in the reperfused myocardium. Moreover, a direct relationship has been noted between this oxidative stress and contractile dysfunction.[60,61] Additionally, increased production of nitrating agents such as nitric oxide and peroxynitrite has been demonstrated following reperfusion.[38]

It has been shown that the immature myocardium is at particular risk for injury from ROS due to significantly decreased antioxidant activity.[62] As the newborn needs to transition from a relatively hypoxic environment *in utero* to a normoxic environment at birth, a healthy infant has to combat increased levels of ROS. It has been shown that the generation of superoxide and hydroxyl radicals increases linearly with an increasing partial pressure of oxygen (pO_2).[63,64] This is further evidenced by the fact that enzymatic antioxidant activity is increased in infants.[65] However, the ability of the newborn to combat this oxidative stress is diminished as compared to adults. This has been shown by the fact that, ferric reducing ability of plasma, a marker of the ability to combat oxidative stress, is decreased in infants.[65] The increased development of ROS following hypoxia-reoxygenation may thus overwhelm the neonate's antioxidant defenses.

Not surprisingly, premature infants are at even greater risk of oxidative stress as their antioxidant abilities are even less developed. They have a limited capacity to increase the antioxidant activity in response to oxidative stress.[66] The activity of superoxide dismutase, catalase and glutathione peroxidase are decreased as compared to full-term infants.[67] Additionally, cord levels of superoxide dismutase and serum levels of glutathione are decreased in premature infants.[68,69]

Perinatal hypoxia causes cardiac dysfunction from damage to sub-endocardial tissue, to papillary muscles and to the myocardium.[55] It has also been proposed that the formation and the accumulation of ROS contribute to cardiac dysfunction via its activity on myosin, a critical contractile protein. Myosin consists of 2 heavy chains and 2 types of light chains, myosin light chain-1 and myosin light chain-2. The light chains help with the structure and function of the molecule. A proposed mechanism for the myocardial dysfunction is that of oxidative stress by nitration of myosin light chain-2 protein via an increase in peroxynitrite ($ONOO^-$). This then leads to the degradation of the protein via matrix metalloproteinase-2, a proteolytic enzyme involved in many physiological and pathological processes.[70]

A recent observational study by Sehgal et al, attempted to observe the association between coronary perfusion and cardiac output in the setting of perinatal asphyxia. They found that asphyxiated infants had significantly lower coronary blood flow via Doppler ultrasound evaluation and that this was in fact associated with a decreased cardiac output.[71] Thus, this study adds decreased coronary perfusion to the pathophysiology of myocardial injury in perinatal asphyxia.

Various cardiovascular clinical scenarios associated with birth asphyxia include: respiratory distress, heart murmur and cardiomegaly. Asphyxia is also one of the most common etiologies of perinatal myocardial ischemia, which can lead to mitral regurgitation. Hypoxia may also cause an increase in the pulmonary arterial blood pressure, leading to tricuspid regurgitation.[72] Cardiogenic shock and congestive heart failure occur in severe asphyxia.[73]

In the neonatal population, cardiac injury can present a diagnostic challenge, as the electrocardiographic (ECG) findings are more difficult to interpret as opposed to adults owing to the small thorax and non-specific ST-T changes often seen. Experience of the examiner is needed. [57,74] If adverse ECG findings are present however, they are significantly associated with poor prognosis.[74] Echocardiography is much more utilized in this age group and may be useful but is also limited and experience is essential. Moderate to severe tricuspid regurgitation can be a sign of increased pulmonary pressure, but mild regurgitation across the tricuspid valve can also be seen in unasphyxiated newborns. Left ventricular dysfunction may be a late change. The utility of an echocardiogram has been questioned in this age group.[74] Serum markers, such as troponin and creatinine kinase-MB, have been extensively studied recently and ease the diagnosis as compared to other clinical parameters.

Troponins are cardiac structural proteins that have three subunits: C, which is not useful in an assay, T and I, which are cardiac-specific. Myocardial injury causes the release of the troponin subunits that are found within the cytosol. Neonatal troponin levels are unlikely to be affected by the mother, as the protein is too large to cross the placental barrier,[75] therefore adding to their specificity.

The troponin T and I subunits have been shown to be strong indicators of myocardial injury in asphyxiated neonates[74] although cutoff levels are needed for clinical application.[57] Troponin I has also been found to be increased in asphyxiated neonates, and is strongly correlated with the clinical grade of encephalopathy and duration of inotropic support.[55] Additionally, an increased troponin I has also been associated with a 33% sensitivity and an 80% specificity in predicting mortality in asphyxiated newborns.[74]

Much like in adults, creatinine kinase-MB has also been found to be elevated in moderate-severe hypoxia, and is of potential clinical marker of injury.[59] N-terminal pro-brain natriuretic peptide has also been studied in neonatal asphyxia and predicts poor neurological outcomes in preterm infants with a patent ductus arteriosus.[59]

Histological findings of hypoxic injury include cytoplasmic eosinophilia, nuclear pyknosis, waviness, and edema as well as necrosis and phagocytosis and contraction bands. Cardiac lactate levels have been found to be elevated in a fetal lamb asphyxia model, while the kidney and liver lactate levels were not.[53]

The neonatal myocardium is therefore at increased risk of injury following hypoxia-reoxygenation as seen in perinatal asphyxia. Protecting the newborn myocardium from oxidative stress and cellular injury may lead to preservation of cardiac function in asphyxiated newborns.

8.5 Lungs:

Like in other organ systems, the extent of the pulmonary injury associated with perinatal asphyxia is variable, and ranges from brief oxygen requirements to pulmonary hypertension and hemorrhage. The lungs are at particular risk for injury, as not only is the injury caused by ischemia and reoxygenation, but other factors also complicate and worsen the injury. These include meconium aspiration, ischemia, myocardial dysfunction and barotrauma associated with mechanical ventilation.[76]

Neonatal pulmonary hypertension can be due to cardiac, pulmonary or diaphragmatic malformations leading to high output heart failure or

persistent pulmonary hypertension of the newborn (PPHN).[77] PPH is a syndrome where the fetal pulmonary circulation and its inherent increased blood pressure persists after birth. This syndrome has significant consequences, including hypoxia and the worsening of cardiac function. In this condition, disorders in the perinatal period lead to increased pulmonary vasculature resistance through vasoconstriction.[77] Asphyxiated infants are at particular risk of this syndrome as hypoxia and acidosis, hallmarks of perinatal asphyxia, are major etiological factors for PPHN.[76]

8.6 Intestine:

During neonatal life, the intestine requires energy for a variety of functions, and the blood flow is approximately 4-fold greater than its need. Blood flow to the intestine is usually maintained but not increased in response to maternal hypoxemia or a reduction in uterine or umbilical blood flow. The intestines maintain their metabolism by increasing oxygen extraction and can maintain oxygen consumption for a variety of arterial oxygen concentrations. A 45% drop in oxygen availability is needed before anaerobic metabolism occurs.[78] A severe drop in fetal partial pressure of arterial oxygen (PaO_2) will eventually drop the mesenteric perfusion, and, in spite of increased extraction, intestinal oxygen consumption falls followed by ischemia.[49] In a study of newborns with HIE, it was found that the severity of the encephalopathy, thus presumably of the ischemic insult,

had an effect on mesenteric blood flow. Severely affected neonates had significantly decreased superior mesenteric artery blood flow on the second day of life, while mild to moderate cases had an increased blood flow to the intestines. This finding identifies a short potential therapeutic window in severe hypoxia-reoxygenation.[79]

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonatal period, and is a devastating and poorly understood complication. The most commonly quoted risk factors include prematurity, early feeding of an immature bowel, bacterial overgrowth, hypothermia, cardiovascular complications and ischemic injury. It has been proposed that ischemia-reperfusion may play a role,[80] and the finding that supports this etiology is that of coagulation necrosis of the bowel found in both conditions. Early reports of NEC described this clinical entity as being due to ischemia.[81,82] However, ischemia alone has not produced NEC in animal models. Also, infants who develop this condition rarely have birth asphyxia and it rarely develops in the first week of life in premature infants. Also, the release of catecholamines, seen in perinatal asphyxia, does not cause decreased mesenteric flow or intestinal tissue hypoxia in the neonate. Therefore, the evidence does not support ischemia as a primary cause of this disease.[48,83]

Ischemic enteropathy does occur however, and a NEC-like disease has been described and causes clinical problems such as intestinal ischemia and spontaneous intestinal perforations. Where this differs from classic NEC is in the clinical presentation and the pathophysiology. Therefore, as with other conditions that predispose to intestinal injury such as congenital heart disease, it is now believed that these are separate disease processes and should not be classified as NEC.[48]

8.7 Kidney:

Renal involvement in perinatal asphyxia is common, and has been quoted as the second most common organ involved after the brain. [18] Additionally, renal failure has been significantly associated with neurological injury in asphyxiated infants, making the diagnosis of kidney injury important for prognostication.[84] However, this diagnosis is complicated by physiologic particularities of the neonate which include the fact that serum creatinine levels reflect maternal levels for up to 3 days after birth and that up to 50% of infants with renal failure do not present with oliguria. As such, there does not exist a consensus definition of acute kidney injury in the neonate.[85] Renal failure in the newborn age group is usually due to shock, hypoxemia or both[86] and 11% of all neonatal acute kidney injuries are caused by asphyxia.[87] Asphyxia-associated acute kidney injury is associated with a high mortality rate, although differentiating it from

hypovolemia-associated injury is challenging.[85] It has been shown that decreased renal flow rates via Doppler examination on the first day of life are predictive for the later development of renal failure.[88] Fractional excretion of sodium is useful in the evaluation of acute kidney injury, but it is only valid after 48 hours of life. Biomarkers of kidney injury in the first two days of life are needed.[85]

Maternal hypoxemia causes a decrease in renal blood flow and maintenance in glomerular filtration rates. The decrease in renal blood flow is spread evenly throughout the cortex.[89] This suggests that the vasoconstriction occurs at the efferent rather than the afferent arteries. Hypoxemia and decreased perfusion causes an increase in renin and vasopressin levels, leading to a reabsorption of fluid,[49] as well as increased catecholamine secretion and the release of prostaglandins.[90]

Histological changes include tubular necrosis with relative sparing of the glomeruli. This occurs even when injury is not very severe in other organ systems, suggesting that it is one of the earliest changes seen in asphyxia.[53] However, in a neonatal intensive care setting, it has been shown that acute renal failure is more common in severe rather than moderate ischemia.[90]

These hypoxic insults are significant, as they can result in renal cortical necrosis, which decreases the effective number of nephrons. They are therefore at risk of not only acute renal failure, but also put them at risk of late complications such as hypertension, proteinuria and chronic renal failure.[90]

8.8 Liver:

Because of where it is situated within fetal circulation, the liver receives the largest amount of oxygenated blood.[49] At least in part because of this, it is one of the most resistant organs to asphyxial insults. In fetal circulation, approximately 55% of the hepatic blood flow is shunted away through the ductus venosus. During various clinical scenarios, the proportion of shunted blood can be adjusted. In animal studies, when umbilical blood flow was decreased or during maternal hypoxia, the fraction shunted increases to 65%,[49] resulting in 40-60% reduction in hepatic blood flow[91] although the hepatic artery flow is relatively preserved.[92] Acute cord compression results in an even greater diversion, with the hepatic blood flow being reduced by 75%.[91]

In an unstressed fetus, there is no glucose uptake or production by the liver. However, hypoxemia has been shown to stimulate the liver to

produce glucose,[93] likely secondary to glycogenolysis.[91] This is then diffused to the rest of the body through the inferior vena cava.[49]

The clinical presentation of hepatic injury following perinatal asphyxia includes ischemic hepatitis, neonatal cholestasis and coagulopathy.[94] The development of ischemic hepatitis is associated with a poor prognosis and increased mortality. With significant injury, possible histological changes can include fatty change and congestion as well as cytoplasmic eosinophilia, canalicular cholestasis, centrilobular necrosis, and inflammatory cell infiltration.[49,94]

9. CONCLUSIONS

Perinatal asphyxia is a significant global health problem and is an important cause of morbidity and mortality in the neonate. It causes significant dysfunction, both systemically and at the level of various individual organs, with both short and long-term sequelae.

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Chapter 2: Management of Perinatal Asphyxia

1. INTRODUCTION

Child health and survival is an important worldwide concern. Socioeconomic developments and directed interventions to address this have been largely successful, yet a great number of children die of preventable causes every year. The United Nation's millennium development goals, a multinational agreement instituted to improve health and decrease poverty worldwide, included a provision for child survival and to decrease under-five mortality by 50% from 1990 to 2015.[1-5] While the incidence of certain infectious diseases are decreasing sufficiently to achieve this goal, perinatal asphyxia is not.[6] In fact, at the current rate of reduction, the rate must improve by a factor of 6 in order to reach the predefined target within that timeline. Being that the proportion of neonatal deaths is increasing within the under-five age group, increasing attention is being given to neonatal health and specifically to perinatal asphyxia, as it is the second most common cause of death in the neonatal age group.[2,7]

Improved antenatal care and skilled delivery personnel are needed for infants at risk of perinatal asphyxia, as it has been estimated that 39% of deaths associated with perinatal asphyxia could be prevented, which represents 359 000 deaths per year worldwide.[8] The prevention of secondary injuries is key in the management of this condition. The early identification of perinatal asphyxia, supportive care and specific therapies

all serve the purpose of avoiding further damage. Early identification is particularly critical for this condition, as the therapeutic window is likely short.[9]

2. MANAGEMENT

It is estimated that approximately 5-10% of infants require assistance with their first breath, while less than 1% require resuscitation.[10,11] A careful assessment of risk factors is needed as most of the time, recognition of the need for resuscitation can be accomplished prior to birth.[10]

Supportive management includes skilled antepartal and intrapartal care, adequate ventilation, avoidance of hypotension, judicious fluid management, avoidance of hypoglycemia and the treatment of seizures.[9]

Well-trained perinatal care is taken for granted in developed countries. A majority of intra-partum related neonatal deaths occur in countries with a low coverage of skilled birth attendants[12] which has been identified as one of the most important factors in preventing neonatal deaths in developing countries. China took this approach whereby the rate of hospital deliveries increased from less than half to near universality from 1988 to the present. While it has been known that maternal mortality has improved significantly, only recently have we had any data concerning the neonatal population. In a recent article in the Lancet, it was confirmed that the neonatal population

benefited greatly, as mortality in this age group decreased by 62% from 1996 to 2008.[13]

Neonatal resuscitation programs are an important aspect in the care of the asphyxiated infant, as they provide guidelines as to how and when to provide appropriate care. The most recent American Heart Association's neonatal resuscitation guidelines emphasize that one appropriately skilled healthcare professional be responsible for the neonate at every birth, and be ready to initiate resuscitative efforts if the need arises.[10]

Neonatal resuscitation education programs have had some success. In a retrospective trial from Turkey, it was shown that after one such training program, the initial Apgar score, the rate of resuscitation, the length of hospitalization and computed tomography (CT) of the head findings were statistically significantly improved, while survival trended toward significance. This study was limited by its small study population with as little as 13 patients in one group.[14] Additionally, the introduction of a neonatal resuscitation program in 14 teaching hospitals in India did show a significant improvement in asphyxia-related deaths, although overall neonatal death was unchanged.[15] In a large multicentre trial however, while the rate of stillbirths were decreased with improved training, it was shown this did not have an improvement in overall 7-day mortality.[16]

There is limited evidence for improved long-term outcomes with standardized resuscitation programs. A recent prospective trial from Turkey has reported an improvement on neurological outcomes with children of 4-6 years of age. The main drawback of this study however is the limited number of participants, with only 40 subjects.[17]

2.1 Oxygen management:

Up until recently, it was advised that supplemental oxygen be given in the setting of neonatal resuscitation due to concerns over continuing tissue hypoxia, in spite of evidence indicating the potential benefits of room air resuscitation.[18] The main concern with this approach is the oxygen free radical generation in hyperoxygenated resuscitation,[19] along with evidence that supplemental oxygen reduces cerebral blood flow in preterm infants.[20] Multiple experimental and clinical studies have shown a benefit to the use of a smaller fraction of oxygen. The use of room air resuscitation has been associated with less vasoconstriction, less oxygen free radical formation, improved apgar scores and a faster recovery of independent breathing.[21] Two meta-analyses have shown improved survival with the use of room air resuscitation[10] within the first week of life and at one month and beyond, with odds ratios of 0.70 and 0.63 respectively.[11,22] It was determined that the treatment of twenty asphyxiated infants with normoxic rather than 100% resuscitation would prevent one death. While the American Heart

Association (AHA) laments the lack of data comparing different doses of supplemental oxygen,[10] previous studies have shown worsened regional perfusion at lower supplemental doses.[20,23] Thus, the most recent AHA neonatal resuscitation guidelines recommend the use of normoxic resuscitation, with supplementary oxygen use being guided by the time-specific preductal pulse oximeter oxygen saturations (SpO_2) outlined in table 2.1.[10]

Table 2.1: American Heart Association Target SpO_2 For Neonatal Resuscitation[10]

Time after birth (minutes)	Target SpO_2 (%)
1	60-65
2	65-70
3	70-75
4	75-80
5	80-85
10	85-95

2.2 Hypothermia

Whole-body or specific head cooling are the only established therapy for neonatal encephalopathy[24] and is currently recommended by the most recent neonatal resuscitation guidelines.[25] A recent systematic review concluded that therapeutic hypothermia significantly reduced risk of death and disability at 18 months.[26] It is estimated that only six to nine infants

with moderate to severe hypoxic-ischemic encephalopathy need to be treated in order to prevent one death or severe neurological disability.[26,27]

The proposed mechanisms for this are numerous, the best known of which are the inhibition of glutamate release and the slowing of metabolism.[9] It is estimated that cerebral metabolism decreases by 5% for every degree that the brain temperature is reduced from a baseline of 38.2^{o}C[28] and that the metabolic rate for glucose and oxygen decreases along with the degradation adenosine triphosphate (ATP).[29] This thus allows an increase in the concentration of high energy phosphates, an improvement in acidosis and a reuptake of excitatory neurotransmitters.[30] The decreased oxygen metabolism has also been linked to possible decreased formation of oxygen free radicals in animal trials.[31,32]

Most studies report few adverse events from this treatment, although a small pilot trial from Uganda did report a higher mortality rate in infants treated with hypothermia (7/21 vs. 1/15).[33] The adverse effects of hypothermia can be limited by performing selective head cooling with a mild decrease in body temperature.[34]

Not unlike most therapies, the sooner it is initiated, the better the results. The results of two randomized trials in humans suggest that hypothermic

therapy for 72 hours was beneficial, especially if the encephalopathy was less severe.[9] It has been shown that hypothermia confers a greater benefit in moderate as compared to severe hypoxic-ischemic encephalopathy (HIE).[26]

Even with a small degree of hypothermia, either systemic or selective, has been shown to decrease the potential for strokes or cardiac events. In a recent meta-analysis, it was shown that cooling within 6 hours of birth was associated with improved survival and less neurological impairment at 18 months.[26] While this is a significant finding, it has been shown that hypothermia does not offer complete neuroprotection and the benefit is less for those most severely affected.[24] It is thought that combining cooling with other modalities could provide further benefit.[35]

It is also possible that the benefits of hypothermia are not limited to the brain. While the evidence is limited, therapeutic hypothermia may improve oxygenation and ventilation in intubated patients, thereby having the potential to reduce barotrauma.[36] Additionally, in a recent study of hypoxia-reoxygenation in a piglet model, it was shown that immediate hypothermia significantly improved serum troponin levels lessened the histological myocardial injury.[37] A second trial has shown that brain natriuretic peptide was lower in the hypothermia-treated group, suggesting a potential

beneficial effect on myocardial function. There was no difference in the troponin level in this trial however.[38] Hypothermia may therefore also be cardioprotective in perinatal asphyxia, although further study is needed.

2.3 Glucose management

Glucose is the main substrate used for energy in the human brain.[39] It has been shown that infant hypoglycemia worsens the brain injury after a hypoxic-ischemic injury. In a retrospective review, hypoglycemia was the factor that was found to be the most significantly associated with adverse outcome, more so than severe acidemia, a low Apgar score or the use of intubation or cardiopulmonary resuscitation.[40] On the other hand, hyperglycemia has the potential to increase the production of lactic acid and thus worsen acid-base balance.[41] In adults, hyperglycemia has been found to worsen neuronal injury, but this has not been the case in immature animal studies.[42] Since hyperglycemia has not been shown to be deleterious in the neonatal population, glucose infusions have been advocated to avoid hypoglycemia.[25] Because of the theoretical risk associated with hyperglycemia however, until further evidence is obtained, it is advised to keep glucose levels within normal concentrations.[43]

2.4 Excitatory amino acid antagonists

Evidence suggests that excitatory neurotransmitters, such as glutamate, have an important role in the hypoxic-ischemic brain injury when found at high extracellular concentrations. With lower concentrations, neurons become less sensitive to injury. Multiple methods are used to achieve a lower concentration, which include anticonvulsants such as phenytoin, the activation of pre-synaptic adenosine receptors as well as glutamate receptor antagonists.[34] Glutamate receptor antagonists have been shown to be beneficial in reducing cerebral injury in both neonatal and adult models.[9]

Magnesium sulfate, a non-competitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist has been studied extensively in the setting of perinatal asphyxia. The neuroprotective benefit has been linked with the blocking of NMDA-mediated calcium cellular influx, thus decreasing excitatory neurotransmitter release.[44] Until recently, animal models had delivered conflicting results, [45,46] with some evidence that it has some benefit in moderate as opposed to severe neurological injury.[44] A recent placebo-controlled randomized trial comparing infants with moderate to severe HIE has shown some promising results. The asphyxiated infants who had received magnesium sulfate were found to have less neurological injury, as measured by abnormal neurological examination findings and

oral feeding at discharge with a trend towards abnormal CT head findings at 14 days, which did not reach significance (p=0.07).[47]

Xenon, an inert noble gas, is another potential treatment as a non-competitive NMDA receptor antagonist. Its advantages include the fact that it has no clinical side effects, it has been used in neonates, it is reversible, it easily crosses the blood-brain barrier, it is non-teratogenic and it does not cause cardiovascular instability. [48,49] In a rat model of neonatal hypoxia-reperfusion, it was found that the combined treatment of xenon with hypothermia was associated with greater neuroprotection than either treatment alone.[49] This provides us with a promising combination treatment, although the dose needed for clinical trials is unclear at this time.[48]

Excitatory amino acid release in asphyxiated infants places them at high risk for seizures, which in turn may aggravate the underlying injury and increase the risk of future epileptic activity. While the treatment of active seizures is an accepted treatment strategy,[9] numerous studies have attempted to assess whether there is a benefit to the prophylactic treatment of severely asphyxiated infants with anticonvulsants. A Cochrane review on the subject examined all randomized and quasi-randomized trials on the subject and failed to show a benefit, although the

studies were lacking in methodological quality and had insufficient power to assess this question appropriately. The authors concluded that the evidence is currently insufficient to promote the use of anticonvulsants on clinical practice.[50]

2.5 Calcium channel blockers

The calcium balance in neuronal cells is also affected by hypoxia-reoxygenation (H-R), as the decrease in adenosine triphosphate (ATP) production causes an influx of calcium, leading to neurotransmitter release and exitotoxicity. This worsens the energy imbalance and ultimately leads to neuronal cell death.[51] Calcium channel blockers have been shown to improve neurologic outcomes and decrease cerebral edema in an animal model.[52] A clinical trial had to be discontinued due to hypotension however, severely limiting its use in the clinical setting.[35]

2.6 Erythropoietin

This hormone, responsible for blood cell synthesis, is already being used in infants for anemia.[24] It has also been shown that giving this endogenous hormone has some neuroprotective effects in experimental models.[9] Different mechanisms have been proposed, which include its anti-inflammatory, anti-apoptotic and neurotrophic effects. Other properties that may confer protection include the reduction of glutamate, limiting nitric

oxide toxicity and enhancing neurogenesis and angiogenesis. Its neuroprotective properties have been proven in animal models, as well as protecting the retina, the placenta and the liver in certain models.[24] Small clinical trials have been performed in infants and have not shown significant adverse effects, while a recent trial showed that death and disability were improved.[53]

2.7 Oxygen free radical inhibitors and scavengers

The use of oxygen free radical inhibitors and scavengers is a therapeutic approach to lessen secondary injury. Superoxide dismutase and catalase are antioxidant enzymes that have been studied in this context. Unfortunately, in animal studies, therapeutic effect has only been achieved if given several hours prior to the insult.[54]

Xanthine oxidase, which produces superoxide and hydrogen peroxide in its interaction with purines, is inhibited by allopurinol.[48] In a clinical trial, allopurinol was shown to decrease serum concentrations of oxygen free radicals in infants.[55] Allopurinol has the added benefit of, at high doses, acting as a free-radical scavenger and a free iron chelator.[56] In a recent follow-up study of two previous randomized trials, it was found that in the moderately asphyxiated group, the use of allopurinol resulted in a significantly lower severity of adverse outcomes. This has to be interpreted

cautiously, as the study populations were small.[56] However, a systematic review of randomized trials failed to demonstrate a benefit in terms of mortality, or the incidence of seizures for allopurinol in perinatal asphyxia. However, there was a total of only 114 infants in all three included trials, and the authors concluded that the data was insufficient at this time.[57]

N-acetyl cysteine has been studied in this population due to its antioxidant properties, which includes being a scavenger of hypochlorous acid as well as reacting with the hydroxyl radical and H_2O_2.[58] Additionally, it inhibits the production of nitric oxide synthase and pro-inflammatory cytokines.[24] In animal studies, its neuroprotective potential has been revealed,[24] and it has also been shown to improve cardiac function and renal perfusion.[59,60]

Many other antioxidants have been studied for this clinical situation. They include the chelating agent deferoxamine, when given during reperfusion, has been shown to decrease brain injury in animal models.[9] Melatonin has been shown in clinical trials to reduce oxidative stress with a trend towards improved survival, albeit in a small trial.[24] Cyclosporine has been shown in a swine hypoxia-reoxygenation model to improve cardiac function and mesenteric perfusion and to decrease intestinal injury.[61,62]

2.8 Theophylline

As discussed in the previous chapter, renal failure is a common and challenging aspect of perinatal asphyxia management. Adenosine has been shown to accumulate in the kidney secondarily to a hypoxic insult, and has been implicated in the pathogenesis of renal failure in this setting through its effect on renal vascular tone and tuboglomerular feedback mechanism.[63] A recent systematic review of the literature has shown that a single bolus dose of theophylline, an adenosine receptor antagonist, shortly after birth significantly reduced the incidence of hypoxia-induced renal failure in severely asphyxiated neonates. The concerns include the narrow therapeutic window of this medication and that the short and long-term adverse events are not well documented.[64] This is thus a potential targeted therapy for renal failure in this clinical setting, with careful monitoring.

3. CONCLUSIONS

While the treatment of perinatal asphyxia remains mostly supportive, the greatest therapeutic potential remains in providing mothers and infants with appropriate care in developing countries through education and implementation of standardized resuscitation programs. Supportive therapy, controlled reoxygenation and therapeutic hypothermia are also important facets in the care of the asphyxiated neonate. Many novel

therapies are on the horizon and further research is necessary to help decrease future mortality and morbidity.

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Chapter 3: Review of the Cardiac Effects of Vasopressin

A version of this chapter has been submitted for publication. Pelletier et al., 2013.

1.INTRODUCTION

Vasopressin is a structurally simple but physiologically complex hormone, and is currently in clinical use, predominantly in the adult population, for a variety of conditions ranging from diabetes insipidus to upper gastrointestinal bleeds to vasodilatory shock. This varied range of therapeutic targets highlights its ubiquitous influence. Its impact on cardiac function has been shown and exploited in certain clinical situations, most notably in the treatment of cardiac arrest. However, its concurrent effects on other organ systems have limited our understanding and its potential use as a cardiac-specific agent. Certain animal models have elucidated interesting physiologic cardiac-specific effects of vasopressin and underline the need to better understand these effects for potential therapeutic benefit. This review aims to briefly present the effect of vasopressin on cardiovascular homeostasis and to summarize the known literature regarding vasopressin and its cardiac effects.

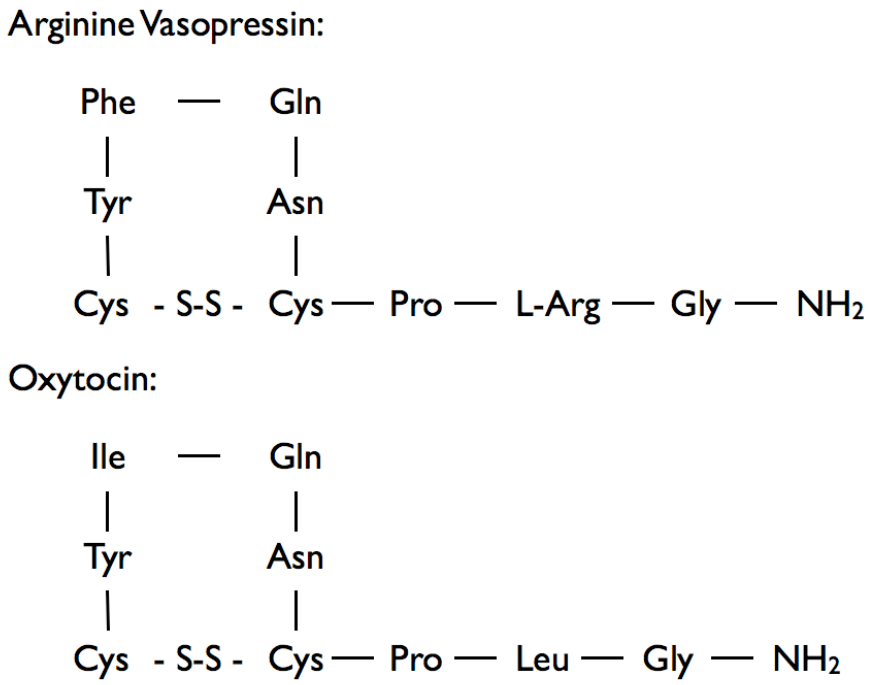
2. PHYSIOLOGY

The vasopressive properties of posterior pituitary extract was first observed in 1895[1] and attempts to isolate and purify the hormone responsible for this effect, vasopressin, proceeded thereafter and was achieved in 1951.[2] As such, it is one of the first described hormones and it has been in use clinically for over five decades, predominantly for diabetes

insipidus, variceal bleeding and, more recently, for the treatment of shock. A version of this hormone is found in both vertebrates and invertebrates[3] and is found in greater than 120 species.[4] It is present in the plasma of fetal sheep and in the brain of fetal rats,[5,6] as well as in human plasma at birth.[7]

Vasopressin's structure shares 80% homology with oxytocin, with the only differences being the 3rd and 8th amino acid. (Figure 3.1) This hormone consists of a nonapeptide with a disulphide bridge between two cysteine amino acids.[8] Vasopressin's prohormone is produced primarily in the magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus, and then migrates to the pars nervosa of the posterior pituitary. There it is stored in granules, of which only 10-20% of total stores are available immediately.[9] In addition, it has been shown that the heart also has the capacity to produce vasopressin, and in concentrations able to act locally and potentially systemically.[10] The release of vasopressin occurs primarily when plasma osmolarity or plasma sodium concentrations increase, and is sensitive to changes as small as 2%. A 10% drop in blood pressure will also stimulate its release.[11] Vasopressinases, which break down the hormone, are found in both the kidney and the liver. The half-life has been found to be 10-44 minutes, with exogenous vasopressin having a slightly longer half-life.[12]

Figure 3.1 – Structure of Arginine Vasopressin and Oxytocin



Shock states cause an endogenous release of vasopressin from 20 to 200 times its normal serum levels. Prolonged shock leads to a decrease in these levels, likely from depletion[13] although other factors, such as inhibition of its release by nitric oxide, may also play a role.[11]

Inherent to its physiological complexity, vasopressin aids in cardiovascular homeostasis though various mechanisms, some of which oppose others. Its most recognized end-effects include: an increase in the vascular tone, increased water reabsorption, the stimulation of adrenocorticotropic hormone release and glycogenolysis.[14] Although it is a weak

vasoconstrictor in healthy patients,[15,16] its effects are more pronounced when pathologic vasodilatation is present.[17] Sepsis is the classic example of this, but vasodilatation occurs in prolonged severe shock of any type.[11] The effects of vasopressin are related to its effects on specific receptors. These G-protein coupled receptors are subdivided into the V_1 (formerly V_{1A}), V_2, V_3 (formerly V_{1B}) and oxytocin-type receptor.[13] Additionally, vasopressin has been related to P_2 purinergic receptor activation, with adenosine triphosphate (ATP) serving the possible role of intermediary between the V_1 and P_2 receptors.[18] The V_2 receptor (V2R), notable for its antidiuretic effect in the renal collecting duct, and the V_3 receptor do not directly modulate heart function and will not be discussed in this review.

3. VASOPRESSIN AND THE HEART

The effects that vasopressin induces on the heart are complex. They are also difficult to interpret as the increased afterload secondary to the vasoconstrictive effects may impact cardiac performance. The effect on coronary vascular tone is not entirely elucidated and some of the results are contradictory.[17] In addition, both positive and negative inotropy has been reported in response to this hormone.[19] We will attempt to summarize the known cardiac effects.

3.1 Effect on coronary vasculature

The effect of vasopressin on the coronary vasculature is complicated and contradictory, as both vasodilatation and vasoconstriction have been reported in different trials. Variables that seem to affect the coronary vasculature's response to vasopressin include the dose, the experimental conditions, and the species studied.

3.1.1 Mechanism of Action on the Coronary Vasculature

The cellular mechanism of action of vasopressin on vascular smooth muscle cells in the coronary vasculature is not definitively elucidated. Little research has been performed on these muscle cells directly and much of what is understood is derived from studies of vascular myocytes of other origins. The presumption is that the intracellular mechanisms are similar, and will be presented in detail.

Vasopressin's predominant effect on the vasculature is mediated by the V_1 receptor (V1R). This receptor, associated with a $G_{q/11}$ protein, converts phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG) via the activation of phospholipase C (PLC).[20] In this context, IP_3 has been shown to induce the release of intracellular stores of calcium,[21,22] which in turn opens store-operated calcium channels (SOCC) which causes its influx into the cell.[23] Concurrently, DAG

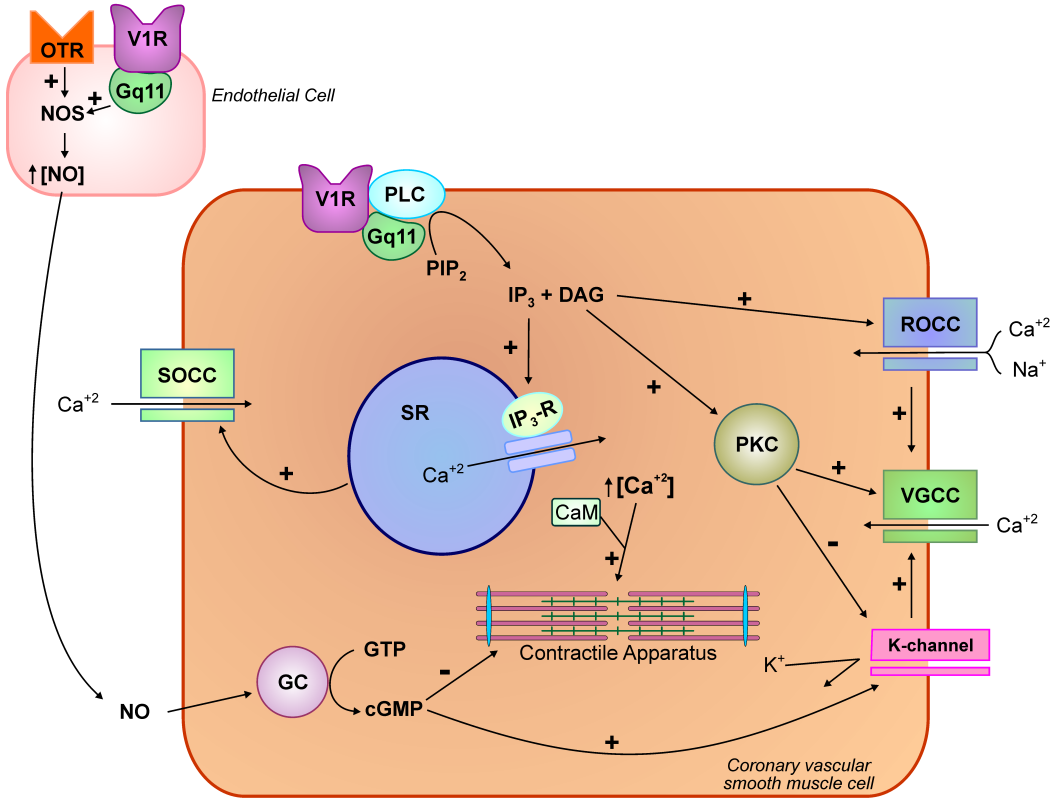
activates protein kinase C (PKC) which serves the dual function of directly opening voltage-gated calcium channels (VGCC)[21] and of closing potassium channels (K-channel) which allows cellular depolarization and calcium influx through VGCCs.[22] DAG has also been shown to directly modulate receptor operated cation channels (ROCC), causing an influx of sodium and calcium into the cell, which eases cellular depolarization and the opening of VGCCs.[22] The end effect of all of this is an increase in intracellular calcium that, following its binding to calmodulin (CaM), activates myosin light chain kinase and causes muscular contraction.[20] (Figure 3.2)

The vasoconstrictive effect of vasopressin differs depending on the vascular bed, with the greatest vasoconstriction being achieved in cutaneous vessels.[24,25] Even within a single organ, such as in the heart, large and small vessels react differently.[26] It has been proposed that the differing action of vasopressin on various vascular beds may be due to differences in the vasodilatory ability of vasopressin.[25] It has been shown that vasopressin-induced coronary vasoconstriction is worsened when exposed to a nitric oxide synthase (NOS) inhibitor, implying that nitric oxide (NO) mediated vasodilatation occurs with vasopressin.[27] In isolated segments of coronary vessels, it has been shown that the coronary vasodilatation associated with vasopressin occurs in a dose-dependent

fashion and is mediated by the V1R. NOS inhibitors and the removal of the endothelium block the dilatation, supporting the theory of an endothelial release of NO as the effector. [28] Oxytocin receptors (OTR), which have equal affinity to vasopressin and oxytocin, have also been shown to cause vasodilatation by activating endothelial NOS and provide another target for vasodilatation.[29] (Figure 3.2)

NO causes relaxation of vascular smooth muscle cells by activating guanylate cyclase (GC), which converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP),[30] which directly inhibits muscular contraction by activating myosin light chain phosphorylase.[31] Additionally, cGMP has the ability to open K-channels, thus hyperpolarizing the cell and inhibiting the influx of calcium through VGCCs.[32] (Figure 3.2)

Figure 3.2 – Vasopressin and the Coronary Vasculature



Purines have the ability to modulate cellular effects through the activation of purinoreceptors, which have been implicated in a multitude of actions including the regulation of vasopressin's release.[33] These are sub-classified into P_1 receptors, which are stimulated by adenosine or adenosine monophosphate (AMP), and P_2 receptors that are sensitive to purines such as ATP or adenosine diphosphate (ADP).[34,35] P_{2X} receptors, that activate ligand-gated ion channels, and P_{2Y} receptors, that activate G-protein coupled intracellular mechanisms, have also been described.[35] Complicating the study of these receptors is the fact that there are seven

P_{2X} and five P_{2Y} receptor types currently described which are differentiated by their relative sensitivity to different circulating purines as well as their physiological action.[36] Furthermore, circulating purines can be broken down, such as ATP into adenosine, and may activate both P_2 and P_1 receptors.[37] As such, the study of these receptors is challenging.

Both P_1 and P_2 receptors have been implicated in the regulation of the coronary vasculature, and both vasoconstriction and vasodilatation have been described. In experiments on coronary vessels, it has been established that P_1 receptors and P_{2Y1} receptors are involved in vasodilatation,[34,38,39] while P_{2X} and P_{2Y2} receptors are involved in coronary vasoconstriction.[40,41] In a guinea pig working heart model, vasopressin was rendered intravascular by its covalent coupling to dextran. Reactive blue and suramin, P_{2Y} receptor inhibitors, as well as V1R antagonism inhibited the vasodilatation elicited by both free vasopressin and vasopressin-dextran. This study has suggested that endothelial V1Rs use purines as secondary messengers to act upon purinoreceptors in the vascular smooth muscle. It is also important to note that while vasodilatation was elicited in this model, the authors indicate that preliminary studies on isolated rat hearts induced coronary vasoconstriction, suggesting a species-specific effect.[18]

As such, vasopressin has the ability to both vasoconstrict and vasodilate coronary vessels, with the end-effect likely consisting of a balance of the two effects. The varied clinical responses to vasopressin, which will be discussed in the following section, are likely a reflection of this balance.

3.1.2 Clinical Effects on the Coronary Vasculature

High dose vasopressin does appear to cause vasoconstriction of the coronary vasculature, which has been shown in separate working rat heart models.[42,43] This effect was shown to be mediated by the V1R but not the V2R.[43] This has also been shown in humans, as cadaveric heart tissue exposed to vasopressin demonstrated dose-dependent vasoconstriction, an effect that was blocked by V1R antagonists.[27,43,44]

The vasoconstriction achieved with very high doses in a normal physiological environment is even sufficient to cause ischemia, as demonstrated by increased intramural pH. The increased vascular resistance leading to ischemia in this model was found to be mostly due to higher resistance in small arteries, which accounted for 94% of the coronary vascular resistance.[26] Once again, V1R antagonists have reversed this, and these compounds have been proposed as a potential therapeutic strategy for myocardial ischemia.[45]

Complicating the issue is a porcine model of cardiovascular resuscitation, where very large bolus doses of vasopressin were given during normal sinus rhythm and following the induction of ventricular fibrillation. They found that at the supraphysiological dose of vasopressin used, 0.4units/kg, there was significant coronary vasodilatation induced in both of these clinical situations as well as after the return to spontaneous circulation. This was found in conjunction with an increase in coronary perfusion pressure.[46] These findings were unchanged after irreversible NOS inhibition in a subsequent study,[47] suggesting an alternate mechanism to vasopressin-induced vasodilatation.

The effect on the coronary vasculature also appears to vary depending on the pathophysiology studied. In a swine ischemia-reperfusion study, a vasopressin infusion titrated to return the mean arterial pressure back to baseline was found to decrease blood flow to the coronary arteries, which returned to baseline after vasopressin was stopped.[48] Additionally, in a working rat heart model, the exposure of the animals to a hypoxic environment was found to attenuate the coronary vasoconstriction and animals treated with vasopressin prior to hypoxia retained their coronary vasodilatory function.[49]

3.2 Effect on inotropy

The literature studying this topic is limited by the difficulty in separating the inotropic effects from the effects on the coronary vasculature and the systemic circulation. As well, like with the coronary vasculature, the effects are contradictory and complicated. Once again, the dose, the experimental conditions and the species studied seem to affect the results.

3.2.1 Mechanism of Action on Inotropy

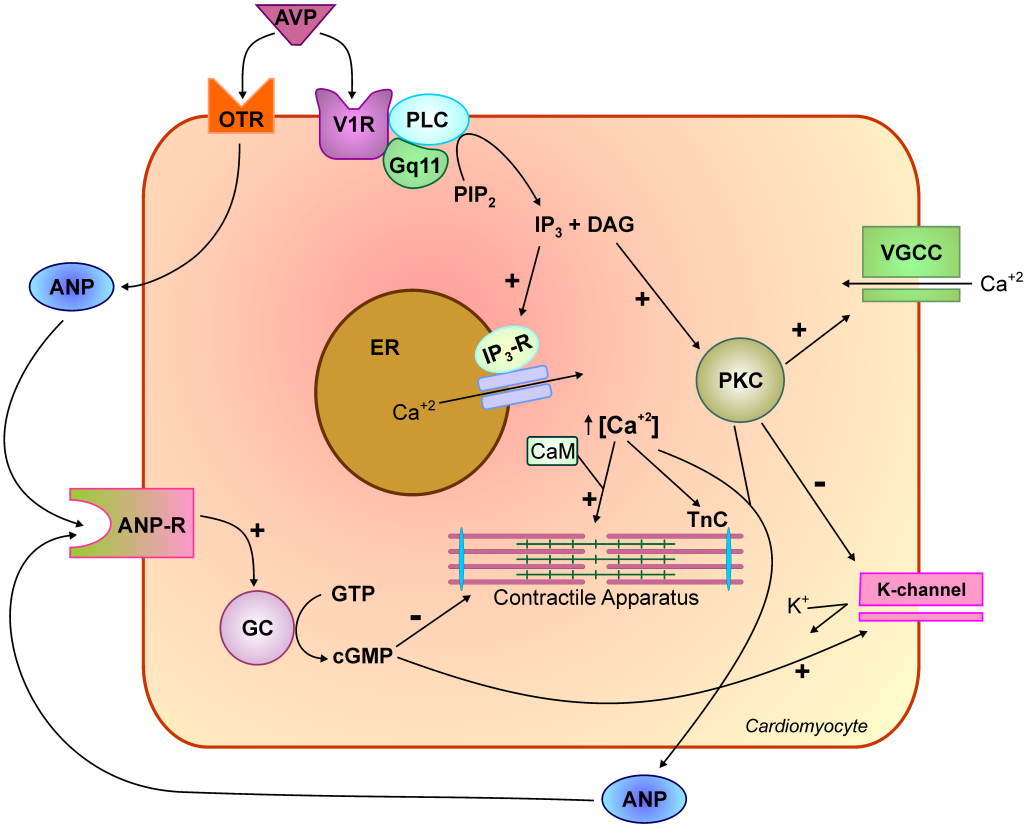
Vasopressin has been known to increase intracellular calcium in the cardiomyocyte via the V1R,[50] an effect that was later found to be dependent on PLC and the formation of IP_3.[51,52] IP_3 then induced the release of intracellular stores of calcium,[51] from what has been suggested to be the endoplasmic reticulum (ER).[53] It has also been shown that PKC activation is implicated in vasopressin-mediated myocardial contraction,[54] VGCC activation,[55] and the inhibition of K-channels.[56] Increased cytosolic calcium in myocardial cells has the dual role of binding to CaM to activate myosin light chain kinase, as well as attaching to troponin C (TnC) to remove the inhibition of the tropomyosin complex on the contractile apparatus.[57] (Figure 3.3)

Atrial natriuretic peptide (ANP) is released from cardiac cells and induces a diuretic and natriuretic effect,[58] and has also been shown to have a

negative inotropic effect.[59] To do this, myocardial ANP receptors increase cGMP, which has been shown to reduce contractility, likely by the phosphorylation of troponin I, VGCCs, phospholamban and titin[60] as well as the activation of K-channels.[61] (Figure 3.3)

Vasopressin has been shown to cause the release of ANP, an effect that was blocked by V1R antagonists, PKC inhibitors as well as calcium channel blockers. This suggests that a V1R-mediated activation of PKC and concurrent increase in cytosolic calcium cause a release of ANP from cardiomyocytes.[62] Additionally, OTRs have been shown to induce the release of ANP from cardiac cells, further inducing a negative inotropic effect.[63,64] (Figure 3.3)

Figure 3.3 – Vasopressin and Inotropy



Along with their effect on the coronary vasculature, purinoreceptors have also been implicated in vasopressin's effect on inotropy. In a guinea pig working heart model, both V1R and P_2 receptor antagonists blocked the dose-dependent negative inotropic effect of vasopressin and dextran-vasopressin, suggesting that purines may act as an intermediary for this effect. The negative inotropic effect following the activation of purinoreceptors demonstrated in this model is not conclusive and may be species-specific however, as the investigators found a positive inotropic

effect in the same model in rat hearts, which was blocked by P_2 receptor antagonists.[18]

3.2.2 Clinical Effects on Inotropy

Early studies on posterior pituitary extract found that this compound exerted negative effects on inotropy.[42] However, these studies are inherently limited by the inexactitude of the concentrations of vasopressin used, as most predate the ability to purify and quantify the hormone. More recently, Boyle et al, in a working rat heart model, and Tipayamontri et al, in an in vivo canine model, confirmed a dose-dependent association between vasopressin and cardiac depression.[42,65] Different reasons to explain this include the fact that large doses have been associated with a decrease in the heart rate and the stroke volume, effects that are blocked by V1R inhibition.[66]

It must be underlined that the negative inotropic effect found in these studies is dose-related, and that the opposite has been shown when low doses have been used. Walker et al, in a working rat heart model, found that vasopressin was actually associated with increased inotropy at lower doses, as evidenced by increased peak ventricular pressures and the first derivative of left ventricular pressure. At higher doses, they confirmed the negative inotropic effect of vasopressin. These effects were blocked by

V1R antagonists, and unaffected by V2R inhibition.[43] It was later suggested that the decreased inotropy found in earlier studies may not be due to a negative effect on cardiac function, but is rather a function of the markedly increased afterload.[67] Adding weight to the argument that vasopressin could have a positive inotropic effect, it was found that in rat cardiomyocytes, vasopressin increased cytosolic calcium via the V1R.[62,67]

Similar to the effect on the coronary vasculature, the physiological environment also modulates the inotropic effect of vasopressin. In a working heart model, response to high-dose arginine vasopressin (777pg/mL) in normoxic and hypoxic conditions were observed. In normoxia, significantly depressed myocardial function was observed, but was associated with improved myocardial efficiency. Hypoxia attenuated the myocardial depression of vasopressin. Additionally, when comparing hypoxia and vasopressin given in normoxic conditions, they induced a similar decrease in O2 delivery/consumption. However, the vasopressin group had significantly less cardiac depression, which the authors attributed to improved cardiac efficiency. The authors commented that this improved cardiac efficiency was similar to what is seen with inotropic agents, suggesting a potential positive inotropic effect of vasopressin. The authors discuss that their results were hard to reconcile with the numerous reports of decreased inotropy associated with vasopressin, even though a

cardio-depressant effect had not been proven independent of coronary vasoconstriction.[49]

While hypoxia seems to attenuate the negative inotropic effect of vasopressin, it appears as though ischemia-reperfusion (I-R) worsens it. In a swine I-R model, vasopressin depressed the cardiac output, an effect which was reversed following its withdrawal.[48] In a mouse I-R model, vasopressin had deleterious effects on the left ventricular ejection fraction and was associated with increased mortality.[17] While further study in needed, the use of vasopressin in the setting of I-R may be harmful.

Underlining its cardiac-specific therapeutic potential, vasopressin has been used in the setting of cardiovascular resuscitation for many years. In a porcine model of cardiovascular resuscitation, high-dose vasopressin given during normal sinus rhythm resulted in a decreased cardiac output, which was worsened following irreversible NOS inhibition.[47] However, when the same dose was given following the induction of ventricular fibrillation, the contractility and relaxation indices improved significantly following the return of spontaneous circulation.[46]

In an ovine burn/lung injury model where a 40% body surface area burn and smoke inhalation was given, a low-dose vasopressin infusion was

started 1 hour after the injury. Along with improved respiratory function, vasopressin improved myocardial function (improved stroke volume index and left ventricular stroke work index) and decreased nitrosative stress without significantly increasing the mean arterial pressure and the systemic vascular resistance as compared to controls.[68]

Vasopressin has been found to be beneficial to myocardial function in clinical trials. In a study on 41 patients with catecholamine-resistant post-cardiotomy shock, a fixed dose infusion of vasopressin was initiated. The result of this was to decrease other inotropic medications (milrinone and norepinephrine), to decrease the biomarkers of ischemia, and to increase the left ventricular stroke work index, the mean arterial pressure and the systemic vascular resistance. The authors concluded that vasopressin improved myocardial performance in this clinical situation.[69] In a small study of seven patients with severe heart failure treated with milrinone, a vasopressin infusion significantly increased the systemic vascular resistance, without negatively affecting the cardiac index. The authors suggested that a positive inotropic effect may have caused this.[19]

Considering the conflicting results of the above trials, it is difficult to come to definitive conclusions. While it appears as though a positive inotropic effect can be achieved with low doses, and the opposite with high doses,

this depends on the physiologic condition and the species studied. Further study is needed however, as the cardiac-specific therapeutic potential with vasopressin is significant.

3.3 Effects on cardiac maturation and hypertrophy

In addition to its direct vascular and functional effects on the heart, vasopressin also influences the maturation of cardiac cells. In a study on isolated neonatal rat cardiomyocytes, it was found that the vasopressin levels as well as the expression of both V1R and V_2 receptors were elevated. Vasopressin was found to induce a cardiomyocyte differentiation pathway, underlining its influence on the maturation of cardiac cells in the neonatal period.[70] This was confirmed in a study on cardiac stem cells, where vasopressin induced cardiomyogenesis via both V1Rs and V2Rs.[71]

It has been shown that vasopressin is capable of stimulating certain cells to proliferate and grow. This includes stimulating the proliferation of fibroblasts and glomerular mesangial cells, and inducing hyperplasia in vascular smooth muscle cells.[72] In cardiac cells, similar effects have been found. In neonatal cardiomyocytes cultured from mice, exposure to vasopressin significantly increased their surface area and induced the release of ANP, a marker of cardiac hypertrophy.[73] This hypertrophic effect has also been shown mature cells. In cultured rat cardiomyocytes, it

has been shown that vasopressin stimulates protein synthesis via PLC.[72] This effect is independent of hypertension-induced hypertrophy and is mediated by the V1R.[74]

3.4 Vasopressin and Cardioprotection

In a recent publication, the ability of vasopressin to act as a cardioprotective agent has been proposed. In a rat model of cardiac ischemia-reperfusion, several bolus doses were given prior to the initiation of ischemia and were found to be beneficial. This was evidenced by a decreased infarct size, improved biochemical parameters, and a decrease in the severity and number of arrhythmias as compared to controls. The administration of a V1R antagonist decreased this effect. The authors suggest that vasopressin may be involved in the process of preconditioning.[75] While the model used in this study is not easily translatable to the clinical setting, it does demonstrate an interesting clinical effect worthy of further investigation.

4. CONCLUSION

The influence that vasopressin has on the heart is complex and difficult to separate from its other end-organ effects. It does appear however that in certain pathological situations, vasopressin has the potential to be of

therapeutic benefit. Further clinical studies are needed to further elucidate this.

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Chapter 4: Clinical Uses Of Vasopressin And Dobutamine

A version of this chapter has been submitted for publication. Pelletier et al., 2013.

1. INTRODUCTION

Vasopressin and dobutamine are vasoactive and inotropic medications, with different mechanisms of action. The following is the known evidence concerning their clinical use and their potential future uses based on animal and clinical trials.

2. VASOPRESSIN

2.1 Mechanism of Action of Vasopressin

Vasopressin and its analogues have been used to treat various conditions for greater than 6 decades.[1] The range of conditions that it currently treats, from severe vasodilatory shock and cardiac arrest to nocturnal enuresis, underlines its ubiquitous influence. This is related to its actions on the vasopressin receptors. These G-protein coupled receptors (guanine nucleotide-binding proteins) classified into different subtypes, which include V_1 (formerly V_{1a}), V_2, V_3 (formerly V_{1b}), and oxytocin type. [2] Additionally, vasopressin has been shown to indirectly activate purinergic receptors.[3]

Vasopressin receptors are found on numerous organs, and accomplish a multitude of tasks, some of which counteract others. The V_1 receptor (V1R), best known for its effect on vascular smooth muscle, causes a muscular contraction by increasing intracellular calcium concentrations.

Activation of this receptor accomplishes this by emptying intracellular stores and by increasing the influx of calcium. The V1R is associated with the $G_{q/11}$ protein that activates phospholipase C (PLC), which in turn hydrolyses phosphatidylinositol 4,5 biphosphate (PIP_2) into inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG). IP_3 is responsible for the mobilization of intracellular stores of calcium, namely from the sarcoplasmic reticulum (SR) and the endoplasmic reticulum (ER).[4] DAG activates protein kinase C (PKC), which has been shown to activate voltage-gated calcium channels (VGCC) to increase the cellular influx of calcium.[5] PKC has also been shown to inhibit potassium channels (K-channels), preventing cellular hyperpolarization and allowing the opening of the VGCCs.[2,6] PKC has the additional effect of increasing the contractile apparatus' sensitivity to calcium.[7] DAG has also been shown to activate receptor-operated cation channels (ROCC), which has the effect of inducing an influx of cations, namely sodium and calcium. In addition to directly increasing the influx of calcium, the increase in cytosolic cations may have the effect of activating the VGCC while the influx of sodium could reverse the Na^+/Ca^{++} exchange pump (NCX).[8] (Figure 4.1) Vasopressin has also been shown to potentiate the vasoconstrictor effects of other agents including angiotensin II via an unknown mechanism,[9] and norepinephrine via the V1R. [10,11] This effect is one of the reasons why the

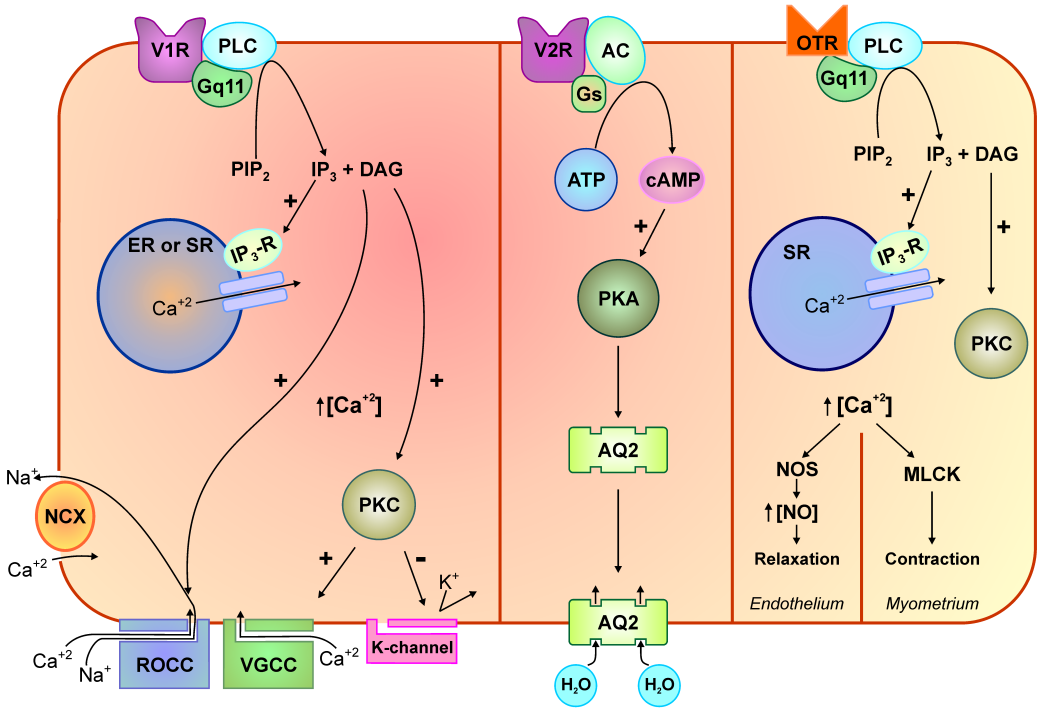
combination of vasopressin and norepinephrine is better than norepinephrine alone in septic shock.[12]

The antidiuretic effect of vasopressin is due to its activation of the V_2 receptor (V2R), the malfunction of which is the cause of nephrogenic diabetes insipidus.[13] This receptor is associated with a G_S protein, which induces adenylate cyclase (AC) to convert adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP).[14] This structure in turn activates protein kinase A (PKA), which then phosphorylates the c-terminal Ser-256 of aquaporin-2 (AQ2) and induces their insertion into the renal collecting duct's apical membrane.[15] (Figure 4.1) This facilitates the reabsorption of water and helps to maintain blood osmolality and volume,[16] an important aspect in the maintenance of cardiovascular homeostasis.

The V_3 receptor is relatively scarce and therefore, has only recently been characterized.[2] This receptor has been associated with the activation of multiple G proteins, which vary based on the expression of the receptor.[17] It is best known for its involvement in the release of adrenocorticotropic hormone through the activation of PKC via the phosphatidyl-inositol cascade.[18] This receptor has also been found on pancreatic islet cells, and is involved in regulation of insulin secretion.[19]

The oxytocin receptor (OTR) is non-specific, having equal affinity for vasopressin and oxytocin.[20] Similar to the intracellular action of the V1R, this $G_{q/11}$ protein-associated receptor activates the phosphatidylinositol cascade and induces the release of intracellular stores of calcium from the sarcoplasmic reticulum. DAG also activates PKC, which phosphorylates as of yet unidentified targets.[21,22] Notably, the increased cytosolic calcium has a variable effect depending on the tissue. In endothelial cells, it produces an activation of nitric oxide synthase (NOS) and the subsequent release of nitric oxide (NO) to cause vasodilatation. Conversely, in the myometrium, the cytosolic calcium binds to calmodulin and activates myosin light chain kinase (MLCK) to cause muscular contraction.[22] (Figure 4.1) Of note, the OTR can be found in the heart, where it can be responsible for the release of atrial natriuretic peptide which is involved in natriuresis, blood pressure regulation and cell growth.[2,23] It has been suggested that larger doses are needed to stimulate these receptors, as they have a lesser affinity for vasopressin.[23]

Figure 4.1 – V_1, V_2 and Oxytocin Receptors



Purinergic receptors are a class of membrane bound receptors that are activated by either adenosine (P_1 receptor) or high-energy phosphates such as ATP, UTP and ADP (P_2 receptors). P_2 receptors are further subdivided mechanistically, as P_{2X} receptors are ligand-gated ion channels, while P_{2Y} receptors are G-protein bound.[24] To date, there are 7 known subunits of P_{2X} and 5 of P_{2Y}.[25] Vasopressin has been shown to indirectly activate P_{2Y} purinoreceptors. In isolated guinea pig hearts, the vasodilatation and negative inotropic effect seen with vasopressin was completely blocked by both V_1 receptor as well as P_{2Y} receptor antagonists. This suggests that in this situation, ATP may act as an intermediary between endothelial V_1 receptors and the effector cells in the

myocardium and the vascular smooth muscle.[3] Additionally, it is known that purinoreceptors are associated with the regulation of the release of vasopressin and oxytocin,[26] as well as counteracting vasopressin's antidiuretic effect.[27]

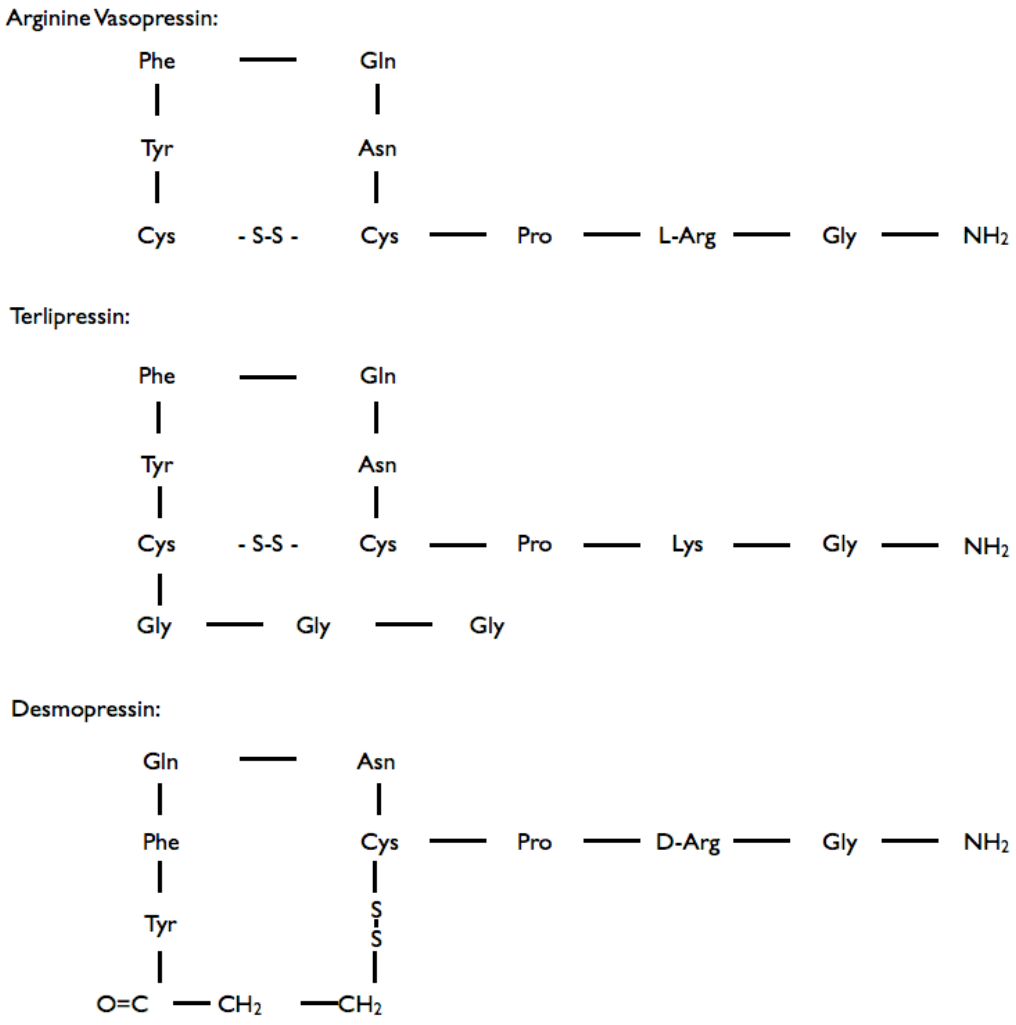
In addition to the five receptors described, it has been suggested that other as of yet unidentified receptors for vasopressin may exist.[2]

2.2 Vasopressin Analogs

Vasopressin analogues are compounds that act upon different vasopressin receptors with variable affinity and are in clinical and experimental use. The two most commonly used include terlipressin and desmopressin, also known as dDAVP. Terlipressin is a slightly larger protein than vasopressin as it has twelve amino acids, and is converted to lysine-vasopressin after the cleavage of its N-terminal glycine. It has a delayed onset of action, but is associated with the longer half-life of 4-6 hours.[28] Clinically and experimentally, it is used to target the V_1 receptor, to which it has a greater affinity,[29] although its vasopressive effect is less pronounced than that of arginine vasopressin.[28] On the other hand, dDAVP has a much greater affinity for the V_2 receptor, with a relative V_2-V_1

receptor binding of 2054:1.[30] It is therefore used for renal-specific effects or to avoid the vasoconstrictive action of vasopressin. (Figure 4.1)

Figure 4.1 – Structure of Vasopressin and its Analogs



3. CLINICAL USES OF VASOPRESSIN

3.1 SHOCK

In response to the drop in vascular resistance in vasodilatory shock, agents are used to maintain and improve the blood pressure. At this point

in time, the agents most commonly used are catecholamines, but these also have the effect of increasing the heart rate, thus increasing myocardial oxygen demand.[31] The use of vasopressin has been limited by concerns over its negative effects, namely intestinal ischemia and deleterious effects on cardiac output and global oxygen delivery.[32] The systemic vasoconstricting effect of vasopressin in normal states is minor and large doses are needed to see a clinical effect. However, in adult shock states, its clinical effect is more easily seen as the sensitivity to vasopressin is markedly increased.[33] Vasopressin has primarily been used in the treatment of septic shock, but other vasodilatory shock states such as post-cardio-pulmonary bypass vasoplegia and vasodilatation due to phosphodiesterase inhibition have also been shown to benefit from the use of vasopressin. The late stages of other shock states such as hemorrhagic or cardiogenic shock are also associated with vasodilatation, and as such may also benefit from vasopressin therapy.[33,34]

3.1.1 Septic Shock

Endogenous vasopressin levels have been shown to follow a biphasic pattern in vasodilatory shock in general and septic shock in particular, with an initially high level that progressively inappropriately declines.[35] This may be due to depleted stores, autonomic dysfunction or to nitric oxide mediated down-regulation.[36] It has also been shown that plasma

vasopressin levels are lower in septic shock versus cardiogenic shock.[37] Therefore, in addition to vasopressin's effect on the systemic vascular resistance, there is a theoretical benefit to also replace pathologically low vasopressin levels in vasodilatory shock, as a hormonal replacement therapy.[34]

While catecholamine administration has been a mainstay in the treatment of septic shock for many years, concerns over their adverse effects and decreased vascular effect over time during septic shock prompted the search for other alternative treatments.[38] The findings in a prospective case-control trial that low-dose vasopressin (0.04 units/minute) increased the arterial pressure, the systemic vascular resistance and the urine output incited further research on the matter.[39] Patel et al then conducted a small, randomized trial comparing the short-term effects of vasopressin and norepinephrine in patients requiring high-dose vasopressor support. They found that vasopressin significantly decreased the use of catecholamines and increased the urine output and creatinine clearance while maintaining the blood pressure and cardiac output.[38]

A large multicenter randomized controlled trial studying the use of vasopressin in septic shock was conducted by Russell et al. They randomized patients in septic shock receiving 5 μg/min of norepinephrine

to vasopressin (0.01-0.03 units/min) or to increasing doses of norepinephrine (5-15 μg/min) titrated to maintain a recommended mean arterial pressure of 65-75 mm Hg. A total of 778 patients underwent randomization. They found that the vasopressin group had a lower heart rate and required less additional norepinephrine, but they did not find a difference in their primary outcomes of 28-day mortality and 90-day mortality nor in the rate of serious adverse events. When they separated the groups in terms of severity of disease, they did find that the less severe group had a lower 28-day mortality in the vasopressin group.[40] In a post-hoc analysis of this same trial, it was concluded that vasopressin trended to help reduce the rate of renal failure, renal replacement therapy and mortality, but on multiple regression analysis, this did not reach statistical significance.[41]

Because plasma vasopressin levels in early septic shock are usually appropriately elevated and the relative vasopressin deficiency occurs later, it is not currently recommended to use vasopressin as an initial vasopressor in septic shock. According to the most recent surviving sepsis international guidelines, vasopressin may be added to norepinephrine at a dose of 0.03 units/minute in order to increase the arterial pressure and to decrease the dose of norepinephrine in the treatment of septic shock.[42]

3.1.2 Vasodilatory shock post-cardiac surgery

Cardiac surgery requiring cardio-pulmonary bypass is one of the most common causes of vasodilatory shock.[12] It has been shown that patients undergoing cardio-pulmonary bypass who develop vasodilatory shock have inappropriately low plasma vasopressin levels as compared to those who have cardiogenic shock, underlining its role in the pathogenesis of this condition.[43] Until recently, catecholamines were used almost exclusively to improve the arterial pressure in this clinical situation. The main problems associated with this approach include catecholamine resistance and the side effects associated with escalating doses.[12] The underlying elevated circulating plasma catecholamines and activated renin-angiotensin system found in catecholamine-resistant shock suggests alternate mechanisms for the vasoplegia found in this disease. Proposed mechanisms include the opening of adenosine triphosphate-dependent potassium channels channels, nitric oxide overproduction and vasopressin deficiency,[34] all three of which could be theoretically treated with vasopressin supplementation.[2]

Furthering the findings in a retrospective analysis that found that a low-dose vasopressin infusion was beneficial in vasodilatory shock,[43] Dünser et al performed a randomized controlled trial. Forty-eight patients in post-cardiac surgery catecholamine-resistant vasodilatory shock were

randomized to receive either vasopressin at 4 units/hour in addition to norepinephrine or norepinephrine alone with a goal to maintain the mean arterial pressure greater than 70. Following the study period of 48 hours there was no difference in terms of mortality. However, the patients in the vasopressin group did have improved mean arterial pressures, cardiac indexes, stroke volume indexes, left ventricular stroke work indexes, required less vasopressive support, had reduced toxic side effects of high-dose catecholamines and improved gastric perfusion. The authors thus concluded that vasopressin was beneficial in cardio-pulmonary bypass induced vasodilatory shock.[12]

3.1.3 Trauma and Hemorrhagic Shock

An increase in the systemic vascular resistance initially accompanies hemorrhagic shock in the early compensatory phase, which is due to high circulating levels of catecholamines and vasopressin. However, this is followed by progressive vasodilatation that is unresponsive to the increased sympathetic activity, which may be nitric oxide mediated.[44] It has been shown in animal models that in this late stage of hemorrhagic shock, characterized by unresponsiveness to catecholamines and fluid administration, endogenous vasopressin levels are low and replacement vasopressin effectively increases the arterial pressure.[45] This has also been observed in human case reports.[46]

Even in the early stages of hemorrhage, there is some evidence that vasopressin may be beneficial. In a porcine model of penetrating hepatic trauma, vasopressin improved survival when surgical repair was delayed, while both epinephrine and placebo did not.[47] It has been postulated that vasopressin may be an ideal vasoconstricting agent in penetrating trauma, as it is selective and may shunt more blood to the heart and the brain.[48] While there are no prospective trials using vasopressin in hemorrhagic shock, there is convincing evidence that vasopressin is beneficial in this situation and warrants further attention.[49]

3.1.4 Anaphylactic Shock

While standard treatment of anaphylactic shock, including the discontinuation of the exposure to the causative agent and administration of epinephrine, is effective in the vast majority of cases, there is some evidence that the use of vasopressin is beneficial.[50] In vitro studies have confirmed that vasopressin has a vasoconstrictive effect on internal mammary vessels dilated with histamine, suggesting a therapeutic benefit for anaphylaxis.[51] Additionally, case reports of vasopressin used in epinephrine-resistant anaphylactic shock provide evidence to its benefit.[50,52,53] While currently, vasopressin is not included in the guidelines for the treatment of anaphylactic shock,[54] it has been suggested that it should be,[50] although prospective trials are lacking at this time.

3.1.5 Milrinone-Induced Vasodilatory Shock

Milrinone, a phosphodiesterase III inhibitor, increases cardiac inotropy by decreasing the degradation of cyclic adenosine monophosphate (cAMP), which leads to an increase in myocardial cytosolic calcium and contractility through similar mechanisms as sympathomimetics.[55] In addition it has the added benefits of not increasing myocardial oxygen consumption and of potentiating the effect of other inotropic agents.[56] In vascular smooth muscle, milrinone increases cAMP as well as cyclic guanosine monophosphate (cGMP) via phosphodiesterase IV inhibition at higher doses. However the increase in both of these compounds in blood vessels causes vasodilatation.[57] Milrinone therefore also causes a significant drop it the systemic vascular resistance, limiting its use.[58] An agent to counteract this negative effect was needed, and vasopressin was proposed as it inhibits both cAMP and cGMP in vascular smooth muscle.[57] In a small study, vasopressin was used in adult patients with severe heart failure treated with milrinone who had subsequent vasodilatory shock. In all seven patients, vasopressin was able to increase the arterial pressure via an increase in the systemic vascular resistance. This was accomplished without negatively affecting the cardiac index, which the authors suggest may be related to an inotropic effect from vasopressin at low doses.[57] As such, vasopressin has been proposed as an alternative to norepinephrine in milrinone-induced vasodilatation.[59]

3.1.6 Shock in the Pediatric and Neonatal Populations

While vasopressin has been shown to be beneficial in vasodilatory shock in adults, the results are mixed in the pediatric population.[31] The interest in vasopressin is high in these patients, as shock and hemodynamic compromise has a mortality rate as high as 53%.[36]

A small (n=65), multicenter randomized controlled trial of vasopressin (0.0005-0.002 units/kg/min) versus placebo in pediatric patients with vasodilatory shock did not improve time to vasoactive-free hemodynamic stability, their primary outcome. There was also a concerning trend toward mortality in the vasopressin group.[60] Another single center randomized trial found that while the mortality was unchanged, terlipressin significantly improved the mean arterial pressure and the partial arterial oxygen concentration to fraction of inspired oxygen ratio without significant adverse effect.[61] Both of these studies were small as compared to adult studies, underlining the need for further study in this area.

Similarly to the adult population, a portion of pediatric patients undergoing cardiac surgery requiring cardio-pulmonary bypass have been found to have a relative vasopressin deficiency.[62] Possibly because of this, evidence of benefit from vasopressin supplementation has also been seen in this population. In a neonatal population (n=28) having undergone the

Norwood procedure, the use of vasopressin with a mean dose of 0.0005 units/kg/minute was shown to improve the systolic blood pressure, the urine output, and to decrease the need for fluid administration. Late responses included a drop in the serum lactate and further improvements in the blood pressure and the urine output. These effects were shown without affecting the heart rate,[23,63] which is important, as patients who undergo cardiac procedures are at high risk of arrhythmias, which can be provoked with the use of catecholamines. Vasopressin has been successful in post cardio-pulmonary bypass hypotension in a patient with ventricular ectopy in response to norepinephrine.[64,65] Other studies in neonatal and pediatric cardiac surgery patients in catecholamine resistant shock have confirmed that the use of vasopressin, in doses ranging from 0.0001-0.003, improved the mean arterial pressure, significantly decreased the use of other vasopressors and was without significant adverse effects.[31,65-67]

A recent review of the literature looking at catecholamine-resistant vasodilatory shock of all types in the pediatric population did not find an improvement in mortality with the use of vasopressin.[68,69] This was in spite of the fact that vasopressin did improve the blood pressure, the urine output and the serum lactate levels.[69,70] Vasopressin was however mostly used as rescue therapy, which may skew the results. Another important

point is that in the pediatric and neonatal populations, effective dosing has not yet been established and is usually extrapolated from the adult literature.

Based on the finding that extremely low-birthweight infants have altered cerebral vascular autoregulation,[40,71] an attempt to improve cerebral perfusion with vasopressin was attempted in this population in two case series.[69,71,72] Two series looked at using vasopressin, at doses of 0.01-0.04 units/kg/hour[44] and 0.001-0.02 units/kg/minute,[45] for the treatment of hypotension in extremely low birth weight infants as a second-line or rescue agent. They both found a significant increase in the mean arterial pressure and one found an increase in urine output. Due to the nature of the studies however, they were unable to comment as to whether vasopressin was otherwise beneficial. Further study is needed in this age group.[23,32,71,72]

Because of limited data, especially in terms of adverse effects, the use of vasopressin in pediatric and neonatal patients is only recommended as a rescue therapy.[73-75] It has the potential to treat various forms of vasodilatory shock in children, but larger trials and the determination of specific dosing for the pediatric population are needed before its indications can be expanded.[29,76,77,78,79]

3.2 CARDIAC ARREST

Outcomes after cardiac arrest are uniformly poor, even when following the updated management guidelines. It was discovered that the plasma concentration of vasopressin was higher in patients who were successfully resuscitated from cardiac arrest,[80,81] which spurred interest in the use of exogenous vasopressin in cardiovascular resuscitation.[82,83] Since, animal models have shown benefit in the use of vasopressin in cardiac arrest, including improved myocardial and cerebral blood flow when compared to epinephrine.[84,85] This prompted the interest in its use in humans.

In a randomized trial of 40 patients with out-of-hospital cardiac arrest secondary to ventricular fibrillation, Lindner and colleagues found that a greater proportion of patients survived to 24 hours with vasopressin as opposed to epinephrine.[84,86-88] A large randomized controlled trial of out-of-hospital cardiac arrest was then conducted, where two doses of either vasopressin or epinephrine were given as the initial drug. If spontaneous circulation was not achieved with the two doses of the study drug, epinephrine was then given. They found that patients with asystole who were given vasopressin had higher rates of hospital admission and discharge than those who were given epinephrine. Additionally, while patients with either ventricular fibrillation or pulseless electrical activity had similar outcomes overall, those that were initially given two doses of

vasopressin followed by epinephrine had significantly higher admission and discharge rates than those given epinephrine initially.[89,90] Another randomized trial of in-hospital cardiac arrest failed to find a survival advantage with vasopressin as opposed to epinephrine, questioning its use in this setting.[84,91]

The American Heart Association's consensus statement on cardiopulmonary resuscitation states that they are unable to conclusively say that any vasopressor increases survival for human cardiac arrest, due to a lack of placebo-controlled studies in this area. However, vasopressin is still found in the American Heart Association's Advanced Cardiac Life Support guidelines as an alternative to one of the first two doses of epinephrine in cardiac arrest,[92,93] as a recent systematic review found vasopressin to be equivalent to epinephrine as the initial vasopressor in cardiac arrest.[84,88,94]

Little evidence currently exists for children in cardiac arrest, and as such, vasopressin in not currently recommended for pediatric or neonatal cardiac arrest.[89,95-97] However, in a recent pilot study to assess the possibility of performing a randomized trial, ten pediatric patients in cardiac arrest were given vasopressin if an initial dose of epinephrine was unsuccessful. They found a substantially improved 24-hour survival of

80%, as compared to 30% in a retrospective matched cohort, although the return to spontaneous circulation, survival to hospital discharge and neurological outcomes were unchanged. The authors conclude that a randomized controlled trial in this patient population is warranted.[98,99]

3.3 ESOPHAGEAL VARICEAL BLEEDING

Esophageal varices occur due to the shunting of portal venous blood into the systemic circulation via esophageal veins as a result of portal hypertension. These abnormally dilated veins have a propensity to bleed, which is a medical emergency associated with a high mortality.[100,101] The main treatment modalities include vasoactive medications, endoscopic therapy, balloon tamponade and porto-systemic shunting procedures. Vasoactive medications, which include somatostatin, octreotide, vasopressin and terlipressin, act to reduce portal blood flow or portal resistance.[102,103] While the choice of medication largely depends on local expertise, as no medication has been proven superior, an intermittent dose of terlipressin has some distinct advantages over the other choices. Most prominently, it is the only medication that has been shown to decrease mortality.[104,105]

In a double-blind randomized controlled trial, patients with an upper gastro-intestinal bleed and clinical signs of portal hypertension were given

either terlipressin and a glyceryl trinitrate patch or placebo prior to arriving to the hospital. The group receiving the early administration of terlipressin were found to have improved bleeding control and bleeding-related survival without serious adverse effects.[105,106] Improved bleeding control with terlipressin has been confirmed in other trials.[31,107-110] Another prominent advantage of terlipressin is that it may prevent renal failure, a common occurrence in acute variceal bleeding and a predictor for mortality.[32,101,111-113]

3.4 LOWER GASTROINTESTINAL BLEEDING

While less common now with the advent of super-selective angioembolization, intra-arterial vasopressin infusion into the main trunk of the superior mesenteric artery has been used for the treatment of lower gastro-intestinal bleeding.[33,92,114] Control of hemorrhage due to diverticular disease or angiodysplasia was 91% effective, although this was limited by a high recurrence rate.[114] Intra-arterial vasopressin infusions were also used for initial stabilization as a bridge to surgical resection.[115] While improved techniques in angioembolization have proven to be superior, intra-arterial vasopressin infusion remains a treatment option, especially with diffuse bleeding or if super-selective embolization is not possible.[116]

3.5 BLEEDING ABNORMALITIES

3.5.1 Von Willebrand Disease

Von Willebrand Factor (VWF) is an important protein involved in hemostasis. In primary hemostasis, it is the primary mediator of platelet adhesion, without which the platelet plug cannot form. This protein is also important for secondary hemostasis, as it is the primary transport protein for factor XIII, without which this crucial factor in the coagulation cascade is rapidly broken down.[117] As such, the deficiency in this factor, termed Von Willebrand disease, may have the mucocutaneous bleeding characteristic of platelet disorders, or they may present with spontaneous deep tissue bleeding associated with factor XIII deficiency.[118] The classification of Von Willebrand disease is presented in table 4.1. Vasopressin, through the activation of both V_1 and V_2 receptors is a secretagogue for VWF. Desmopressin, as it is not associated with the vascular effects of vasopressin, is used to treat this condition and causes an increase in both VWF and in factor XIII.

Table 4.1: Classification of Von Willebrand Disease[117,118]

Type 1	Partial quantitative deficiency of VWF
Type 2	Qualitative deficiency in VWF
Type 2A	Decreased platelet-dependent VWF function with selective deficiency of high-molecular-weight multimers
Type 2B	Increased affinity for platelet glycoprotein Ib, leads to excessive platelet activation and clearance
Type 2M	Decreased platelet-dependent VWF function with high-molecular weight multimers present
Type 2N	Markedly decreased binding of factor VIII to VWF
Type 3	Complete deficiency of VWF

Desmopressin is highly effective in Von Willebrand disease type 1, as it induces the release of VWF from endothelial storage sites. In type 3, the most severe type, it has no effect, as there isn't any stored VWF to be released.[117] In type 2, as it is associated with a functional issue with the VWF, it would be expected that desmopressin would have little effect. However, in types 2A, 2M and 2N, desmopressin can be effective.[119] Desmopressin is contraindicated in type 2B, as it would induce increased platelet destruction and thrombocytopenia.[117]

3.5.2 Hemophilia A

Hemophilia A, an X-linked bleeding disorder caused by a deficiency in factor XIII, has variable severity based on the gene mutation of the X

chromosome. Desmopressin can also be used in mild or moderate cases of hemophilia A, where the mutated factor XIII still has some activity.[117]

3.5.3 Other Bleeding Disorders

Other bleeding disorders that have also been treated with desmopressin with variable success include: congenital platelet functional defects, uremia, cirrhosis and drug-induced bleeding.[120] While exact mechanisms are not established, it is thought that improved coagulation occurs by increasing the amount of available factor VIII, by increasing the efficiency of platelet adhesion and by inducing the release of larger, more adhesive VWF molecules.[117]

3.6 CENTRAL DIABETES INSIPIDUS

Diabetes insipidus (DI) is a disease where large volumes of dilute urine are produced, either due to vasopressin deficiency, central DI, or due to the kidney's insensitivity to vasopressin, nephrogenic DI. Central DI can be congenital or acquired idiopathically or secondary to vascular insults, tumors or infections. All of these causes disrupt the synthesis of vasopressin. Desmopressin is the main treatment modality for central DI, due to its specific action on the V_2 receptors responsible for vasopressin's antidiuretic effect.[121]

3.7 NOCTURNAL ENURESIS

Nocturnal enuresis is a functional disturbance of the lower urinary tract where at discrete time points during sleep, involuntary bladder voiding occurs. It is most common in children, and usually improves with age. The causes include an inability to awaken in response to the micturition reflex, detrusor muscle hyperactivity and abnormally low nighttime vasopressin levels.[122,123] While behavioral therapies are the first line therapy for this condition, desmopressin may be used in cases where polyuria is present and has a 30-40% response rate.[124]

3.8 ANESTHESIA

Vasopressin can be a useful drug to address significant hypotension in particular circumstances that occur in the operating room. During epidural or spinal anesthesia, the sympathetic drive is blocked. It has been shown that endogenous vasopressin is a major mechanism involved in maintaining the systemic blood pressure in this situation.[125] Ornipressin, a vasopressin analog that acts on the V_1 receptor, has been shown to restore arterial blood pressure during combined epidural-general anesthesia, making it an attractive option to treat epidural or spinal anesthesia induced hypotension.[126] Other situations where vasopressin can be useful is in peri-operative hypotension in chronic angiotensin

converting enzyme inhibitor users,[127] following pheochromocytoma removal,[128] and for carcinoid crisis.[129]

3.9 BRAIN DEAD ORGAN DONORS

Cadaveric organ donation is an important part of healthcare to treat end-stage organ failure. Unfortunately, due to high demand, there is a chronic shortage of donors. The hemodynamic instability of otherwise suitable donors worsens the availability of organs and has profound effects on patients awaiting donation. Catecholamines have been utilized to maintain an appropriate blood pressure in these patients, but these agents have undesirable side effects at high doses, including regional hypoperfusion. A defect in the baro-reflex mediated secretion of vasopressin has been shown in brain dead organ donors, and the use of vasopressin in this population has improved the arterial pressure and decreased the need for catecholamines.[130] Vasopressin used in conjunction with low-dose epinephrine has also been shown to substantially extend the survival time after brain death as compared to epinephrine alone, thus prolonging the opportunity for transplantation.[131] As such, following fluid resuscitation, vasopressin is recommended as the initial medication for hemodynamic support in cadaveric organ donors.[132,133]

3.10 PULMONARY HYPERTENSION

Pulmonary hypertension is a heterogeneous disease process with numerous etiologies. It can arise as a primary disorder of the pulmonary vasculature, or can be secondary to left-sided heart disease or pulmonary disease.[134] In animal models, vasopressin has been shown to dilate the pulmonary vasculature at lower doses (10^{-12} – 10^{-7} mol) via a release of nitric oxide through the activation of endothelial V_1 receptors.[135] While not widely used in the treatment of pulmonary hypertension, vasopressin has been beneficial in certain cases of pulmonary hypertension by decreasing the pulmonary arterial pressure. In two patients with obstructed total anomalous pulmonary venous return, the use of vasopressin increased the systemic arterial pressure and concomitantly decreased the pulmonary arterial pressure.[136] Also, in two separate case reports, terlipressin was successfully used as rescue therapy in patients with severe pulmonary hypertension secondary to congenital diaphragmatic hernias refractory to inhaled nitric oxide. Once again, terlipressin had the added advantage of improving the systemic arterial pressure, allowing the authors to wean the catecholamine infusions.[137,138] While vasopressin is not currently a primary treatment modality for pulmonary hypertension,[139] it remains an interesting option in clinical situations where an increase in the systemic pressure and a decrease in pulmonary pressure is required.

3.11 CONCLUSION

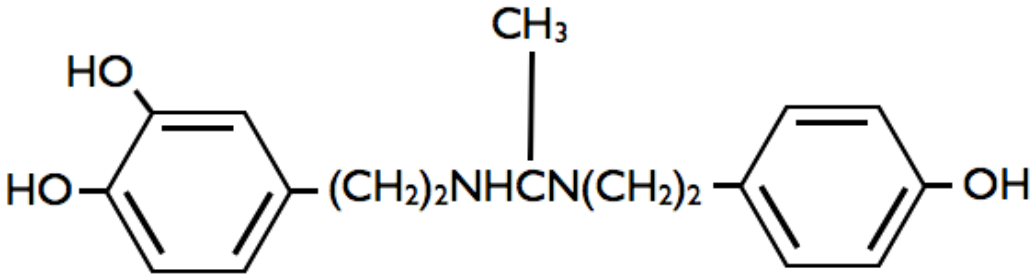
Vasopressin is an essential endogenous hormone that serves several functions in physiological and pathological conditions. It is thus used in the treatment of various conditions, but its hemodynamic properties have been relatively slowly tested and adopted. This is especially true in the pediatric population, where the potential for benefit is great.

4. DOBUTAMINE

4.1 INTRODUCTION

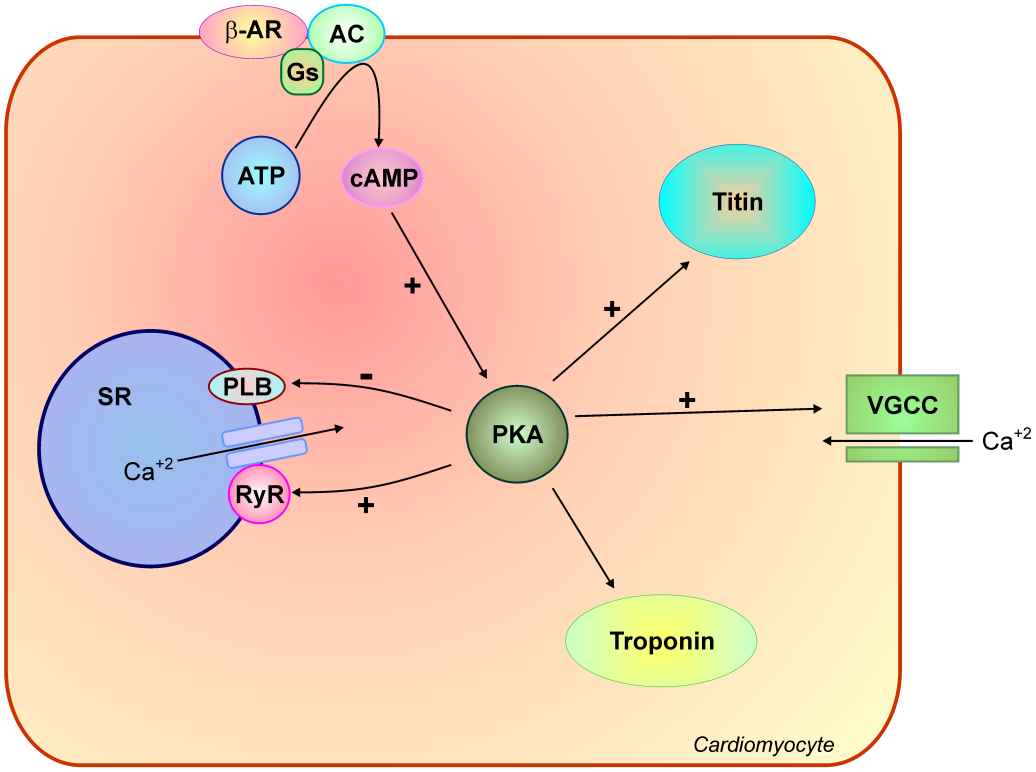
Dobutamine is a synthetic catecholamine developed in the 1970's to offer an alternative to isoproterenol as an inotropic agent in the treatment of heart failure.[140,141] This was done by modifying isoproterenol's chemical structure in an attempt to reduce its chronotropic, arrhythmogenic and vascular side-effects.[141] This was successful in that all of these side effects were lessened with dobutamine,[141] but are still associated with the use of this drug, especially at higher doses.[73,142]

Figure 4.3 – Chemical Structure of Dobutamine



The compound in clinical use is a racemic mixture of (+) and (-) enantiomers, both of which act upon different receptors, with the (+) activating β_1 and β_2 receptors and the (-) activating the α_1-receptor. Its chemical structure is presented in Figure 4.2. Dobutamine activates myocardial β_1 and, to a lesser extent, β_2 adrenoreceptors (β-AR) to improve inotropy,[73] which increase intracellular calcium via cAMP-mediated activation of PKA. PKA has been shown to activate VGCCs and cause the release of intracellular stores of calcium through the activation of ryanodine receptors (RyR) on the SR. [143] The increased intracellular calcium then improves the magnitude of the contraction.[144] In addition, the activation of cardiac β_1 adrenoreceptors inhibit phospholamban (PLB) and remove its inhibitory effect on the SR's CaATPase channels with the end-effect being increased inotropy and chronotropy.[145] The activation of these cardiac receptors also activates titin, which maintains myofilament structure,[146] and troponin to decrease calcium sensitivity of the contractile apparatus.[147] This decreased calcium sensitivity aids in myocyte relaxation during diastole.[148] (Figure 4.4)

Figure 4.4 – β-Adrenoreceptor Receptor Activation by Dobutamine



Evidence does suggest that myocardial α_1-receptor activation may contribute to the inotropic effect,[140] as these receptors are known to increase contractility by increasing cytosolic calcium by potentially directly activating calcium channels.[73]

The systemic vascular resistance decreases with the use of dobutamine as a direct result of β_2 receptor activation, and also because of a reflex vasodilatation in response to the increased cardiac output. This is countered by α_1-receptor activation, which causes vasoconstriction. The decrease in vascular resistance is countered by the increased cardiac

output, which improves the systemic blood pressure. As such, a significant drop in arterial pressure is rare with the use of dobutamine,[140] unless large doses are used.[73] Dobutamine does not act upon dopamine receptors[149] and as such, does not elicit a release of norepinephrine.[150]

The half-life of dobutamine is relatively short at approximately 2 minutes with the principle routes of excretion via urine and bile.[151] Clinical effect can be seen within two minutes of starting the drug, with maximum effect reached by ten minutes.[142]

The net effects of the use of dobutamine include an increase in contractility and stroke volume, and a decrease in systemic and pulmonary vascular resistance.[73] At usual doses (2.5-10µg/kg/min), this is accompanied by modest tachycardia. When doses exceed 10µg/kg/min however, significant tachycardia and hypotension may ensue.[73] Prolonged use (24-72 hours) has been associated with tachyphylaxis, and long-term use is also associated with sudden cardiac death and decreased survival.[142]

Dobutamine has been associated with increased myocardial oxygen consumption and decreased energy efficiency.[152] This poor energy efficiency leads to an increase in oxygen demand known as the “oxygen-

wasting effect". This is related to cellular calcium supersaturation, which then requires active transport out of the cell.[144] These negative effects may be somewhat countered by the increase in coronary blood flow.[73] However, with excessive tachycardia, myocardial perfusion decreases and ischemia is induced. As such, it has therefore been suggested that dobutamine may not be ideal for an ischemic myocardium[144] or for patients with coronary artery disease, although this is the rationale for the dobutamine stress test. Large doses may also cause ischemia-induced necrosis and apoptosis.[73]

In terms of regional effects, it has been suggested that dobutamine may improve mesenteric blood flow in septic shock, although it is unclear whether there is a benefit on the intestinal microcirculation.[104,153] Regional circulation has been seen to improve in healthy animal models, but to not have a significant effect on asphyxiated piglets.[154] Dobutamine has been known to cause an increase in the pulmonary circulation pressure at high-doses, likely due to α-stimulation.[154] A vasodilatory effect on the pulmonary vasculature has also been reported.[151]

4.2 CLINICAL USES OF DOBUTAMINE

The most common use of dobutamine is for acute exacerbations of congestive heart failure. Other indications include severe heart failure

complicating myocardial infarction or cardiac surgery, cardiogenic shock and as a bridge to cardiac transplantation.[73,155] Dobutamine is also currently the first-line choice in septic shock for a poor cardiac output in spite of adequate filling pressures in the adult population.[76]

4.2.1 Adult Heart Failure

Heart failure is a major health problem in the western world associated with exorbitant costs, high relapse rates and poor outcomes. While the mainstay of treatment revolves around loop diuretics and vasodilators, inotropes such as dobutamine are important for severe cases associated with cardiogenic shock. Its use is not without controversy however.

In a randomized multicenter trial, concerning adverse events were associated with the use of dobutamine. In this trial, dobutamine was compared to nesiritide (B-type natriuretic peptide) in patients with acute decompensation of chronic congestive heart failure. While both medications were similarly effective at improving the symptoms, dobutamine was associated with significant pro-arrhythmic and chronotropic effects. The authors suggest that dobutamine may not be the safest drug in this clinical situation.[156]

Overall, the use of dobutamine in congestive heart failure has been questioned, as the results are mixed.[157] However, when dobutamine has been compared to other medications used to provide inotropic support, it is as effective as milrinone and levosimendan without an apparent increase in adverse events.[158,159] Currently dobutamine, milrinone or dopamine may be used initially for patients with a low blood pressure associated with a poor cardiac output.[142]

4.2.2 Septic Shock in Adults

Septic shock is associated with a low systemic vascular resistance and a high cardiac output. Because of this, dobutamine is not an intuitive choice to treat this condition. However, studies in septic shock have shown that dobutamine significantly improved myocardial function as well as oxygen supply.[160] It was also found to improve microcirculatory flow via its action on β_2 receptors, which was independent of systemic hemodynamic parameters.[161]

Because of these findings, Rivers et al included the use of dobutamine in their landmark paper of early goal-directed therapy for adult septic shock. They recommended that dobutamine be started for a central venous saturation of less than 70% following the optimization of the central venous pressure, the hematocrit and the mean arterial pressure.[100] While difficult

to quantify the benefit of dobutamine individually in this algorithm-based therapy, overall early goal-directed therapy has resulted in significant benefit.[162]

It has also been shown that the response to dobutamine in septic shock is a prognostic factor. In a prospective trial, it was shown that responsiveness to dobutamine in a population of patients suffering from septic shock was correlated with survival.[163]

4.2.3 Pediatric And Neonatal Population

As in adults, dobutamine is used in the pediatric and neonatal populations to treat heart failure and septic shock associated with low cardiac output and adequate systemic vascular resistance.[74,77]

Early studies of dobutamine in the pediatric population suggested that the effects were lessened in children less than one year of age. It has been found to be effective at improving the cardiac output without major side effects[164] although the pediatric population seems to have an increased chronotropic effect, which may represent the fact that the inotropic effect easily reaches its limit.[151]

Robel-Tillig et al performed a study in pre-term infants with documented myocardial dysfunction and found that dobutamine not only improved the cardiac output, it also was found to significantly improve the blood pressure.[165] The authors conclude that in cases with impaired cardiac function and near normal blood pressure, such as some cases of perinatal asphyxia, dobutamine may be a good option.[165] In a review of hypotensive pre-term infants however, it was shown that dobutamine was less effective than dopamine at improving the blood pressure, in spite of dobutamine being more effective at improving the cardiac output.[166]

A special circumstance in the neonatal population where dobutamine may be useful is in persistent pulmonary hypertension of the newborn. In this situation, it is important to support the systemic hemodynamics, but to decrease the pulmonary hypertension. As such, dobutamine may be the first-line choice in this situation when inotropic support is needed.[77]

4.3 CONCLUSION

Dobutamine is a commonly used inotrope in both the adult, pediatric and neonatal populations. Its main indication is as an inotropic medication in cardiogenic shock. While initially developed with the goal of avoiding the chronotropic effects of available inotropic medications, it does increase the

heart rate, especially in the pediatric population. This contributes to its energy inefficiency, which is its main disadvantage.

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Chapter 5: Piglet Hypoxia-Reoxygenation Model For Neonatal Asphyxia

1. INTRODUCTION

Animal models for perinatal asphyxia were developed, as studying the condition in neonates is challenging and imprecise.[1] The use of animals to study this condition has a long history, dating as far back as 1813 and most of our knowledge of this condition has been as a consequence of such studies.[2] These models help us understand mechanisms and the progression of the associated injuries, and allow us to develop and test novel treatments.[3] Many inconsistencies exist however when comparing human pathophysiology to an animal model. These include inter-species anatomical and functional differences, differences in maturity and the physiologic accuracy of the asphyxial insult. Because of this, care must be taken when translating the findings from animal models of perinatal asphyxia to humans.[2]

It has been proposed that an appropriate model to study this condition should conform to certain characteristics. These include a similar maturation pattern to humans, an adequate size to allow for instrumentation as well as an asphyxial model that has translational value.[1] An issue with animal models of perinatal asphyxia is that they focus on a single asphyxial injury, not taking into account the different etiologies of asphyxial injuries nor the possibility for chronicity.[2]

Different animal models have been developed to study this condition, and no model is ideal.[4] These include rat pups, fetal or neonatal lambs, neonatal piglets or rhesus monkeys. Rat pups are the most commonly used and are associated with certain advantages, including lower cost and large litters, allowing for a greater number of subjects. Due to their small size however, instrumentation and accurate hemodynamic monitoring is challenging, making it difficult to compare groups according to these parameters. Conversely, the fetal lamb's size allows for accurate instrumentation, but the cost and availability of these animals limit their use.[1] Non-human primate models are thought to have a high fidelity for human neonatal brain injury, for which hypoxia-reoxygenation is the most common etiology. However, ethical and financial issues limit the use of primates in biomedical research.[3,5] Due to their large litter size, fetal piglets are an impractical model for perinatal asphyxia.[1] Fetal sheep and neonatal piglets are ideal for the study of acute and sub-acute endpoints, while rats and primates are better suited to assess neurological and long-term behavioral outcomes.[4] Our neonatal swine model of perinatal asphyxia has been published in its entirety in video form.[6]

2. NEONATAL PIGLET MODEL

The European wild boar, domesticated 9000 years ago, is the common ancestor for all pig breeds.[7] Pigs in general are appropriate for biomedical

and surgical research, owing to their strong anatomical correlation to humans.[8] Neonatal piglets have the development of a 36-38 week human fetus, which is an advantage compared to other common models, as rats are altricial (born immature – the equivalent of a 24-26wk infant) while lambs and rhesus monkeys are precocial (more advanced maturity as compared to humans).[1] This is an important point in an asphyxial model, as it has been shown that immature animals are more resistant to the effects of hypoxia.[2,9] Another advantage of swine research is their large and predictable size and their short cycle of reproduction. [1,10] The main disadvantage of a neonatal swine model is that the cost and the amount of time taken for instrumentation makes the obtaining of large sample sizes difficult.[1]

2.1 Comparative Anatomy

2.1.1 Cardiovascular

The pig's cardiovascular system is very similar to the human's, the coronary vasculature has up to 90% homogeneity in anatomy and the cardiac function is also similar. Additionally, analogous to humans, the cardiac blood supply and conduction system are both right side dominant [8] and the histological appearance of the myocardium is identical.[10] An important note on the instrumentation of swine is that their blood vessels and atria tend to be more friable, most notably in neonates.[8] Of particular

importance to our field of study, in response to asphyxia, the neonatal piglet has similar circulatory redistribution characteristics as humans.[9] It has been reported that swine are prone to circulatory instability due to a poor tolerance to stress.[11] Congenital abnormalities, such as septal defects, patent foramen ovale, and a patent ductus arteriosus are seen in piglets.[8]

2.1.2 Pulmonary

The pigs' lungs have three lobes on the left and four lobes on the right, which includes the accessory lobe. The right apical lobe has a bronchus that branches off of the trachea proximal to the carina.[8] This is an important note, as an endotracheal or tracheostomy tube could potentially block this bronchus if positioned too distally. Additionally, a prominent larynx with a large vestibule and ventricles contributes to the narrow laryngeal passage, making intubation challenging in this model.[8]

2.1.3 Gastrointestinal

The pig's gastro-intestinal tract has some important anatomical differences as compared to humans, but its function is similar. This is likely owing to the fact that, like humans, swine are true omnivores.[10] The most appreciable difference is in embryological intestinal rotation, which leads to the swine's spiral colon being entirely on the left side of the abdominal

cavity. Its mesenteric blood flow is very similar, which makes the study of intestinal low-flow states ideal.[8]

2.1.4 Renal

The swine's kidney closely resembles a human's, more so than any other species including primates. For this reason, they have been used in many urologic surgical models.[10]

2.2 ANESTHESIA

Many different anesthetic protocols have been used in pigs and should be coherent with the goals of the research. Inhalational agents are a good choice for complex surgical procedures due to the depth of anesthesia. Isoflurane is considered less cardio-depressive than other inhalational agents and may be superior if hemodynamic parameters are to be measured.[10] It does depress the cardiac output, mean arterial pressure, heart rate and contractility as well as dilate the coronary vasculature however,[12,13] so care must be used not to give unnecessarily high doses. It may be used in combination with nitrous oxide, which confers a slightly faster induction of anesthesia.[14] Nitrous oxide should not be used in isolation, as pigs do not benefit from its analgesic effects.[10] Of interest to animal models studying hemodynamic variables, inhalational anesthetics

have been shown to have a cardio-protective effect in pigs and humans.[15,16]

Limiting the use of isoflurane in humans is its odor and association with laryngospasm. Newer inhalational agents are therefore used in the human pediatric population, and some have also been tested in piglet models. Sevoflurane, used for both pediatric and veterinary procedures, was not found to be superior to isoflurane in terms of induction and recovery time, and is associated with a higher cost as well as agitation and seizure activity if used for anesthesia maintenance.[17] Isoflurane is not an ideal agent for anesthesia maintenance in hypoxic models, as it further depresses the arterial pressure as compared to intravenous agents.[18] Additionally, as in humans, malignant hyperthermia or cardiac arrhythmias may occur with the use of these agents,[10] with pigs being particularly prone to the former.[11]

Intravenous anesthetic agents can also be used in animal models. Propofol is a commonly used induction and maintenance agent in humans, and is characterized by a rapid onset and recovery.[11] The use of this drug in asphyxia models is problematic however, as hypoxia induces increased plasma concentrations which can subsequently lead to cardiac depression.[18]

Narcotic agents are commonly used in swine, but higher doses are usually needed as they are relatively insensitive to their effects.[10,19] Fentanyl is a popular narcotic, due to its rapid effects. In swine, fentanyl given in addition to inhalational anesthesia has been shown to moderately increase the systemic vascular resistance at the high dose of 100µg/kg, but not at 50µg/kg. It had no effect on the blood pressure, cardiac output and stroke volume and is therefore an appropriate agent for experimental models looking at hemodynamic variables.[20]

Midazolam is a benzodiazepine that possesses anesthetic, amnesic and anxiolytic effects. It has a rapid onset of action and may be used for induction or maintenance, but not as a single agent. It has been successfully used in swine as a combination agent with fentanyl with and without propofol.[11,21]

All forms of anesthesia are associated with some effect to the animal. As such, as in our model, it is important to utilize sham-operated piglets and randomization to minimize the effects of the surgical/anesthetic model on the results.

3. BLOOD FLOW MEASUREMENTS

In order to measure hemodynamic variables within experimental protocols, it is imperative that a simple, safe and reliable system is used to measure blood flow. The first technology used to measure blood flow without opening the vessel or introducing a foreign body is the electromagnetic flowmeter.[22] Essentially, this technique measured the voltage generated within blood vessels, which is proportional to intraluminal velocity, thus being able to calculate the blood flow.[23] This technique grew out of favor, as it was prone to errors in measurements. The probe not being perpendicular to the vessel would affect the measurements, as would a lack of conducting fluid, hematocrit levels and vessel thickness. Additionally, the calibration of the system was difficult.[24]

In order to avoid the problems associated with the above technique, flowmeters using ultrasound to measure the transit time were developed. To accomplish this, two piezoelectric crystals direct pulsed ultrasound waves at a perpendicular angle through the vessel from the same side, one upstream and one downstream. These waves hit a reflector and return to the probes. The difference between the upstream and downstream ultrasonic transit time is proportional to the transit time of the blood flow, which is subsequently calculated.[24,25] This technique has the advantage of not requiring calibration, and of not being affected by hematocrit and

vessel thickness.[25] It has been used successfully in animals and humans and allows for continuous blood flow measurements.[25,26]

While transit time flow rate technology is effective, there are other options to measure blood flow. One of these techniques is that of injected radiolabeled microspheres. For this technique, the microspheres are injected, and arterial blood samples are compared with the tissue for radioactivity. An advantage of this technique is that, with different isotopes, multiple measurements can be performed. Additionally, regional flows and cardiac output can be measured at the same time, and end-organ tissue perfusion can be estimated. Unfortunately, this technique has poor accuracy and cannot perform continuous measurements.[27]

Laser-Doppler flowmetry is a technique developed in the 1970s to monitor microvascular flow and tissue perfusion. With this technique, a monochromatic light is directed into tissue and is influenced by moving particles, especially as blood cells. The changed frequency of the light is recorded and, while it cannot quantify the velocity of blood flow, the strength of the Doppler signal is proportional to blood flow. The main limitations of this technique revolve around tissue changes that occur with the change in blood flow, which include changes in vascular geometry and microhematocrit. Also, it is vulnerable to the influence of external

conditions, such as changes in illumination.[28] Additionally, this technique can only measure an area of approximately 1mm^3, and is thus limited by spatial variability and poor reproducibility.[29,30]

To improve upon this, laser Doppler imaging was developed. With this technique, the monochromatic light is delivered to a large surface is measured using the same principle. While still limited by poor depth, up to 1.5mm into the tissue, it does provide important information on tissue perfusion over a larger area. A drawback as compared to the previous technique is that the measurement is not continuous.[30]

A method was thus developed to improve upon these techniques by utilizing laser speckle. This phenomenon is produced by random interference when a laser's light is reflected off of an irregular surface.[31] Laser speckle contrast imaging was developed to take advantage of the fact that speckle fluctuates more rapidly with increased blood flow velocity. With this technology, continuous video recording measures the speckle produced by the shining of a laser onto a large area, and is able to monitor changes in tissue perfusion.[29] It thus combines the advantages of the two previously described techniques. While a drawback is that the depth of penetration is less with this technique (approximately 300μm),[32] it has

been found to be superior to laser Doppler imaging and flowmetry in certain situations.[33,34]

Another technique used to assess tissue perfusion is near-infrared spectroscopy. Light, when passed through a colored compound, is absorbed. In the infrared range, light is poorly absorbed by tissue, but is absorbed by deoxyhemoglobin, oxyhemoglobin and oxidized cytochrome oxidase. By using differential wavelengths, these different compounds can be measured, and with continuous measurements their velocity of flow can be monitored. As such, blood flow and oxygen concentration can be measured using this technique.[35] The main limitations include the interference of other tissues, the effect of a change in blood volume and the difficulty in precisely localizing the measured blood flow.[36]

Thermodilution is used to measure flow rate as well as cardiac output. To accomplish this, a bolus of cold liquid is injected into a vessel and the temperature the blood downstream is measured. While accurate, it is not recommended to measure flow in arteries, as the fluid can unpredictably affect them. As such, this technique is only valid for veins and to calculate cardiac output.[37]

Magnetic resonance imaging is a commonly used diagnostic tool. It relies upon powerful magnets that spin electrically charged atoms, and detects the rotating magnetic field in order to produce an image. This technique can also be applied to blood vessels, and is able to measure the flow within.[38] The main disadvantages are the cost and availability of the equipment.

4. NORMOXIC REOXYGENATION PROTOCOL

It was postulated that, due to the neonate's vulnerability to a reoxygenation injury, that room air resuscitation would be beneficial in the treatment of perinatal asphyxia. In 1992, Rootwelt and colleagues performed a neonatal swine model of hypoxia-reoxygenation, and showed equivalency between normoxic and hyperoxic resuscitation,[39] which was consistent with previous studies in rabbits and lambs.[40,41] The Resair 2 trial, a large multicenter prospective cohort study was then performed and confirmed that room air resuscitation was equally effective, with a significantly shorter time to first breath.[42] Furthermore, in a subsequent randomized controlled trial, it was found that infants resuscitated with room air displayed less oxidative stress, as evidenced by increased oxidized glutathione levels in infants resuscitated with 100% oxygen, a finding that persisted up until 28 days postpartum.[43] Further study found that

asphyxiated piglets given 100% resuscitation had increased mesenteric oxidative stress, which correlated with pathological intestinal findings.[44]

While no single trial has shown a mortality benefit from normoxic resuscitation, two meta-analyses have.[45,46] As such, the current Canadian and American Academy of Pediatrics guidelines for neonatal resuscitation are to begin resuscitation with room air, and to only give supplemental oxygen if needed during cardiopulmonary resuscitation, for persistent bradycardia, or to treat hypoxemia according to the pulse oximeter oxygen saturation (SpO_2) targets outlined in table 5.1.[47] Therefore, room air resuscitation in animal models is translatable to current clinical practice.

Table 5.1: Preductal SpO_2 Targets in Neonatal Resuscitation[47]

Time After Birth (min)	Preductal SpO_2 Target
1	60 - 65%
2	65 – 70%
3	70 – 75%
4	75 – 80%
5	80 – 85%
10	85 – 95%

5. CONCLUSION

The heterogeneous condition that is perinatal asphyxia is challenging to study in vivo. Animal models allow us to gain knowledge in the pathogenesis and advance the treatment of this condition. While imperfect, the swine model for perinatal asphyxia confers many benefits as compared to other animal models. Blood flow measurements using ultrasound transit time technology is useful to obtain real-time continuous measurements. Normoxic resuscitation is now standard of care for neonatal resuscitation and as such, should be used in translational animal models.

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Chapter 6: Low-Dose Vasopressin And Dobutamine In A Neonatal Swine Model Of Hypoxia-Reoxygenation

A version of this chapter has been submitted for publication. Pelletier et al., 2013.

1. INTRODUCTION

Neonatal asphyxia is a major worldwide health concern, and is the second most common cause of neonatal death worldwide.[1] It is also responsible for significant morbidity including cerebral palsy and developmental delay.[2,3] The deleterious effects of neonatal asphyxia are not only due to hypoxia, but also the resultant ischemia and the reoxygenation/reperfusion injury.[4] This damages multiple organ systems including the brain, the heart and the intestines. The associated cardiac injury is significant as it occurs in 50-80% of asphyxiated infants.[5] The severity of cardiac dysfunction is correlated with the degree of encephalopathy[6] suggesting that the treatment of cardiac dysfunction may improve outcomes in other organ systems.

Currently, the management of neonatal asphyxia and its related complications includes judicious fluid and oxygen management, blood pressure support, avoidance of hypoglycemia, cooling and the prevention of seizures.[7] Inotropic and vasopressive medications including dobutamine, dopamine and epinephrine, are widely utilized, but an ideal supportive regimen for newborns has yet to be established.[8]

Vasopressin is a neurohypophysial hormone essential for cardiovascular and osmotic homeostasis, and its roles include the maintenance of

vascular tone and circulating volume via the V_1 and V_2 receptors respectively.[9] There is also some evidence that it has an inotropic effect at low doses.[10] It has been used for various adult conditions for over six decades,[9] but its use in neonates has been limited by concerns over regional hypoperfusion[11] as well as insufficient evidence justifying its use.[12] To date, its use in pediatric shock has been focused on its vasopressive effects.[12]

Our understanding of the cardiac effects of vasopressin is evolving. Its effects on coronary vascular tone and inotropy in particular have been contradictory[10,13-15] and are dependent on the model used and the doses studied. It is worth noting however that recent animal models using low-dose vasopressin have demonstrated significant myocardial functional improvement[13] and a potential cardioprotective effect.[16] Additionally, in a recent study, we have shown that a low-dose vasopressin infusion given in a neonatal swine model of hypoxia-reoxygenation (H-R) improved myocardial tissue lactate levels, indicating reduced myocardial anaerobic metabolism in this setting. This was achieved without negatively affecting regional blood flow.[17]

We speculated that a low-dose vasopressin infusion initiated earlier in the above model would result in a demonstrable cardiac functional

improvement without deleterious regional hemodynamic effects. We chose to compare the hemodynamic effects with those of dobutamine, a commonly used inotrope in neonatal shock.

2. MATERIALS AND METHODS

2.1 Animal Instrumentation

Our protocol was approved by the institutional research ethics office and conformed to the Canadian Council of Animal Care (2000) standards. Mixed breed newborn piglets (1-4 days old; 1.4-2.5 kg) were obtained from the University. As described previously,[17,18] individual piglets were induced into anesthesia using 5% isofluorane followed by maintenance using midazolam (0.2–1 mg/ kg/h) and fentanyl (5–20 mcg/kg/h) once intravenous access was achieved via the right femoral vessels. These were isolated and catheterized with 5-French catheters (Sherwood Medical Co., St. Louis, MO) for continuous mean arterial blood pressure (MAP) and central venous pressure measurements, as well as for intravenous access. Additional bolus doses of fentanyl (10 mcg/kg) and acepromazine (0.25 mg/kg) were given as needed to maintain anesthesia and analgesia. D5W at 10 mL/kg/hr and 0.9% NaCl at 1 mL/kg/h were used as fluid maintenance.

Following tracheostomy, pressure-controlled ventilation was initiated using a Sechrist infant ventilator model IV 100 (Sechrist Industries Inc. Anaheim, CA). Flow probes were placed around the left common carotid, superior mesenteric, left renal, and main pulmonary arteries. The ductus arteriosus was ligated and an angiocatheter was secured within the pulmonary artery. A 10 mL/kg bolus of 0.9% normal saline was given to all piglets after instrumentation to ensure normovolemia. Flow and pressure measurements were digitized by a DT2801-A analogue to a digital converter board (Data Translation, Ontario, Canada) and recorded into a computer using Asyst programming software.

2.2 Animal Monitoring

A pulse oximeter (Nellcor, Hayward, California) was used to measure percutaneous oxygen saturation, and a Hewlett Packard 78833B monitor (Hewlett Packard Co., Palo Alto, CA) was used for continuous electrocardiographic and blood pressure measurements. Inspired oxygen levels were measured using an Ohmeda 5100 oxygen monitor (Ohmeda Medical, Laurel, MD). Temperature was regulated between 38.5-39.5 ^{o}C using lamps and heating pads and monitored with a rectal thermometer.

Blood was sampled repeatedly at pre-determined intervals throughout the experiment for blood gases analysis, hemoglobin and biochemical assays. Plasma samples were obtained, flash frozen immediately and stored at -80°C for subsequent analysis.

2.3 Hypoxia-Reoxygenation Protocol

Following the instrumentation, the animals were afforded an approximate 1-hour recovery period in order to achieve hemodynamic stability, which was defined as changes in hemodynamic parameters of less than 10% over 10 minutes. Normocapnic hypoxia was induced by reducing the fractional inspired oxygen concentration (FiO_2) to 0.10-0.15 for 2h through nitrogen dilution, which resulted in decreased cardiac output to 50-55% of baseline, MAP of 30-35mmHg and pH of 7.00-7.05. Further stress to the animals resulted in high mortality rates. Normoxic (FiO_2=0.21-0.25) reoxygenation was then initiated to keep the SaO_2 >88%. After 4h of reoxygenation, all piglets were euthanized using pentobarbitol (100 mg/kg iv).

2.4 Drug Administration

Based on our dose-response study,[17] the vasopressin infusion dose of 0.01 units/kg/h was chosen and started 30min after the commencement of reoxygenation to mimic the initiation of an infusion in asphyxiated

newborns after resuscitation. The dose of dobutamine (20 mcg/kg/min) was chosen as it had the greatest inotropic effect in a previous study using a similar model of H-R.[19] We initiated this infusion 2h after the start of reoxygenation as, based on our previous experiments using this animal model, this was when cardiogenic shock was most prominent.[17,18]

2.5 Group Allocation

Piglets were block-randomized as: sham-operated controls (n=5), H-R controls (n=8), vasopressin-treated (n=8) and dobutamine-treated (n=8) animals. A technician prepared the medications and either a drug or a saline placebo was given at both timepoints. The first author (JSP) performed the experiments, collected the data and was blinded to group allocation until they were completed.

2.6 Histological and Biochemical Analysis

Samples of myocardium and ileum were fixed in 10% formalin, and others were flash frozen in liquid nitrogen and stored at -80^{o}C. A pathologist (CS) blinded to group allocation performed the histological analysis using the Rose Criteria and Park's classification to score the myocardial and intestinal injuries, respectively.[20,21]

Myocardial and intestinal lactate levels were determined from the crushed tissues using enzymatic spectrometric methods measuring the absorbance of NADH at 340nm. The protein content was determined using a bicinchoninic acid assay kit (Sigma). The crushed tissues were also used in the determination of the glutathione (GSH), glutathione disulfide (GSSG) and lipid hydroperoxydes (LPO) levels (Cayman Chemical Company, Ann Arbor, MI).

Plasma vasopressin (Peninsula Laboratories, #S-1356, San Carlos, CA) and intestinal fatty acid binding protein (Hycult Biotech, HK406, Plymouth Meeting, PA) levels were measured using commercially available enzyme-linked immunosorbent assays at set time points. An enzyme-linked immunosorbent assay was also used to measure plasma concentrations of troponin-I (Life Diagnostics, #2010-4-HS; West Chester, PA) which is an established cardiac biomarker in swine.[22]

2.7 Statistical Analysis

All results are expressed as mean ± standard error of the mean. Hemodynamic variables and biochemical data were analyzed using 2-way repeated measures and one-way analysis of variance, respectively, followed by Fisher *post hoc* testing (SigmaPlot v11, Systat Software Inc., San Jose, CA). Significance was defined as $p<0.05$.

3. RESULTS

Twenty-nine newborn piglets were used for the experiments (age: 3.1±0.3 days; weight: 1.9±0.03 kg). There were no differences in blood gases and hemodynamic variables at baseline among groups (Tables 6.1 and 6.2).

Cardiogenic shock was induced in all H-R piglets with the cardiac output (CI) of H-R groups being 51-54% of baseline (Table 2) All H-R groups had an improved CI immediately upon reoxygenation (88-100% of baseline), which gradually decreased thereafter. In the vasopressin-treated group, CI continued to deteriorate for 1.5h following the commencement of infusion and then increased continually until the end of experiment up to 88% of baseline. When the dobutamine infusion was started, CI increased immediately. At 4h of reoxygenation, CI for both vasopressin and dobutamine-treated groups were higher than that of H-R control group ($p<0.05$), but not different from each other or the sham-operated group. (Figure 6.1)

All H-R piglets were tachycardic following hypoxia, without a significant difference among groups. After the commencement of the dobutamine infusion, there was an increase in the heart rate, which was significantly higher than that of sham-operated piglets, but not other H-R groups (Table 6.2).

All H-R piglets became severely hypotensive following hypoxia, with their MAP at 30-32 mmHg, which recovered upon reoxygenation. The MAP then progressively decreased without difference between groups by the end of experiment. The pulmonary arterial pressure increased significantly during hypoxia and then slowly normalized to baseline by the end of experiment, without significant differences among H-R groups (Table 6.2).

The plasma troponin-I and left ventricular myocardial lactate levels were lower in the vasopressin and dobutamine-treated H-R groups than those of H-R controls. For the markers of oxidative stress, the left ventricular myocardial LPO levels of vasopressin and dobutamine-treated H-R groups were not different from that of H-R control group (25.4±1.5 and 20.1±3.5 vs. 24.2±2.4 μmol/mg protein, respectively, $p>0.05$), whereas the left ventricular GSSG/GSH ratio was not significantly different among groups (Table 6.3).

The superior mesenteric arterial flow index (SMAFI) decreased significantly with hypoxia and immediately normalized upon normoxic reoxygenation. SMAFI gradually deteriorated during 4h of reoxygenation in H-R controls, but was improved in the vasopressin and dobutamine-treated groups (vs. H-R control group, $p<0.05$) to levels not statistically different than the sham-operated group (Figure 6.2). The carotid arterial

flow index was lower in all H-R groups as compared to the sham-operated group, and although there was a trend to improved flow in the vasopressin and dobutamine-treated groups, this did not reach statistical significance as compared to the H-R control group. There was no significant difference in renal artery flow index among groups (Table 6.2).

There were significantly lower intestinal GSSG/GSH ratio and LPO levels in the vasopressin and dobutamine-treated groups compared to H-R controls. Intestinal tissue lactate and plasma intestinal fatty acid binding protein levels showed a trend toward being lower in the vasopressin and dobutamine-treated groups than those of H-R controls, but these did not reach statistical significance (Table 6.3).

Plasma vasopressin levels were measured in the H-R control and sham-operated groups at baseline, 2h of hypoxia and 4h of reoxygenation. The plasma vasopressin levels were not significantly different between these two groups (data not shown).

Systemic and mesenteric oxygen delivery was significantly increased by the end of the experiment in both the vasopressin and the dobutamine groups as compared to H-R controls (Figures 6.3 and 6.4). Systemic and regional oxygen consumption was similar in all piglets that underwent H-R

(data not shown). The systemic vascular resistance index was similar in all treatment groups as compared to sham-operated piglets (data not shown).

Regarding the histological features of H-R injury in the left ventricular myocardial and intestinal tissues based on the Rose Criteria and Parks Classification, respectively, it was not significantly different among groups (data not shown).

4. DISCUSSION

In this study we have demonstrated that in a neonatal swine model of myocardial H-R injury a low-dose vasopressin infusion significantly improves CI. To our knowledge, this is the first report of vasopressin in this context. Interestingly, this finding was only seen 1.5h after the infusion started. Although the mechanism is unclear, the clinical implication would be that a low-dose vasopressin infusion would need to be started without immediate effect, likely in conjunction with other therapies. The benefit of such a therapeutic strategy would have to be evaluated.

Although vasopressin has some cardiac-specific effects, including increased inotropy at low-doses (maximal response at 50 pg/mL), the most compelling evidence has been shown in-vitro.[10] However, in a recent in-vivo study of ovine lung injury, low-dose vasopressin (0.03 units/kg/h)

was found to improve stroke volume and left ventricular stroke work indices.[13] Evidence of the same in the clinical scenario is sparse, and it includes a study by Gold et al, where vasopressin infusions reduced inotropic support and heart rate suggesting an improved myocardial performance in seven patients with milrinone-induced cardiogenic shock.[23]

In our study, the hemodynamic effect of vasopressin was similar to that of dobutamine, a potent inotrope with β-adrenoreceptor agonistic properties. Dobutamine is known for its inotropic and chronotropic abilities, but is also associated with increased tissue oxygen demands and the potential for impaired myocardial perfusion,[24] making it a less than ideal choice for inotropic support. Although we did not specifically study the myocardial oxygen metabolism, the piglets in the dobutamine group had lower myocardial lactate levels than H-R controls. Vasopressin-treated piglets had similarly reduced myocardial lactate levels compared to H-R controls. Therefore, both treatment groups decreased the anaerobic metabolism within myocardial tissue. This is at least in part consistent with the study by Ornato who reported that vasopressin does not increase myocardial oxygen demand.[25]

Our hemodynamic findings in vasopressin and dobutamine-treated groups were associated with decreased plasma cardiac troponin-I and myocardial

tissue lactate levels, although we did not find significant histopathologic features of H-R injury due to the short observation period. We did not find a specific mechanism to explain the alleviation in biochemical markers of cardiac injury. Of interest, Nazari et al recently published results of a rat model of cardiac ischemia-reperfusion pre-treated with different bolus doses (0.03-2.4 units/kg) of vasopressin. They found that vasopressin at lower doses (with a maximally effective bolus dose of 0.06 units/kg) had a protective effect as evidenced by decreased infarct size, biochemical parameters and arrythmogenicity.[16] Further investigations are warranted to examine this “cardioprotective” effect of vasopressin.

While it may seem counterintuitive to use vasopressin prophylactically in neonates, it isn’t unprecedented. In a recent trial in neonates undergoing complex cardiac surgery, a low-dose vasopressin infusion was initiated in the operating room and continued in the intensive care. They found that, as compared to historical controls, the infants treated with vasopressin required less fluid and catacholamines.[26]

In adults with vasodilatory shock, fixed low-dose vasopressin infusions (0.02-0.04 units/min) are currently being used as it has been shown that plasma vasopressin is inappropriately low in this clinical situation.[27] In our model of neonatal H-R, we did not find a significant difference in

vasopressin levels between the H-R control and sham-operated groups. This suggests that, at least in the acute phase of H-R, vasopressin deficiency may not be a significant factor in H-R induced shock as it has previously been shown in other forms of cardiogenic shock.[27,28] Interestingly, Devane et al found high levels of vasopressin in asphyxiated fetal sheep.[29] Our acute model does not capture if there is a subsequent vasopressin deficiency.

A key issue associated with the use of vasopressin is its potential negative (vasoconstrictive) effects on regional perfusion. We demonstrated an improvement in mesenteric perfusion and oxygen delivery with a low-dose vasopressin infusion. This was associated with decreased intestinal oxidative stress, as evidenced by lower intestinal LPO levels and GSSG/GSH ratio, and modestly lower tissue lactate and plasma intestinal fatty acid binding protein levels, than those of H-R controls. While vasopressin has been associated with decreased mesenteric perfusion, this is usually with the use of supra-physiological doses[30] or with inadequate fluid resuscitation.[31] In fact, it has been shown that vasopressin may actually improve splanchnic perfusion,[32] when fluid resuscitation is adequate.[31] Further, we found that vasopressin did not negatively affect regional flow to the carotid and renal vascular beds, which is consistent with our previous study.[17] Therefore we believe that

the concern over regional perfusion is implausible with a low-dose vasopressin infusion with adequate fluid resuscitation.

Another key implication of this study is that we have demonstrated a delayed beneficial effect to the use of low-dose vasopressin without a significant change to the systemic blood pressure. This is very important as most clinical trials studying the use of vasopressin or terlipressin, a long-acting vasopressin analog, in neonates titrates the dose to a significant effect on the blood pressure.[33,34] This study suggests that a baseline low-dose infusion of vasopressin may be of greater benefit, and may guide clinical trials in the future.

The limitations of our study include that it is an animal model, and that there has been significant inter-species variability reported in vasopressin receptors, with only 80% homology between human and rat V_1 receptors.[35] However, the swine's cardiovascular system and function is very similar to the human's and the histological appearance of the myocardium is nearly identical.[36,37] A neonatal swine model also does not take into consideration the unique circumstances at birth resulting from the transition from a fetal to neonatal environment, although the neonatal swine's development at birth is akin to a 36-38 week old human fetus.[38] As such, it is an appropriate cardiovascular large animal model. Additionally, as our study

duration was set at six hours, we can only comment on acute events. However the acute resuscitation stage is very important and influences long-term prognosis. The causes of birth asphyxia are numerous and our model does not represent all potential etiologies. Further, a dose of 20 mcg/kg/min dobutamine was chosen based on our previous experience in this model.[19] The dose is high and fixed, which will limit the translation of our findings to clinical practice. In addition, it is difficult to compare two medications with such differing mechanisms, and this is made more complicated by the fact that they were started at different time points in an effort to improve its translatability. We must stress that our goal was not to demonstrate superiority, but rather to have a point of reference in the evaluation of the cardiac action and regional perfusion of vasopressin.

5. CONCLUSIONS

This study is the first to demonstrate that a low-dose vasopressin infusion used in the setting of a neonatal swine model of H-R is associated with an improvement in the cardiac output and the mesenteric perfusion.

6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

7. Tables and Figures

Table 6.1 – Arterial Blood Gases and Plasma Lactate Concentrations During Hypoxia and Reoxygenation

	Baseline	End of Hypoxia	30 Minutes Reoxygenation	4 Hours Reoxygenation
pH				
H-R controls	7.41 ± 0.01	7.06 ± 0.05	7.18 ± 0.05	7.32 ± 0.04
Vasopressin	7.40 ± 0.01	7.06 ± 0.02	7.21 ± 0.03	7.34 ± 0.03
Dobutamine	7.41 ± 0.02	7.06 ± 0.03	7.20 ± 0.03	7.36 ± 0.02
Sham-operated	7.41 ± 0.03	7.38 ± 0.04 *	7.39 ± 0.02*	7.35 ± 0.02
PaO_2 (torr)				
H-R controls	72 ± 4	46 ± 6	75 ± 4	68 ± 4
Vasopressin	70 ± 3	43 ± 1	74 ± 7	63 ± 4
Dobutamine	69 ± 2	37 ± 2	76 ± 3	66 ± 2
Sham-operated	69 ± 2	65 ± 3*	67 ± 3*	65 ± 3
$PaCO_2$ (torr)				
H-R controls	40 ± 2	38 ± 2	37 ± 1	37 ± 1
Vasopressin	41 ± 1	37 ± 1	35 ± 1	44 ± 1
Dobutamine	40 ± 1	38 ± 2	35 ± 1	42 ± 1
Sham-operated	42 ± 4	40 ± 2	39 ± 1	42 ± 1
HCO_3 (mM)				
H-R controls	25 ± 1	10 ± 1	14 ± 1	21 ± 2
Vasopressin	25 ± 1	10 ± 1	14 ± 1	22 ± 1
Dobutamine	25 ± 2	10 ± 1	14 ± 1	23 ± 1
Sham-operated	24 ± 1	23 ± 1*	24 ± 1*	22 ± 1
Lactate (mM)				
H-R controls	2.7 ± 0.3	11.2 ± 1.0	9.3 ± 1.2	3.0 ± 0.9
Vasopressin	2.6 ± 0.2	11.5 ± 0.7	8.8 ± 0.5	2.3 ± 0.5
Dobutamine	3.0 ± 0.3	10.2 ± 0.8	8.3 ± 0.5	2.2 ± 0.3
Sham-operated	2.4 ± 0.3	1.9 ± 0.2*	1.9 ± 0.1*	1.7 ± 0.3

H-R, hypoxia-reoxygenation
* $p<0.05$ vs. H-R controls

Table 6.2: Hemodynamic Data During Hypoxia and Reoxygenation

	Baseline	End of Hypoxia	30 Minutes Reoxygenation	4 Hours Reoxygenation
Cardiac Index (mL/kg/min)				
H-R controls	194 ± 14	99 ± 16	138 ± 17	118 ± 15
Vasopressin	190 ± 18	103 ± 11	160 ± 17	167 ± 17 *
Dobutamine	205 ± 18	109 ± 9	163 ± 15	183 ± 18 *
Sham-operated	160 ± 11	159 ± 7 *	171 ± 15 *	159 ± 6 *
MAP (mmHg)				
H-R controls	73 ± 3	32 ± 1	50 ± 5	42 ± 3
Vasopressin	76 ± 3	30 ± 1	46 ± 2	44 ± 3
Dobutamine	74 ± 3	30 ± 1	43 ± 2	42 ± 3
Sham-operated	78 ± 6	61 ± 2 *	60 ± 2	49 ± 3
HR (beats/min)				
H-R controls	187 ± 11	240 ± 14	210 ± 11	220 ± 9
Vasopressin	200 ± 14	245 ± 11	232 ± 12	229 ± 9
Dobutamine	198 ± 10	222 ± 5	209 ± 9	256 ± 8
Sham-operated	199 ± 7	209 ± 10 *	209 ± 5	202 ± 2
PAP (mmHg)				
H-R controls	30 ± 2	41 ± 4	33 ± 2	34 ± 3
Vasopressin	30 ± 2	34 ± 2	33 ± 2	31 ± 2
Dobutamine	30 ± 1	37 ± 3	34 ± 1	33 ± 1
Sham-operated	27 ± 2	28 ± 2 *	29 ± 1	30 ± 1
SMAFI (mL/kg/min)				
H-R controls	37.8 ± 6.4	22.2 ± 2.3	28.9 ± 3.4	21.9 ± 4.7
Vasopressin	34.1 ± 3.6	22.5 ± 2.4	34.2 ± 4.4	40.0 ± 5.6 *
Dobutamine	40.0 ± 4.9	26.7 ± 2.9	40.3 ± 7.1	41.6 ± 5.6 *
Sham-operated	34.8 ± 2.4	29.5 ± 2.1	31.5 ± 1.8	33.0 ± 3.2 *
CAFI (mL/kg/min)				
H-R controls	23.6 ± 1.6	12.4 ± 2.5	13.9 ± 2.7	13.7 ± 3.8
Vasopressin	23.2 ± 2.3	12.8 ± 0.9	14.3 ± 1.1	17.3 ± 3.8 *
Dobutamine	24.0 ± 1.6	14.0 ± 2.8	17.0 ± 2.3	20.5 ± 4.5 *
Sham-operated	24.0 ± 3.0	25.3 ± 3.9 *	26.3 ± 4.7 *	26.0 ± 4.7 *
RAFI (mL/kg/min)				
H-R controls	12.7 ± 4.2	5.0 ± 3.4	7.5 ± 2.9	6.5 ± 3.2
Vasopressin	9.6 ± 0.7	4.3 ± 2.0	8.7 ± 2.7	10.5 ± 3.1
Dobutamine	8.8 ± 0.8	4.1 ± 1.8	5.5 ± 0.9	6.7 ± 1.4
Sham-operated	8.9 ± 1.7	8.0 ± 1.6	7.8 ± 1.5	6.0 ± 1.9

H-R, hypoxia-reoxygenation
MAP, mean arterial pressure
HR, heart rate
PAP, pulmonary artery pressure
SMAFI, superior mesenteric flow index
CAFI, carotid artery flow index
RAFI, renal artery flow index
* $p<0.05$ vs. H-R controls

Table 6.3: Biochemical markers of myocardial and intestinal oxidative stress and injury

	H-R Controls	Vasopressin	Dobutamine	Sham-operated
Plasma troponin-I (ng/mL)	1.4 ± 0.4	0.6 ± 0.1 *	0.5 ± 0.1 *	0.4 ± 0.2 *
Left ventricular LPO (nmol/mg)	24.2 ± 2.4	25.4 ± 1.4	20.1 ± 3.5	15.3 ± 2.8
Left ventricular GSSG/GSH ratio	0.07 ± 0.008	0.06 ± 0.004	0.06 ± 0.001	0.05 ± 0.002
Left ventricular lactate (μM/mg)	2.8 ± 0.3	1.6 ± 0.2 *	2.0 ± 0.2 *	1.7 ± 0.3 *
Intestinal LPO (nmol/mg)	32.8 ± 2.3	21.5 ± 3.6 *	25.0 ± 1.8 *	25.2 ± 2.1
Intestinal GSSG/GSH ratio	0.30 ± 0.03	0.15 ± 0.03 *	0.16 ± 0.02 *	0.16 ± 0.02 *
Intestinal lactate (μM/mg)	0.69 ± 0.15	0.39 ± 0.08	0.40 ± 0.10	0.29 ± 0.10 *
Plasma I-FABP (pg/mL)	1809 ± 363	1031 ± 243	1019 ± 301	327 ± 136 *

LPO, lipid hydroperoxydes
GSSG, glutathione disulfide
GSH, glutathione
I-FABP, intestinal fatty acid binding protein
* $p<0.05$ vs H-R Controls (ANOVA)

FIGURE LEGENDS

Figure 6.1: Cardiac index during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 6.2: Superior mesenteric artery flow index during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 6.3: Systemic oxygen delivery during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 6.4 Superior mesenteric artery oxygen delivery during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 6.1

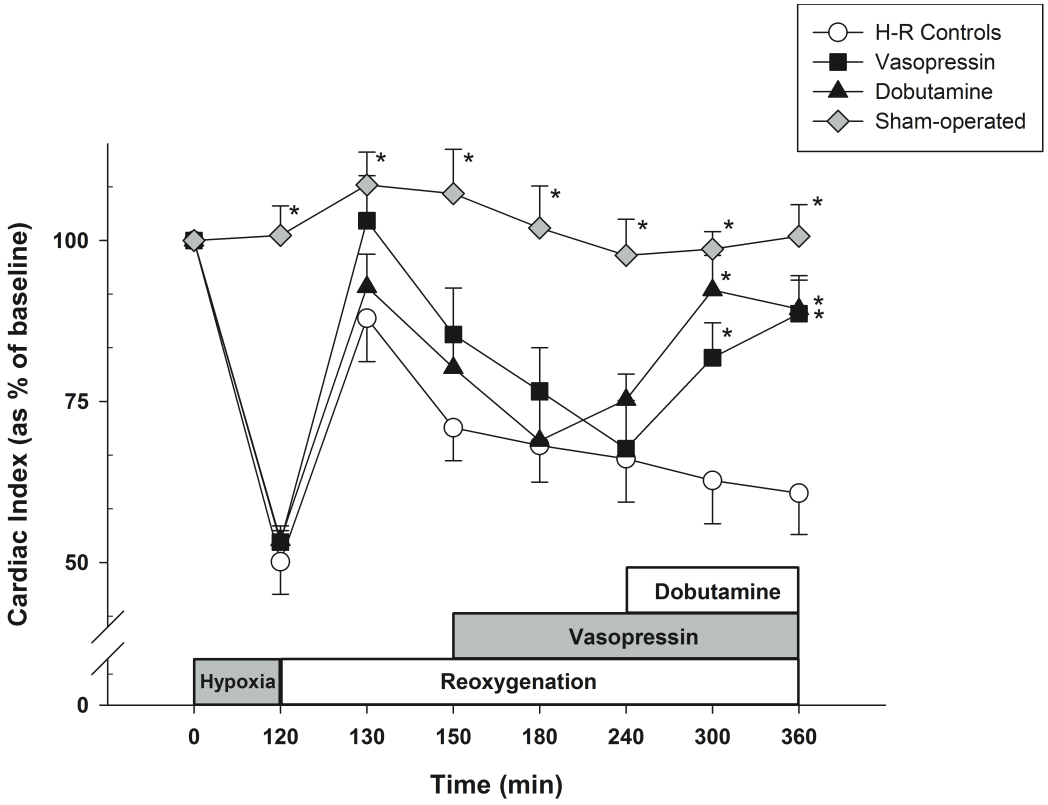

H-R Controls
Vasopressin
Dobutamine
Sham-operated
Cardiac Index (as % of baseline)
100
75
50
0
Dobutamine
Vasopressin
Hypoxia
Reoxygenation
0
120
130
150
180
240
300
360
Time (min)

Figure 6.2

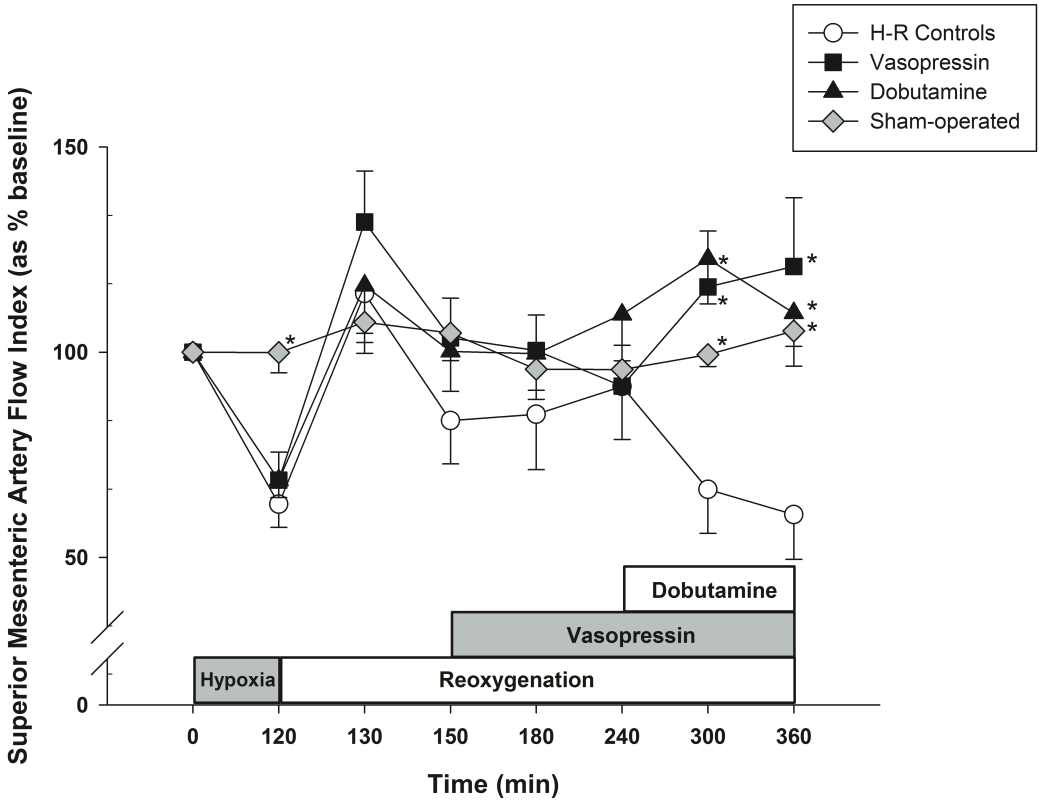

H-R Controls
Vasopressin
Dobutamine
Sham-operated
Superior Mesenteric Artery Flow Index (as % baseline)
150
100
50
0
Dobutamine
Vasopressin
Hypoxia
Reoxygenation
0
120
130
150
180
240
300
360
Time (min)

Figure 6.3

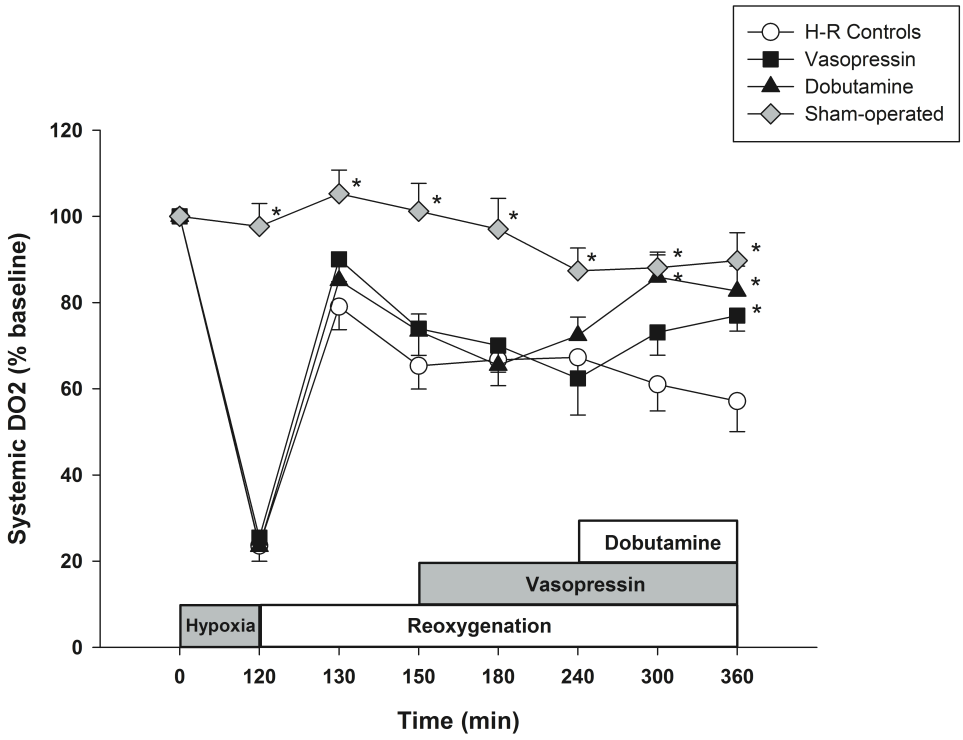
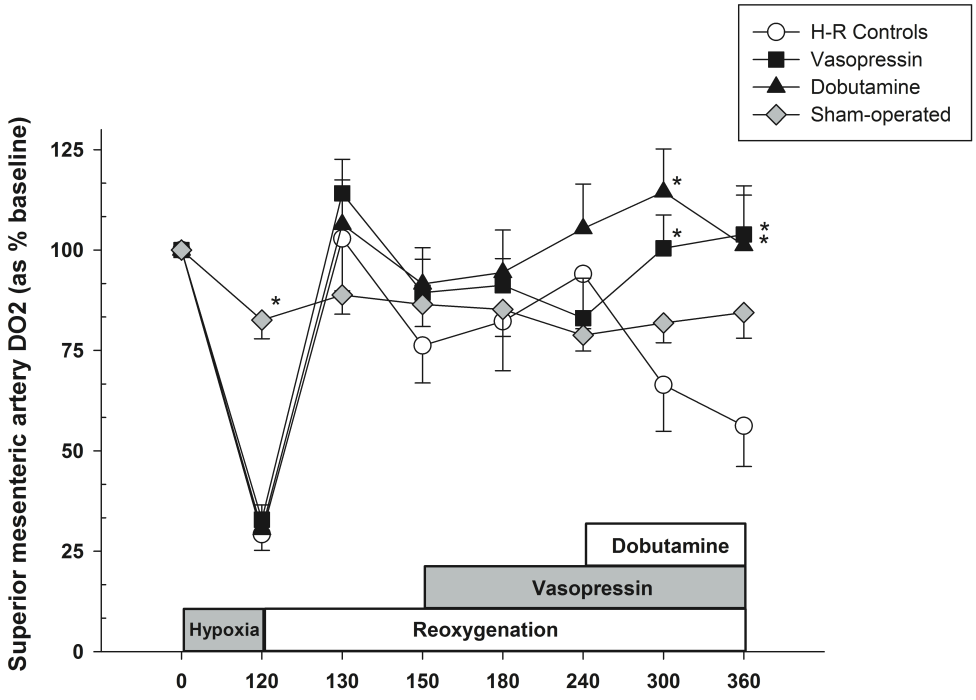


Figure 6.4



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Chapter 7: The Combined Use Of Vasopressin And Dobutamine In A Swine Model Of Hypoxia-Reoxygenation

1. INTRODUCTION

Perinatal asphyxia is a major worldwide health problem and is the cause of approximately one million neonatal deaths annually.[1] Along with hypoxic-ischemic encephalopathy, this condition can cause significant cardiac dysfunction and may lead to cardiogenic shock.[2] In fact, the degree of cardiac dysfunction is correlated with the severity of encephalopathy, suggesting that improving the cardiac output could potentially attenuate the severity of the neurological injury.[3]

Vasopressin is an endogenous hormone that is essential for cardiovascular homeostasis.[4] In the adult population, vasopressin is a commonly used vasoactive agent that has been proven to be beneficial in vasodilatory shock.[5] In the pediatric and neonatal populations, vasopressin is used more sparingly to treat shock, as studies to support its use are lacking.[2,6] Additionally, there are some concerns over its use that include potentially decreasing the cardiac index as well as regional blood flow, which have been reported in some studies.[3,7,8] Nevertheless, the potential for benefit in pediatric and neonatal populations exists, but further trials are needed to delineate potential indications and specific dosing.

Dobutamine is a synthetic sympathomimetic that acts upon β_1, β_2 and α_1 adrenoreceptors with inotropic, chronotropic and systemic vasodilatory

effects.[4,9] It is used in adult, pediatric and neonatal populations to improve cardiac function.[10-12] Its combined use with vasopressin has not been extensively studied, although there is a theoretical benefit to using both of these agents simultaneously. As vasopressin has been associated with cardiac depression in some trials, dobutamine could be used to counteract this, as has been shown in an ovine model of endotoxic shock.[13] Additionally, it has been shown that, in a porcine model of endotoxic shock, their combined used improved splanchnic flow and decreased mesenteric injury.[14] In turn, vasopressin is potentially beneficial in reversing the vasodilatation caused by dobutamine. Additionally, at low-doses, vasopressin has a potential inotropic effect,[15] which may act synergistically with dobutamine.

In a dose-response study of vasopressin use in a neonatal piglet model of hypoxia-reoxygenation, vasopressin was shown to improve myocardial tissue lactate levels, suggesting improved myocardial oxygen transport balance, without negatively affecting regional blood flow.[10] We therefore postulated that the combined use of vasopressin and dobutamine would improve myocardial function with a synergistic effect in the above model, without a negative impact to mesenteric, carotid and renal blood flows.

2. MATERIALS AND METHODS

Our protocol, in terms of the instrumentation, monitoring and hypoxia-reoxygenation and drug dosing and administration, was identical to what was described in chapter 6. Please refer to that chapter for further details.

An important difference, is that we had an additional group who received both low dose vasopressin (0.01 units/kg/h) 30 minutes and dobutamine (20mcg/kg/min) two hours following reoxygenation respectively. All piglets were therefore block randomized into one of five groups: sham-operated non-asphyxiated controls (n=5), hypoxia-reoxygenation controls (n=8), vasopressin-treated animals (n=8), dobutamine-treated animals (n=8) and vasopressin-dobutamine treated animals (n=8).

Histological and biochemical analysis was performed identically to what was described in the previous chapter, but we also measured vasopressin levels using an enzyme-linked immunosorbent assay (Peninsula Laboratories, #S-1356, San Carlos, CA) in the sham-operated and H-R control groups at the start and the end of the experiment to evaluate the effect of our protocol on endogenous vasopressin levels.

The statistical analysis protocol was identical to the one used in the previous chapter.

3. RESULTS

In total, there were thirty-seven newborn piglets used in our experiment (age: 3.1 ± 0.2 days) with a mean weight of 1.9 ± 0.03 kg. There were no differences in blood gases and hemodynamic variables at baseline among groups (Tables 7.1 and 7.2).

All H-R piglets had their cardiac index (CI) decrease to 51-54% of baseline ($p<0.05$ vs. sham-operated)(Figure 7.1) at the end of hypoxia. Their CI improved immediately with reoxygenation (88-100% of baseline) and then decreased thereafter for the remainder of the experiment. Following the commencement of the vasopressin infusion, the CI continued to deteriorate for 1.5h and then increased continually until the end of experiment up to 88% of baseline in the vasopressin only group. When the dobutamine infusion was started, the CI increased immediately. At 4h of reoxygenation, the CI for both the vasopressin and the dobutamine-treated groups were significantly higher than that of the H-R control group ($p<0.05$), but not different from each other or the sham-operated group. In the combination vasopressin-dobutamine group, the CI only modestly increased after the addition of dobutamine. One hour after the combination of the drugs, the CI in this group was significantly higher than H-R controls, but was not different than H-R controls by the end of the experiment. (Figure 7.1)

The heart rate of all piglets that underwent H-R was increased following hypoxia, without a significant difference among groups. After the commencement of the dobutamine infusion, there was an increase in the heart rate in both the dobutamine and the vasopressin-dobutamine groups, which was significantly higher than that of sham-operated piglets, but not other H-R groups (Figure 7.2).

All piglets that were subjected to H-R became severely hypotensive following hypoxia, with their MAP ranging from 30 to 32 mmHg, which recovered upon reoxygenation. The MAP then progressively decreased without difference between groups by the end of experiment (Figure 7.3). The pulmonary arterial pressure increased significantly during hypoxia and then slowly normalized to baseline by the end of experiment, without significant differences among H-R groups (Figure 7.4).

The plasma troponin-I was lower in the vasopressin and dobutamine-treated H-R groups but not in the combination vasopressin-dobutamine group as compared to H-R controls (Figure 7.5). The tissue left ventricular lactate levels were not significantly different among groups who underwent H-R (Figure 7.6). As for the markers of oxidative stress, the left ventricular myocardial LPO levels and the GSSG/GSH ratio were not significantly different among all H-R groups (Table 7.3).

The superior mesenteric arterial flow index (SMAFI) decreased significantly with hypoxia and immediately normalized with room air resuscitation (Figure 7.7). SMAFI gradually deteriorated during 4h of reoxygenation in H-R controls, but was improved in the vasopressin, dobutamine and vasopressin-dobutamine treated groups (vs. H-R control group, $p<0.05$) to levels not statistically different than the sham-operated group (Figure 7.7). The carotid arterial flow index (CAFI) was lower in all H-R groups as compared to the sham-operated group, and by the end of the experiment, there was improved flow in the vasopressin and dobutamine-treated groups as compared to H-R controls, but not in the vasopressin-dobutamine group (Figure 7.8). There was no significant difference in renal artery flow index (RAFI) among groups (Figure 7.9).

There were significantly lower intestinal GSSG/GSH ratio and LPO levels in the vasopressin and dobutamine-treated groups compared to H-R controls, but not in the vasopressin-dobutamine group (Table 7.3). Intestinal tissue lactate and plasma intestinal fatty acid binding protein levels showed a trend toward being lower in the vasopressin and dobutamine and vasopressin-dobutamine treated groups than those of H-R controls, but these did not reach statistical significance (Table 7.3).

Plasma vasopressin levels were measured in the H-R control and sham-operated groups at baseline, 2h of hypoxia and 4h of reoxygenation. The plasma vasopressin levels were not significantly different between these two groups (Table 7.4).

Systemic and mesenteric oxygen delivery (DO_2) was significantly increased by the end of the experiment in both the vasopressin and the dobutamine groups as compared to H-R controls, but not in the vasopressin-dobutamine group (Figure 7.11 and 7.12). Systemic oxygen consumption (VO_2) was similar in all piglets that underwent H-R (Figure 7A). The systemic vascular resistance index was similar in all treatment groups as compared to sham-operated piglets (Figure 7.13).

There was not a significant difference regarding the histological features of H-R injury in the left ventricular myocardial and intestinal tissues based on the Rose Criteria and Parks Classification, respectively (data not shown).

4. DISCUSSION

To our knowledge, we are the first to find an improvement in cardiac function with the use of low-dose vasopressin in a model of H-R. While vasopressin and dobutamine both improved systemic and regional hemodynamics, there was not an additional improvement seen with their

combined use. This therefore shows that in this model and at the doses used, vasopressin and dobutamine do not act in an additive or in a synergistic fashion. It is unclear as to why this is the case, although it may be related to the fact that both of these medications increase inotropy by elevating myocardial cytosolic calcium. These intracellular concentrations may have therefore already been maximally increased, thus negating the benefit.

While their combined use has not been studied elsewhere in an effort to synergistically improve cardiac function, they have been studied in combination before with a goal of combining the predominant vasoactive properties of vasopressin with the positive inotropic effects of dobutamine. As they are readily used together in certain clinical conditions, finding the effect of their combined use is important. An important example where they are used in conjunction is in adult septic shock, where both of these medications are included in the treatment algorithm.[5,16]

This combination was studied in a randomized controlled trial where terlipressin, a long-acting vasopressin analog, was used with and without dobutamine in norepinephrine-resistant shock. The dose of terlipressin used was a single 1 mg dose, while the dose of dobutamine was titrated to the effect of reversing the anticipated decrease in $S_{CV}O_2$ associated with

terlipressin. The average dose of dobutamine used was 20mcg/kg/min. They found that terlipressin increased the MAP and reduced norepinephrine requirements but decreased the CI. The addition of dobutamine in this trial successfully increased the CI, the $S_{CV}O_2$ and the oxygen delivery index.[6,17]

Holt et al were not able to replicate these findings in a porcine model of induced severe endotoxic shock. They studied both medications alone and in combination, with predetermined doses of 0.04 units/kg for vasopressin and 10mcg/kg/min for dobutamine. While vasopressin was successful at improving the MAP, dobutamine did not improve cardiac output or mesenteric flow regardless of whether vasopressin was also given.[7,8,18] It is worth underlining that the dose of dobutamine was half of what was used in the previous trial, suggesting that the lack of improvement may have been dose related.

It is difficult to come to any conclusions based on our study and the two studies above, as different models and doses of medications were used. Therefore, it would likely be beneficial to proceed with further study examining the safety and efficacy of this combination of medications, especially at multiple different doses.

The limitations of this trial include the fact that a neonatal swine model does not fully capture the complexity of perinatal asphyxia, given that the unique circumstances that occur at birth are not present in this model. However, no animal model is perfect for perinatal asphyxia, and the swine model does confer some advantages such as size and ease of instrumentation.[9,19] Also, substituting a piglet for a human infant is not without flaw, as there has been significant inter-species variability reported in vasopressin receptors, with only 80% homology between human and rat V_1 receptors.[11,12,20,21] However, the swine's cardiovascular system and function is very similar to the human's and the histological appearance of the myocardium is nearly identical.[8,13,22,23] As such, it is an appropriate cardiovascular large animal model. Additionally, as our study duration was set at six hours, we can only comment on acute events. However the acute resuscitation stage is very important and influences long-term prognosis. Additionally, the dose of dobutamine of 20 mcg/kg/min was chosen based on our previous experience in this model.[14,24,25] The dose seems to be higher than the usual initial clinical dose, which ranges from 5-20 mcg/kg/min in neonates with cardiogenic shock. Finally, when studying the combined use of vasopressin and dobutamine, we only studied one dose of each and at separate time points. As such, we can only conclude that when used at these times and at these doses, their combined use does not appear to be beneficial.

5. CONCLUSION

In a neonatal swine model of H-R, the combined use of low-dose vasopressin (0.01 units/kg/min) and dobutamine (20 mcg/kg/min) does not confer an additional benefit to cardiovascular hemodynamics as compared to either medication used alone.

6. TABLES AND FIGURES

Table 7.1 – Arterial Blood Gases and Plasma Lactate Concentrations During Hypoxia and Reoxygenation

	Baseline	End of Hypoxia	30 Minutes Reoxygenation	4 Hours Reoxygenation
pH				
H-R controls	7.41 ± 0.01	7.06 ± 0.05	7.18 ± 0.05	7.32 ± 0.04
Vasopressin	7.40 ± 0.01	7.06 ± 0.02	7.21 ± 0.03	7.34 ± 0.03
Dobutamine	7.41 ± 0.02	7.06 ± 0.03	7.20 ± 0.03	7.36 ± 0.02
Vaso-Dob	7.38 ± 0.01	7.08 ± 0.03	7.20 ± 0.02	7.33 ± 0.03
Sham-operated	7.41 ± 0.03	7.38 ± 0.04	7.39 ± 0.02	7.35 ± 0.02
PaO_2 (torr)				
H-R controls	72 ± 4	46 ± 6	75 ± 4	68 ± 4
Vasopressin	70 ± 3	43 ± 1	74 ± 7	63 ± 4
Dobutamine	69 ± 2	37 ± 2	76 ± 3	66 ± 2
Vaso-Dob	63 ± 1	41 ± 2	75 ± 2	75 ± 3
Sham-operated	69 ± 2	65 ± 3	67 ± 3	65 ± 3
$PaCO_2$ (torr)				
H-R controls	40 ± 2	38 ± 2	37 ± 1	37 ± 1
Vasopressin	41 ± 1	37 ± 1	35 ± 1	44 ± 1
Dobutamine	40 ± 1	38 ± 2	35 ± 1	42 ± 1
Vaso-Dob	42 ± 1	37 ± 1	37 ± 1	39 ± 1
Sham-operated	42 ± 4	40 ± 2	39 ± 1	42 ± 1
HCO_3 (mM)				
H-R controls	25 ± 1	10 ± 1	14 ± 1	21 ± 2
Vasopressin	25 ± 1	10 ± 1	14 ± 1	22 ± 1
Dobutamine	25 ± 2	10 ± 1	14 ± 1	23 ± 1
Vaso-Dob	24 ± 1	10 ± 1	15 ± 1	20 ± 1
Sham-operated	24 ± 1	23 ± 1	24 ± 1	22 ± 1
Lactate (mM)				
H-R controls	2.7 ± 0.3	11.2 ± 1.0	9.3 ± 1.2	3.0 ± 0.9
Vasopressin	2.6 ± 0.2	11.5 ± 0.7	8.8 ± 0.5	2.3 ± 0.5
Dobutamine	3.0 ± 0.3	10.2 ± 0.8	8.3 ± 0.5	2.2 ± 0.3
Vaso-Dob	2.6 ± 0.3	11.4 ± 1.0	8.5 ± 0.6	3.4 ± 0.8
Sham-operated	2.4 ± 0.3	1.9 ± 0.2	1.9 ± 0.1	1.7 ± 0.3

H-R, hypoxia-reoxygenation

Table 7.2: Hemodynamic Data at Normoxic Baseline

	H-R controls	Vasopressin	Dobutamine	Vaso-Dob	Sham-operated
Cardiac Index (mL/kg/min)	194 ± 14	190 ± 18	205 ± 18	202 ± 16	160 ± 11
MAP (mmHg)	73 ± 3	76 ± 3	74 ± 3	71 ± 35	78 ± 6
HR (beats/min)	187 ± 11	200 ± 14	198 ± 10	199 ± 8	199 ± 7
PAP (mmHg)	30 ± 2	30 ± 2	30 ± 1	30 ± 2	27 ± 2
SMAFI (mL/kg/min)	37.8 ± 6.4	34.1 ± 3.6	40.0 ± 4.9	40.9 ± 4.6	34.8 ± 2.4
CAFI (mL/kg/min)	23.6 ± 1.6	23.2 ± 2.3	24.0 ± 1.6	24.1 ± 2.4	24.0 ± 3.0
RAFI (mL/kg/min)	12.7 ± 4.2	9.6 ± 0.7	8.8 ± 0.8	8.7 ± 1.6	8.9 ± 1.7

MAP, mean arterial pressure
HR, heart rate
PAP, pulmonary artery pressure
SMAFI, superior mesenteric flow index
CAFI, carotid artery flow index
RAFI, renal artery flow index

Table 7.3: Biochemical markers of intestinal oxidative stress and injury

	H-R Controls	Vasopressin	Dobutamine	Vaso-Dob	Sham-operated
Left ventricular LPO (μM/mg)	24.2 ± 2.4	25.4 ± 1.5	20.1 ± 3.5	20.4 ± 3.2	15.3 ± 2.8
Left ventricular GSSG/GSH ratio	0.07 ± 0.008	0.06 ± 0.005	0.06 ± 0.001	0.05 ± 0.004	0.05 ± 0.002
Intestinal LPO (nmol/mg)	32.8 ± 2.3	21.5 ± 3.6 *	25.0 ± 1.8 *	28.9 ± 1.6	25.2 ± 2.1
Intestinal GSSG/GSH ratio	0.30 ± 0.03	0.15 ± 0.03 *	0.16 ± 0.02 *	0.32 ± 0.06	0.16 ± 0.02 *
Intestinal lactate (μM/mg)	0.69 ± 0.15	0.39 ± 0.08	0.40 ± 0.10	0.51 ± 0.13	0.29 ± 0.10 *
Plasma intestinal FABP (pg/mL)	1809 ± 363	1031 ± 243	1019 ± 301	992 ± 281	327 ± 136 *

LPO, lipid hydroperoxydes
GSSG, glutathione disulfide
GSH, glutathione
FABP, fatty acid binding protein
* $p<0.05$ vs H-R Controls (ANOVA)

Table 7.4: Plasma vasopressin levels

	H-R control	Sham-operated
Pre-hypoxia level (ng/mL)	17.0 ± 5.3	16.7 ± 6.5
Post-hypoxia level (ng/mL)	17.9 ± 6.4	6.6 ± 1.6
End of experiment (ng/mL)	13.6 ± 3.6	8.5 ± 1.4

FIGURE LEGENDS

Figure 7.1: Cardiac index during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.2: Heart rate during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.3: Mean arterial pressure during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R.

Figure 7.4 Pulmonary artery pressure during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R.

Figure 7.5: Plasma troponin-I level at the end of the experiment. Hypoxia-reoxygenation (H-R) control piglets (n=8) received no

vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.6: Left ventricular tissue lactate level at the end of the experiment. Hypoxia-reoxygenation (H-R) control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.7: Superior mesenteric artery flow index during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.8: Common carotid artery flow index during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.9: Renal artery flow index during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.10: Systemic oxygen delivery during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.11: Mesenteric oxygen delivery during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.12: Systemic oxygen consumption during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.13: Vascular resistance index during hypoxia and reoxygenation (H-R). H-R control piglets (n=8) received no vasopressin or dobutamine. Sham-operated piglets (n=5) underwent no H-R. *p<0.05 vs. H-R controls (ANOVA).

Figure 7.1

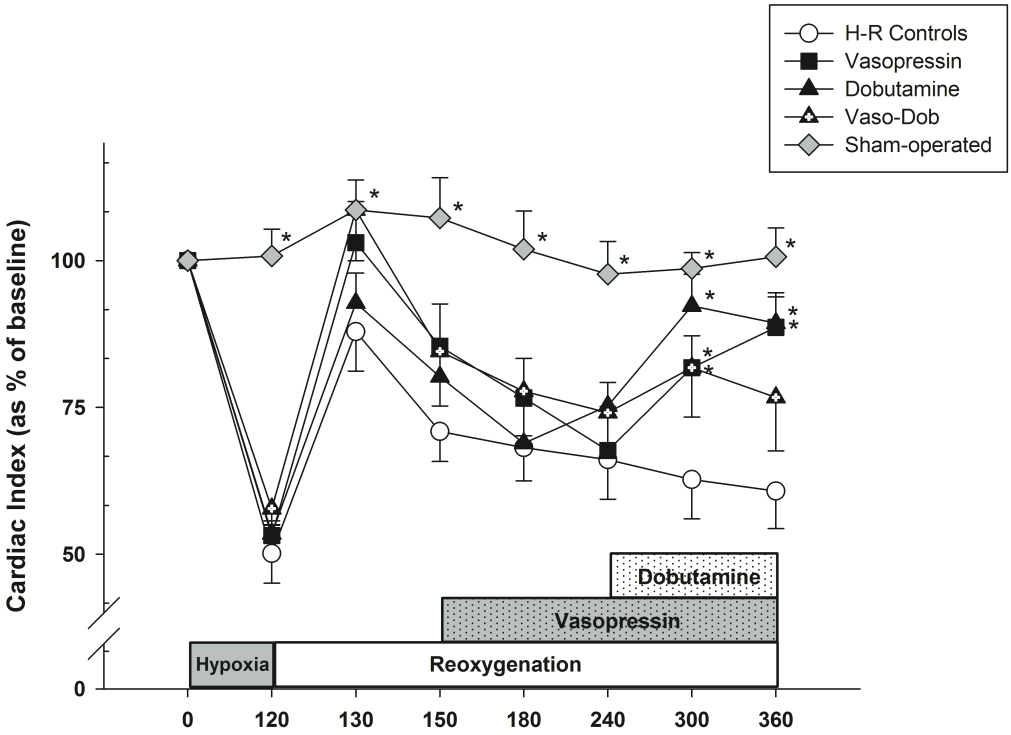
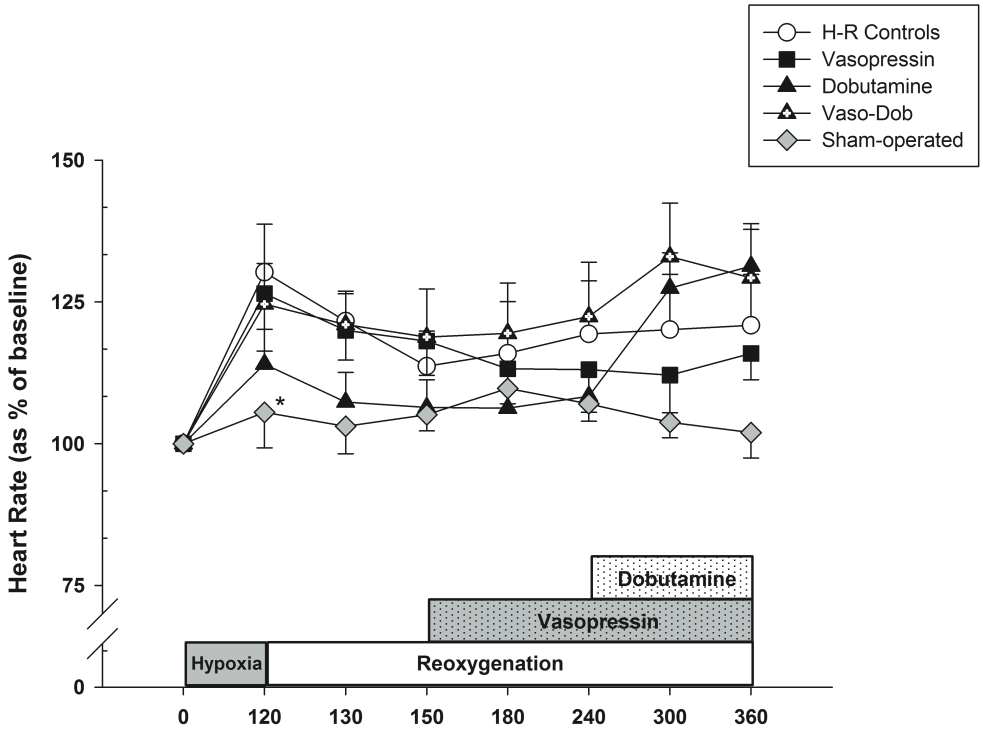


Figure 7.2



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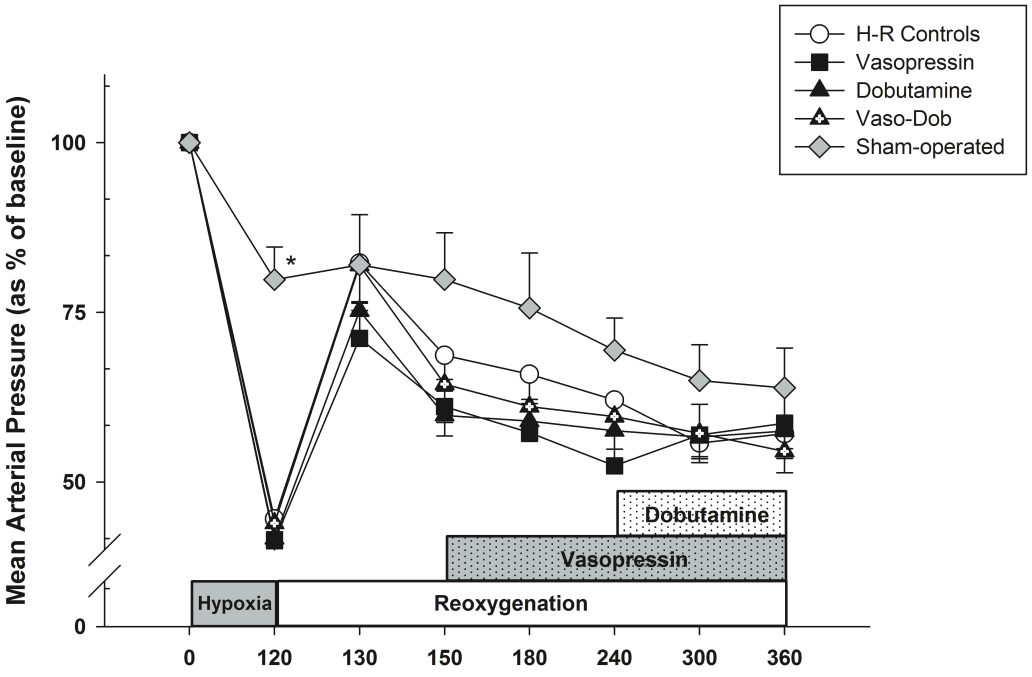

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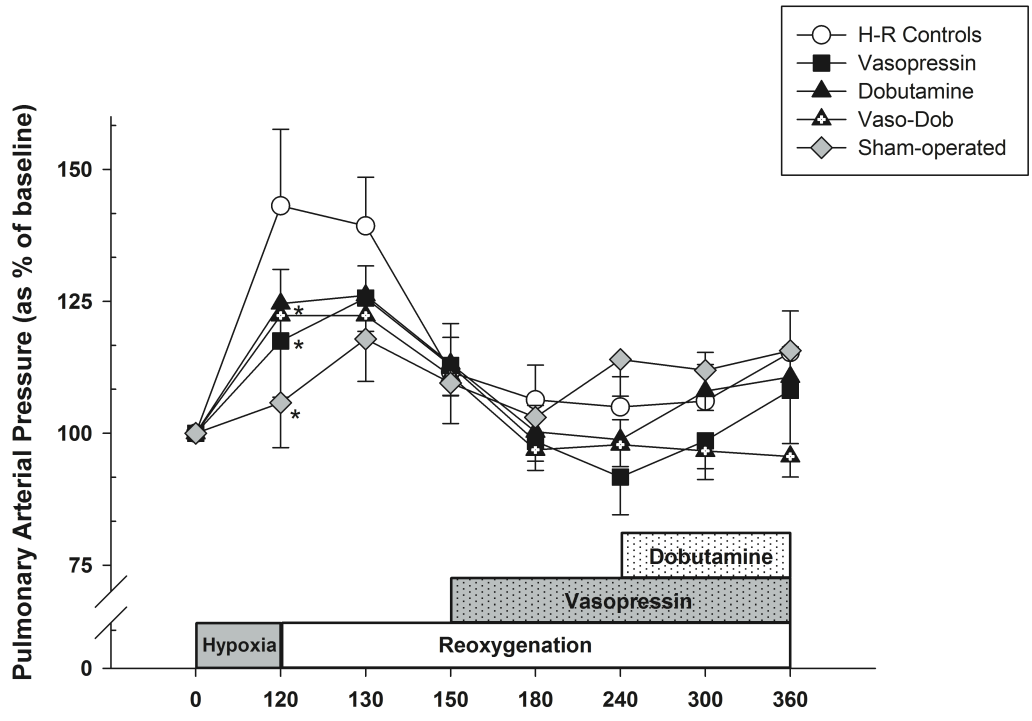

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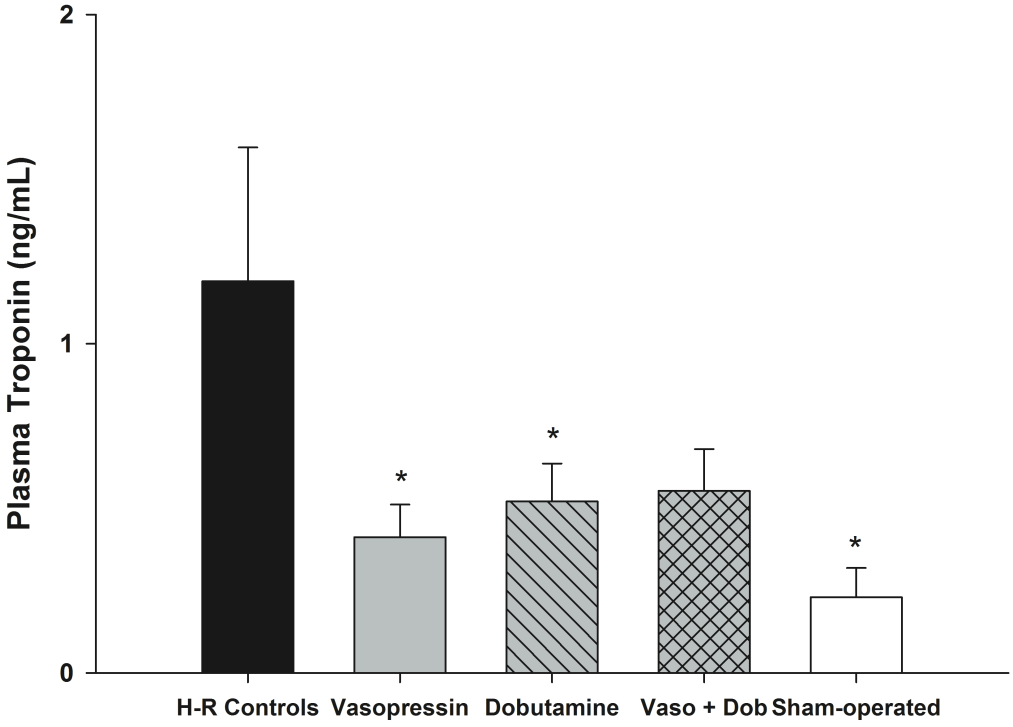

Plasma Troponin (ng/mL)
2
1
0
*
*
*
H-R Controls
Vasopressin
Dobutamine
Vaso + Dob
Sham-operated

Figure 7.6

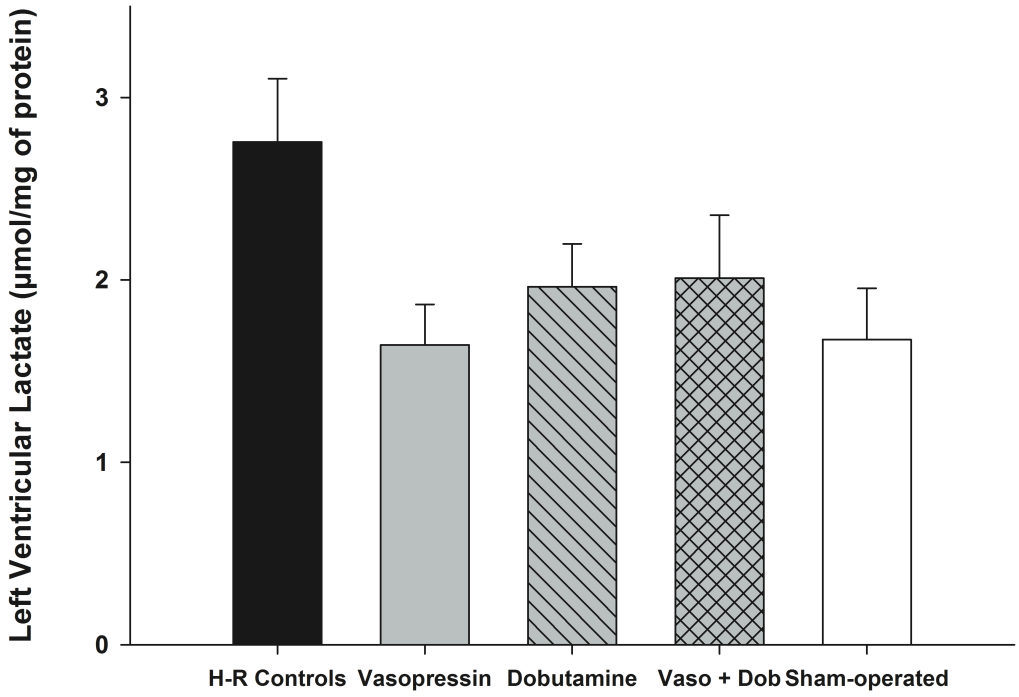
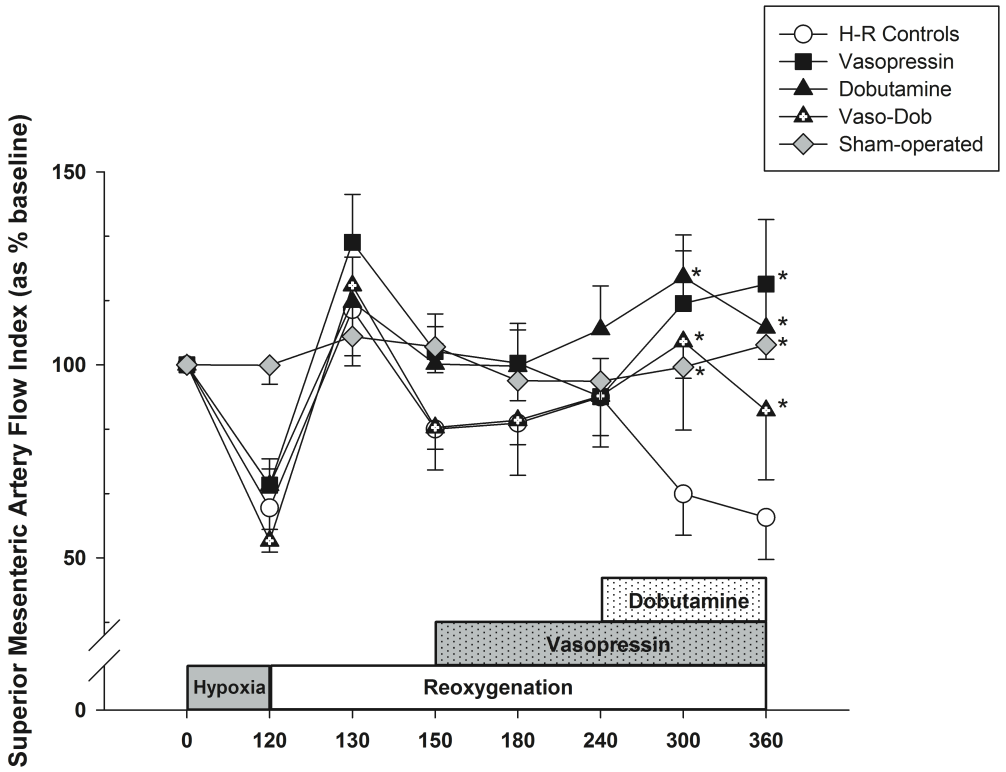

Left Ventricular Lactate (µmol/mg of protein)
3
2
1
0
H-R Controls
Vasopressin
Dobutamine
Vaso + Dob
Sham-operated

Figure 7.7



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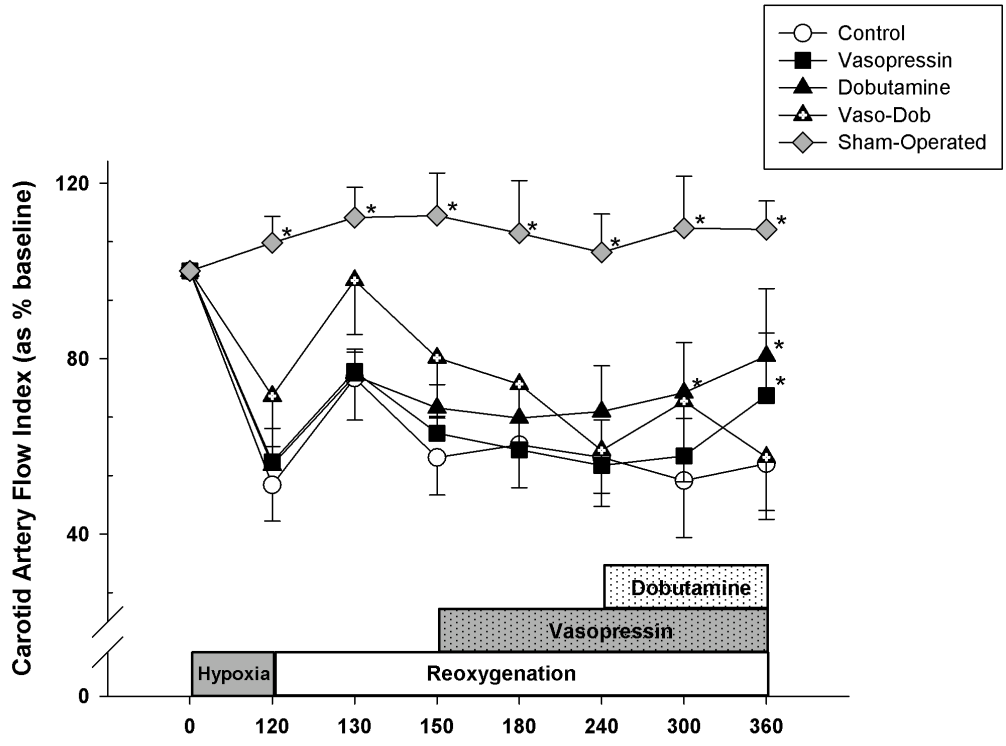

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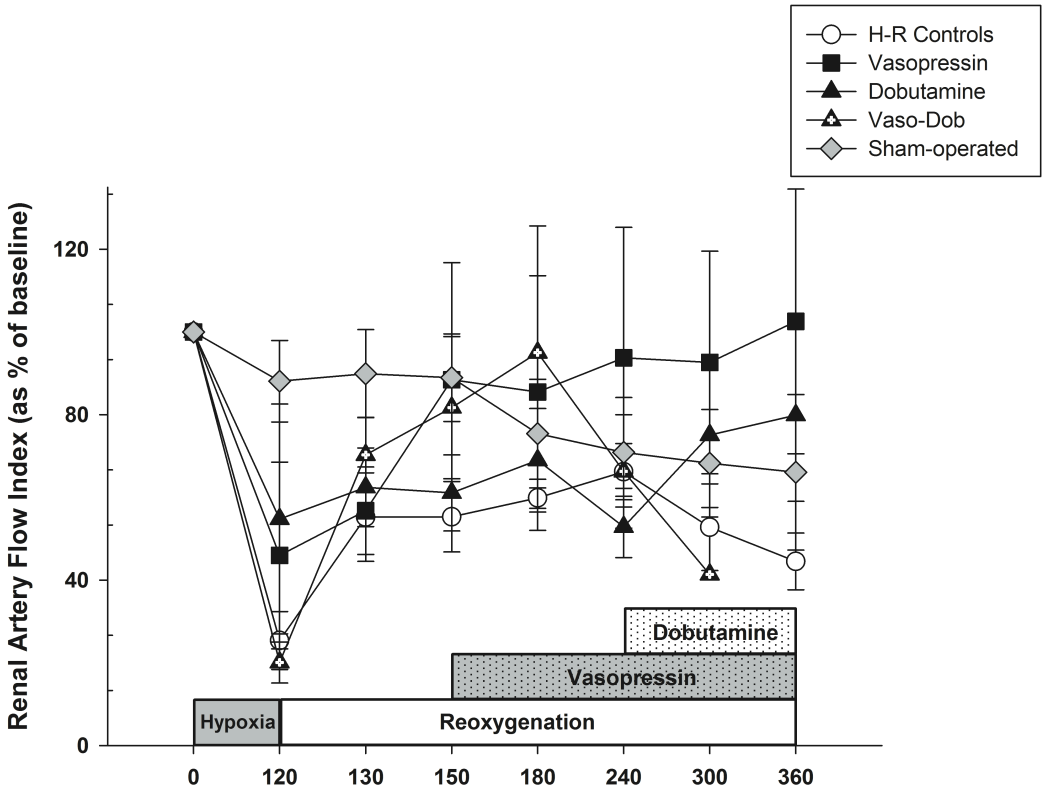

H-R Controls
Vasopressin
Dobutamine
Vaso-Dob
Sham-operated
Renal Artery Flow Index (as % of baseline)
120
80
40
0
Dobutamine
Vasopressin
Hypoxia
Reoxygenation
0
120
130
150
180
240
300
360

Figure 7.10

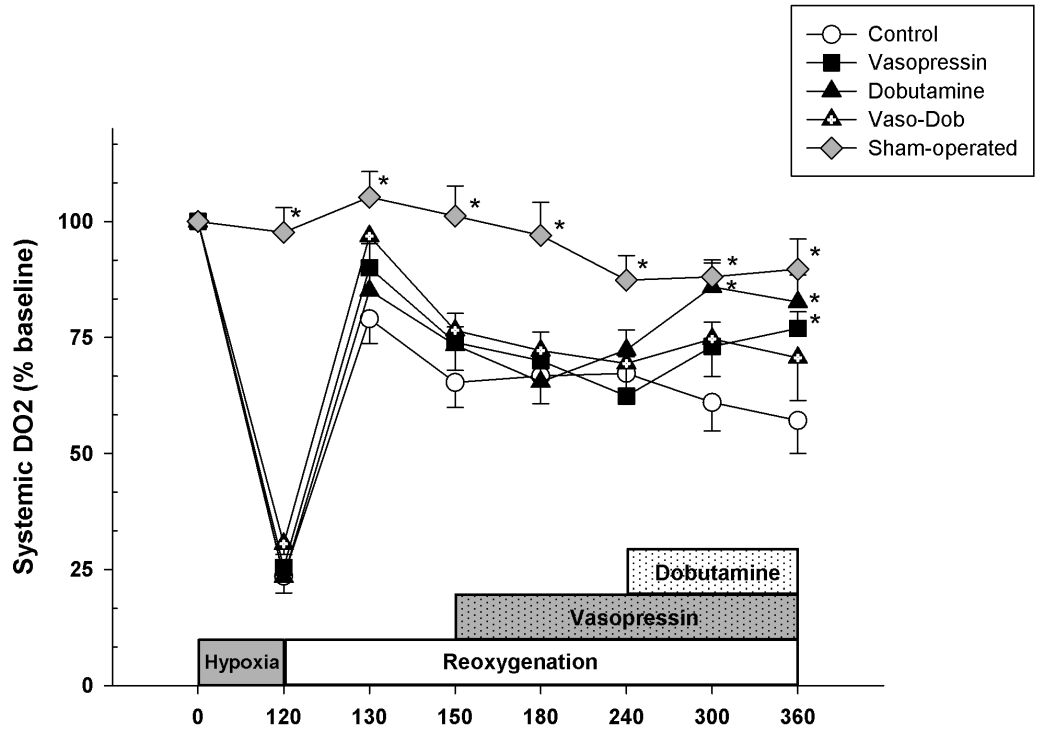
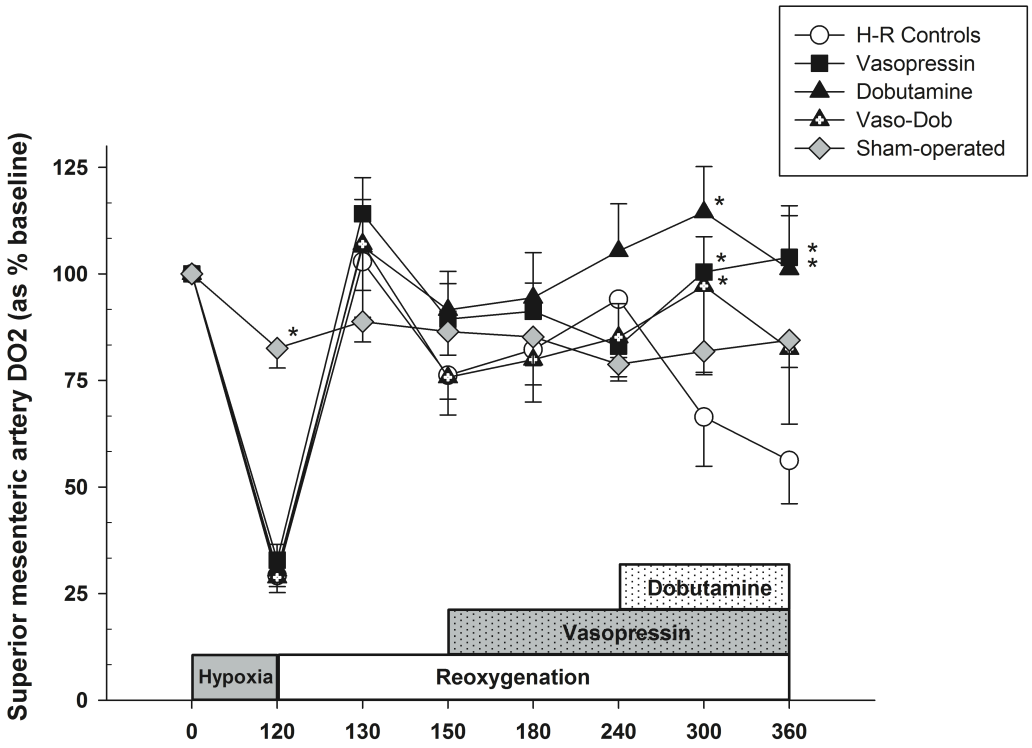


Figure 7.11



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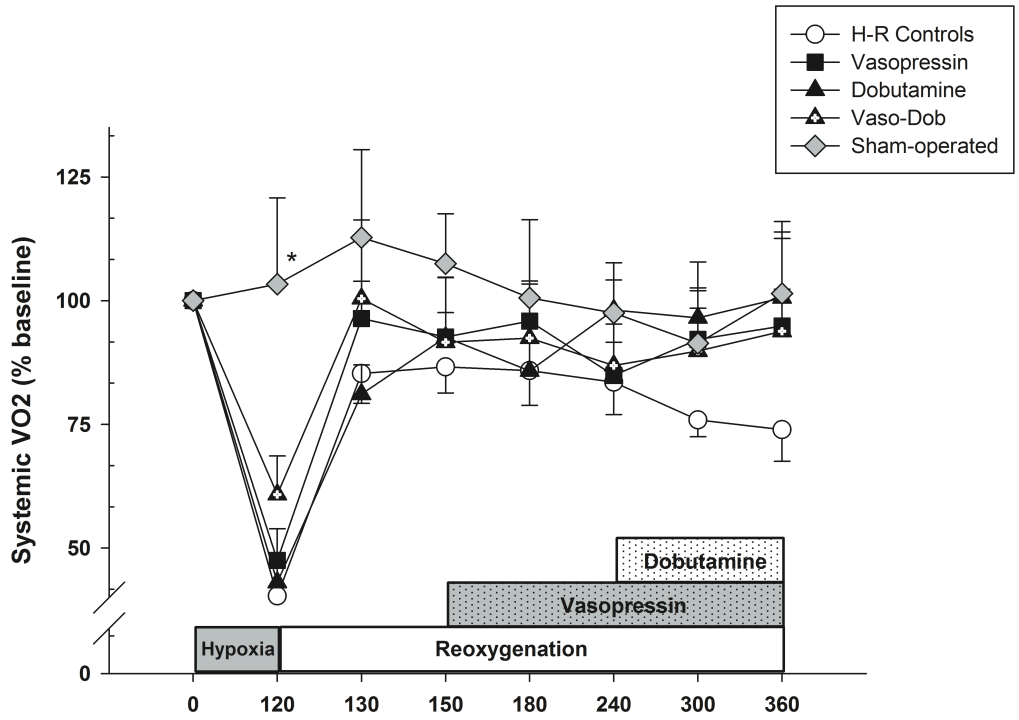
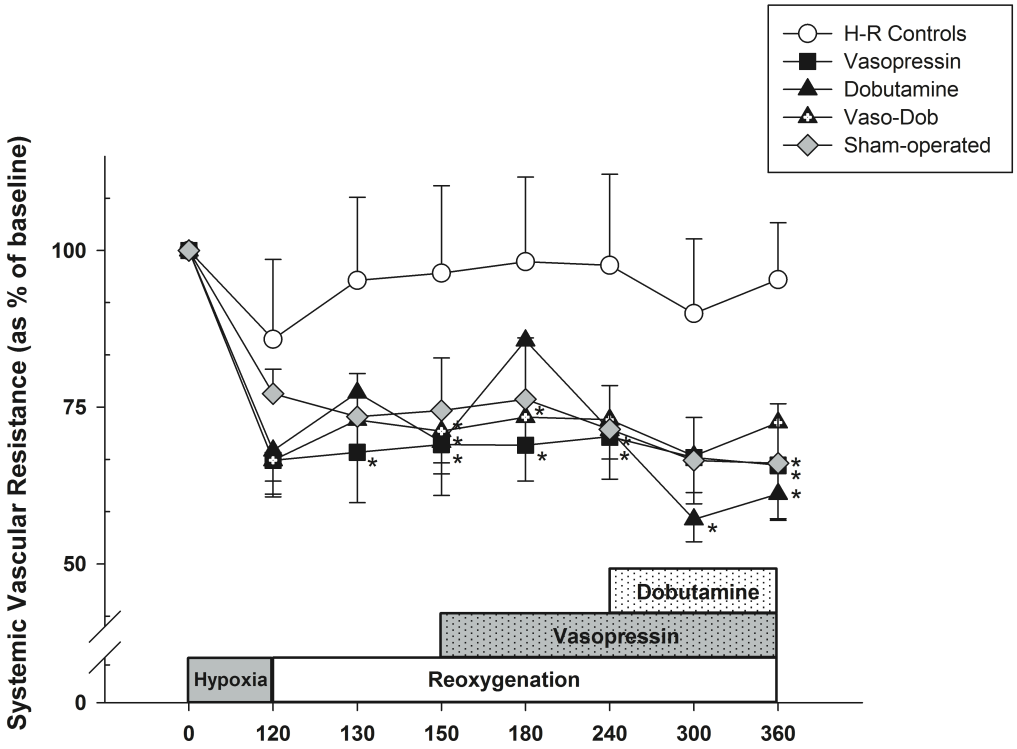


Figure 7.13



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Chapter 8: Conclusions And Discussion

The preceding chapters have provided an in depth analysis of perinatal asphyxia as a significant global health problem and as an important cause of morbidity and mortality in the neonate. While perinatal asphyxia causes significant multi-organ dysfunction, our study focused mainly on the cardiac effects. The current treatment of perinatal asphyxia revolves around supportive care, in addition to specific therapies including therapeutic hypothermia and the judicious use of supplementary oxygen. Myocardial dysfunction is correlated with worsened outcomes yet relatively few therapies exist to treat it, although some novel therapies are on the horizon.

Most of the current knowledge of perinatal asphyxia was initially acquired with the use of animal models, including hypoxia-reoxygenation in neonatal swine. While unable to fully capture the unique physiological changes that occur with birth, this model for perinatal asphyxia confers many benefits as compared to other animal models. It is especially useful when studying cardiovascular effects, as the pig's heart and circulatory system bear numerous similarities to their human counterpart.

Vasopressin is essential to cardiovascular homeostasis and is also used therapeutically for various conditions in all age groups. The influence that vasopressin has on the heart is complex and difficult to separate from its

other end-organ effects. It does appear however that in certain clinical situations, vasopressin has the potential to improve cardiac function. Further clinical studies are needed to more clearly define this role. Dobutamine is a synthetic inotrope used in the adult, pediatric and neonatal populations. It is very useful in low cardiac output states, but does have a chronotropic effect at higher doses, especially in the pediatric population. This contributes to its energy inefficiency, which is its main disadvantage.

Our research group has recently found that vasopressin might be useful in the treatment of perinatal asphyxia in a dose-response study. A hemodynamic improvement was not seen however when low-dose vasopressin was started two hours after commencing reoxygenation. This may have been because the myocardial injury was too advanced by this point, or due to the fact that the animals were only followed for two hours after the initiation of the infusion. Nevertheless, in our model, we used vasopressin for hemodynamic prophylaxis, as it was initiated prior to hemodynamic deterioration. While this is not an entirely novel approach to the use of vasopressin, it is much less common than its use to treat preexisting shock. Additionally, the use of vasopressin without expected vasoconstrictive effect is novel and requires further study for potential clinical applicability.

Our main study findings are that of an improved cardiac output, mesenteric blood flow and oxygen delivery with the use of low-dose vasopressin in asphyxiated neonatal piglets. These positive findings seem to justify further studies for the use of vasopressin in this timeframe and in this context, as a very low dose was able to achieve results similar to dobutamine, a potent sympathomimetic inotrope. This is an important finding, as the ability to use low doses minimizes adverse effects. The improvement was not seen immediately however, which leads to questions regarding the mechanism of action. We were able to show that our results were not due to the replacement of pathologically low endogenous levels. Being that vasopressin has such complex and varied actions, this mechanism may be difficult to fully elucidate in subsequent trials.

We were unable to confirm our hemodynamic findings with signs of injury on histopathology, as there was not a difference between treatment and hypoxia-reoxygenation control groups. Likely, this is a function of the acuity of our model that may not have allowed us to observe gross histological changes within the relatively short study period. Our hemodynamic findings were confirmed however with positive biochemical results. These included decreased plasma cardiac troponin-I and myocardial tissue lactate levels as well as evidence of decreased intestinal oxidative stress. These findings are significant, as they serve as proof of

decreased cardiac and intestinal injury, suggesting that histologic evidence of injury would be lessened over time.

In our model, when vasopressin and dobutamine were used concurrently, there was not an added benefit in spite of both medications being beneficial when used individually. It is unclear as to why this is the case, although it must be stressed that this may only be the case in this model and at the studied doses. Concurrent use of both of these medications has been shown to be beneficial in the past, although the evidence is not entirely conclusive.

We have therefore shown that a low-dose vasopressin infusion has the ability to improve systemic hemodynamics and regional blood flow and oxygen delivery in a neonatal swine model of hypoxia-reoxygenation. While further study into mechanisms is warranted, this has important clinical implications and may help clinicians in the near future in the treatment of perinatal asphyxia.