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HISTAMINE EFFECTS ON THE RAT'S RIGHT AND LEFT ATRIA

by

DAVORKA KRIZAJ-KAPLJIC

A THESIS

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Supervisor

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.....Simon Lee.....

E.J. Sanders

Date.....May 30.....1985.....

To my husband Drago and my two daughters, Aida and Katarina

ABSTRACT

The positive inotropic and chronotropic effects of histamine (10^{-3}M), observed on the right, right and left paced atria, were antagonized by metoprolol, verapamil and low Ca^{++} Krebs solution. Those substances, as well as low Ca^{++} Krebs solution, were used to investigate the possible mechanisms of histamine's actions, on the rat heart. Contrary to the other techniques where drugs were injected into the preparation, we used a continuous infusion technique of administering the above mentioned substances, at a constant rate and time. This allowed us to observe the longer term effects of histamine on the rat heart. It was observed by this technique, that the positive inotropic effect of histamine preceded the positive chronotropic effect on the right atrium. Once the tension reached its maximum value, this was quickly followed by an increase in the rate, reaching its peak value after about 20 minutes of histamine infusion. However, at very high rates the tension has sometimes been observed to decrease slightly. On the paced right and left atria, histamine produced a positive inotropic effect, that often increased the basal tension two fold. Metoprolol (10^{-7}M) blocked the positive inotropic and chronotropic effects on the left and right atrium. The Ca^{++} slow channel blocker, verapamil, antagonized the positive chronotropic effect of histamine on the right atrium, at a concentration of (10^{-8}M). However, the positive inotropic effect on the paced right atrium was blocked with verapamil

(10^{-8}M). The positive inotropic effect of histamine on the left atrium, was antagonized with verapamil (1.5×10^{-8}M). Adrenaline reversed the negative inotropic effect of verapamil. Lowering [Ca^{++}] to one eighth of its normal concentration, antagonized the positive inotropic effect of the histamine on the right and left atria, while the positive chronotropic effect on the right atrium was not blocked.

The results led to the speculations, that histamine could act via catecholamines on the rat heart, as previously [illegible] Laher & McNeill (1980c). The importance of Ca^{++} ions in histamine [illegible] also evident, with the positive chronotropic effect being most sensitive to verapamil. Paced right atrium requires a greater concentration of verapamil then the left atrium, thus indicating the possibility that histamine induces a greater influx of the Ca^{++} ions into its cells. The study also indicates that a decrease in the Ca^{++} ion concentration in Krebs solution, effects mostly the tension but not the rate. One could speculate that in order for histamine to stimulate the rate, it either requires less external Ca^{++} ions or it mobilizes the internal stores of Ca^{++}. Future studies with EDTA, to deplete the muscle of its internal stores of Ca^{++}, could give more light on the subject.

In conclusion, the experiments show that histamine promotes a greater influx of Ca^{++} ions into the cells to produce positive inotropic and chronotropic responses.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF NOTATIONS

hist. = histamine

metop. = metoprolol

verap. = verapamil

kr. = krebs solution

1. INTRODUCTION

From the time Dale and Laidlaw (1910) observed the effects of histamine on mammalian tissue and its main role in anaphylactic shock, there has been great interest in histamine research. In 1927, Best, Dale, Dudley, and Thorpe, isolated histamine from fresh liver and lung tissues thus establishing its presence in various tissues. In 1953 Schachter demonstrated that in sensitized rabbits, horse serum releases considerable amounts of histamine from skin, liver and to a lesser extent, from the gut. Large quantities of histamine are contained in cardiac tissue as well (Rocha e Silva, 1966). Its distribution is not uniform, since it was found by Giotti *et al.* (1965) that guinea-pig heart had the highest content of histamine and also highest concentration of mast cells in the right atrium and lowest in the left ventricle.

The release of histamine into the blood from mast cells can be affected by drugs like pethidine and atropine (Schachter, 1952), or by injury (Lewis, 1924). Histamine causes systemic reactions, observed in anaphylactic shock, in various animals. These reactions have been described by various investigators (Dale & Laidlaw, 1910; Lewis 1924; Schachter 1953; Rocha e Silva 1966). Even from early times it has been observed that histamine can produce positive chronotropic and inotropic effects on the heart. The first such report came from Dale and Laidlaw (1910) who observed the effect of histamine on the rabbit and cat cardiac

tissue. This positive chronotropic and inotropic effect was described many times afterwards in various species such as cat, rabbit and guinea-pig (Trendelenburg 1960; Mannaioni 1960; Bartlet 1963; Pöch & Kukovetz 1967; Rocha e Silva 1966; Flacke *et al*. 1967; Verma & McNeill 1977). Experiments were carried out on the whole perfused mammalian hearts (Levi 1972; Bartlet 1963; Rocha e Silva 1966; McNeill & Verma 1974; Flacke *et al*. 1967), also on the isolated atrium preparations (Trendelenburg 1960; Verma & McNeill 1977) and right ventricular muscle preparations (DeMello 1976; Verma & McNeill 1977; Eckel 1982).

In 1937 when Bovet discovered H_1 antihistamine, 2 isopropyl-5-methylphenoxyethyldiethylamine, which lessened the symptoms of anaphylactic shock in guinea-pig and histamine induced bronchospasm, the future of histamine research was established. Soon new investigations led to the discovery of the most potent antihistamine up to this day - diphenhydramine (Loew *et al*. 1946). This led to the discovery of other antihistamines.

Using those antihistamine drugs as tools to further investigate the effects of histamine, it was eventually observed by Folkow (1948), that diphenhydramine does not abolish the high dose of histamine effect on cat's limb vasculature. This led him to suggest the presence of two distinct receptors for histamine. Trendelenburg (1960) and Bartlet (1963) also observed, that positive chronotropic and inotropic effects of histamine on the heart of certain

species were not antagonized by antihistamines then available. Furthermore, beta adrenergic receptor antagonists failed to inhibit the positive chronotropic effect of histamine. (Trendelenburg 1960; Pöch and Kukovetz, 1967).

Finally in 1966, Ash and Schild presented their classical paper in which they introduced H_1 receptors. Those effects such as vasodilatation, bronchospasm and allergic reactions mediated via H_1 receptors, could be blocked by H_1 antagonists. Other effects of histamine like stimulation of the heart, uterus or gastric secretion that were not blocked by classical antihistamines were termed non-H_1 receptors.

In 1972, Black *et al.* introduced the new antihistamine, burimamide, which selectively inhibits the actions of histamine that were not antagonized by the classical antihistamines, suggesting the existence of a second receptor responsible for these actions. Burimamide was defined as a selective H_2-receptor antagonist (Black *et al.* 1972). Burimamide was found to antagonize the positive chronotropic effect of histamine in the guinea-pig heart, (Levi, Capurro & Lee 1975; McNeill & Verma 1974; Verma and McNeill 1977).

With the discovery of these H_2 receptor antagonists, the interest in the physiological role of histamine intensified. Many investigators demonstrated, on various species, the actions of histamine on the heart and the differentiation of its receptors into H_1 and H_2 types.

During the last decade, a great deal of information has been obtained, as to the distribution and properties of H_1 and H_2 histamine receptors in different species. One of the results of these studies showed that the distribution and the type of these receptors, particularly on the heart, not only depends upon the species of the animal but also upon different parts of the heart within the same species (McNeill 1981; McNeill 1984; Owen 1977). Cook (1984) pointed that even the strain differences within the single species can produce different histamine responses. It seems that of all the organs, heart is the most heterogeneous in histamine receptor type (Cook, 1984).

The following is a review of the distribution of receptors in the heart of the rabbit, cat, guinea-pig, man and the rat. The effects produced by the stimulation of these receptors with histamine will be discussed, as well as their possible mechanism of action, as discovered by different investigators.

In the *rabbit*, the positive chronotropic and inotropic response of the right atrium is not blocked by H_1 antihistamines as shown by Trendelenburg (1960). McNeill and Verma (1978) undertook an investigation on rabbit atrium and ventricular tissue for histamine H_1 and H_2 receptors. They found predominantly H_1 receptors in the rabbit heart, except in the right atrium where H_2 receptors seemed to predominate. Both H_1 and H_2 receptors are responsible for the positive chronotropic response. However rabbit ventricle

and papillary muscle contain only H_1 receptors (McNeill, 1981).

In the *kitten*, Laher and McNeill (1980) observed that the positive chronotropic effect in the right atrium is blocked by cimetidine (H_2 receptor antagonist) and by the beta adrenergic antagonist propranolol. This would indicate that histamine produces its effect via the H_2 receptor and indirectly via catecholamine release. In the kitten's left atrium, right papillary muscle and right ventricle, the histamine responses were blocked by propranolol, indicating that histamine is acting only by catecholamine release. When the kitten was reserpine pretreated, the positive inotropic response to histamine was not observed and the chronotropic effect was decreased. McNeill (1981), also concluded that the effect of histamine on cat's heart is mainly due to release of catecholamines, with a minor direct H_2 mediated positive chronotropic effect.

The *guinea pig* has been the most commonly used species for experimental purposes for decades. Its isolated heart was found to respond to histamine (Levi 1972; Rocha e Silva 1966). The positive inotropic effect of histamine on the guinea pig atrium and its sensitivity to histamine was observed by Trendelenburg (1960). He found that the effects of histamine on guinea pig atrium were not antagonized by H_1 antihistamines. Similar results were reported by McNeill and Muschek (1972), as well as by Pöch and Kukovetz (1967), suggesting the presence of two types of receptors in guinea

pig heart. McNeill and Verma (1974), and Reinhardt (1974), antagonized the positive chronotropic effect of histamine in the guinea pig left atrium with burimamide, an H_2 blocker, while the positive inotropic effect of histamine on the left atrium was blocked by the H_1 antagonist, promethazine. Levi *et al.* (1975), with their experimental findings, support the hypothesis that H_2 receptors mediate the histamine-induced increase in rate and contractility while H_1 receptors mediate the slowing of the atrioventricular conduction. Furthermore, Verma and McNeill (1977), conclude that H_1 receptors are present in guinea pig left atrium and right ventricle, while H_2 receptors are present in right atrium and ventricle. The stimulation of the H_2 receptor accordingly is associated with an increase of cAMP.

The enhancement of automaticity in guinea pig heart treated with histamine seems to be mediated by H_2 receptors (Levi *et al.* 1976). Levi and Zavecz (1979) observe that cimetidine antagonized idioventricular automaticity produced by histamine on guinea pig heart. McNeill (1981), summarizes the effects of histamine and receptor type presence in guinea pig heart. According to his report, the H_2 receptors are present in the guinea pig right atrium and ventricle, where they mediate an increase in both rate and force of contraction. The H_1 receptors are responsible for the positive inotropic effect and are present to a lesser extent in the ventricle. They also concluded that cyclic AMP increases following the H_2 receptor stimulation. Previously,

Reinhardt (1977), investigated the effect of histamine on cAMP in guinea pig heart. His observations showed that cAMP increase is associated with H_2 receptor stimulation while in the H_1 receptor, no increase in cAMP was observed. Activation of adenylate cyclase is dependent on the activation of H_2 receptors in guinea pig ventricle as well, and is blocked by burimamide (McNeill and Muschek 1972; McNeill and Verma 1974). Therefore the H_2 receptor stimulation is connected with an increase in adenylate cyclase and cAMP. This finding led Inoue *et al.* (1978) and McNeill (1984), to suggest the idea that histamine action paralleled those of the adrenergic agents, thus the H_2 receptor being similar to beta adrenergic receptors. Both seem to stimulate the increase of cAMP while H_1 and α adrenergic receptors do not.

Since the concept of cAMP involvement in the H_2 mediated responses by histamine has arisen, the following is a brief outline of the the relationship of adenylate cyclase and cAMP to the H_2 receptors. The first suggestion of an involvement of histamine with cyclic AMP in the heart were made in 1967 by Pöch and Kukovetz. They concluded that histamine increased cardiac contractility by activating adenylate cyclase, closely allied to a specific histamine receptor, thus leading to an increase in cyclic AMP. This view was confirmed in 1971 by Klein and Levey. According to them, histamine could stimulate adenylate cyclase prepared from guinea pig, cat, and human heart. McNeill and Muschek

(1972) were able to show that there was a histamine receptor associated with cardiac adenylate cyclase, that was separate and distinct from the beta adrenergic receptor, which was poorly blocked by H_1 antagonist. They also observed that histamine could activate the enzyme glycogen phosphorylase, a cyclic AMP dependent enzyme. The positive inotropic, chronotropic and phosphorylase activating effects of histamine were all enhanced by theophylline, a phosphodiesterase inhibitor.

It has been reported that in guinea pig heart, cyclic AMP seems to increase prior to the positive inotropic response or increase in phosphorylase (McNeill & Verma 1974; Reinhardt 1977). The phosphodiesterase inhibitor papaverine, enhances the histamine inotropic response on guinea pig papillary muscle, while the same effect is abolished by burimamide (Reinhardt, 1977). Furthermore Groupp *et al.* 1980), reports that RMI 1233A, a lactam imine and an inhibitor of adenylate cyclase on guinea pig heart, completely blocks the histamine positive inotropic effect.

H_1 receptors are also associated with changes in inotropic responses. Studies on the mechanism underlying H_1 receptor mediated changes in cardiac contractility, show that the increase in tension is not associated with any changes in cyclic AMP levels (Reinhardt *et al.* 1977; Verma and McNeill, 1977). Therefore, it indicates that in guinea pig heart the H_2 receptors are predominating and that their stimulation is associated with cAMP increase, while H_1

receptors are not.

There have been few studies carried out on *human* heart for obvious reasons. Klein and Levey (1971), found a 90% increase in cyclic AMP in the right human ventricle pretreated with histamine. It seems that human heart contains only H_2 receptors and that their positive inotropic effect due to histamine stimulation is abolished by the H_2 blocker, cimetidine (McNeill, 1984; Gristwood, 1981).

Studies on the isolated *rat* hearts have been carried out by some investigators, but not to such an extent as in guinea pig. It was Went *et al*. (1952, 1954), who first reported that histamine releases an adrenaline-like substance from the isolated rat heart. According to this study, histamine first decreases and then increases the amplitude of contraction of the rat heart. Bartlet (1963), in his study reports the depressant effect of histamine on the contraction of the isolated rat heart, thus concluding that the effects of histamine could not be due to sympathomimetic action, since noradrenaline would increase the contractility of the preparation. Similar results were presented by Dai (1976), who attempted to clarify the role of H_1 and H_2 receptors in the isolated rat heart. By injecting histamine (4×10^{-7}M), through an aortic cannulae every 10 minutes, he found that histamine produced a dose-dependent decrease in heart rate and force of contraction. Metiamide, an H_2 receptor antagonist and diphenhydramine, an H_1 receptor antagonist did not have any

effect on histamine induced negative inotropic and chronotropic effects. He suggests that there is probably neither H_1 nor H_2 receptors in the rat heart, but rather that some substance is released in the rat heart to produce such a negative effect. Later, Satayavivad *et al.* (1977), indicated that histamine produced a positive chronotropic effect on the rat right atrium with an ED50 of ($1.65 \pm 0.68 \times 10^{-3}$M), however this chronotropic effect can be potentiated by a phosphodiesterase inhibitor. In 1978 Korosec and Erjavec reported that the positive inotropic effect of histamine in the perfused rat heart is due to the direct stimulation of H_2 receptors. They found no change in rate after histamine administration. They also found that rat heart was not very sensitive to histamine. Work done by Laher and McNeill 1980), on the rat isolated atrium, shed some new light on the subject. They found that at very high doses (10^{-4} to 10^{-2}M), histamine produced a positive chronotropic effect on the right atrium which is not blocked by either promethazine or cimetidine, former being the H_1 antagonist and later being the H_2 antagonist. However, the effect was blocked by propranolol or reserpine pretreatment. On the left atrium, large doses of histamine produced a positive inotropic effect. This response was also antagonized by propranolol or reserpine pretreatment. This led them to suggest that histamine in high concentrations causes an indirect stimulation of beta adrenoceptors in the right and left atria, to release endogenous catecholamines

and acetylcholine. Atropine was found to reverse the negative chronotropic effect of histamine in the presence of propranolol. McNeill (1984), suggests that the decrease of contractile force on the heart due to histamine, described by Bartlet (1963), was the effect of acetylcholine released. Johnson (1982), gives further evidence against the presence of histamine receptors in the rat heart, by failing to find an increase in adenylate cyclase after histamine stimulation of right and left ventricular tissue.

It is obvious that the question of histamine and the distribution of its receptors in the heart, as well as in other organs is very complex. Cook (1984), points out that many of its functions and roles as natural component of the body are not yet clear.

Recently, the role of Ca^{++} in eliciting the effect of histamine on the heart, has been increasingly recognized. With the discovery of Ca^{++} blocker substances, such as verapamil and D600 it became possible to study the relationship of Ca^{++} to the histamine effects. The importance of Ca^{++}, in cardiac contraction was discovered by Ringer 1883, but the main importance of Ca^{++} in excitation-contraction coupling came much later, around 1950. Since then, several major discoveries led to the present knowledge of the role of Ca^{++} in:

1. Activation of contractile proteins.
2. Different distribution of Ca^{++} concentrations in the tissue cells.

3. The mechanism involving beta-adrenergic agonists, which causes the activation of cyclic AMP, that promotes Ca^{++} ions entry from the extracellular space into the cell and sarcoplasmic reticulum, to produce a response.
4. The discovery of Ca^{++} channel blockers.

The Ca^{++} channel blockers, verapamil (Isoptin), D600 and nifedipine are valuable in experimental procedures in understanding the physiology of Ca^{++} in muscle. These drugs have been used for some time now to treat angina pectoris, supraventricular arrhythmias, as well as atrial fibrillation in humans (Krupp 1982). It seems that verapamil and its more potent methoxy derivative D600, selectively block the slow inward Ca^{++} current, which flows during the plateau phase of action potential, thus blocking the Ca^{++} transmembrane conductivity (Kolhardt 1972; Fleckenstein 1977). In his experiments on cat ventricular tissue, Kolhardt (1972), using a voltage clamp technique, proved the independence of two separate channels; a fast Na^{+} channel and a slow Ca^{++} channel. Neither verapamil nor D600 significantly block the fast Na^{+} current, although the Ca^{++} slow current was blocked. He also found that the excitability, which depends primarily on the fast Na^{+} current was not altered by these two drugs, while the contractile force was reduced, since the Ca^{++} supply to the contractile system was inhibited. However, the addition of extra Ca^{++} can improve the transmembrane Ca^{++} conductivity, so that verapamil and D600 probably compete with Ca^{++} for a common receptor carrier

system in the cardiac fibre. Furthermore, Nayler and Szeto (1972), were able to show on dog myocardium, that the negative inotropic effect of verapamil, was due to the imparied capacity of sarcolemma to bind Ca^{++}, thus reducing the Ca^{++} uptake. According to them, the cardiac microsomal fraction, representing sarcoplasmic reticulum, did not alter the accumulation, binding or exchange of Ca^{++} under verapamil influence.

It seems that the sarcolemmal Ca^{++} transporting properties dependent on ATP, as well as intrinsic ATP-ase, are blocked by verapamil (Más Oliva & Nayler, (1980). Bayer (1975), unsatisfied with the concept of verapamil being only the transmembrane Ca^{++} blocker, postulates that verapamil also slows the Ca^{++} translocation to the lateral cisternae within the sarcoplasmic reticulum. He based his assumptions on the fact that verapamil treated papillary muscle of a cat is able to produce supranormal contractions, after the reduction of the stimulation rate, thus implying that Ca^{++} is released from the accumulated storage site. He gives an explanation that Ca^{++} is present in the sarcoplasmic reticulum as a primary storage site, but is not allowed to enter the lateral cisternae as long as frequency of stimulation persists and verapamil is present. Such an effect was not observed with other Ca^{++} interventions, such as lowering $[Ca^{++}]$, or addition of Ni^{++}, La^{3+}. According to this study, verapamil does not have to cross the membrane but can communicate with lateral cisternae of sarcoplasmic

reticulum via the TTS-transverse tubular system, both being connected by tight junctions.

However this interesting study is outside of the scope of my project and would require more time and experimental work to verify or to investigate further. Fleckenstein (1977), presents a study on Ca^{++} antagonists on the heart and vascular smooth muscle. He states that verapamil and other Ca^{++}-antagonists interfere with Ca^{++} transmembrane supply, thus diminishing the force of contraction without any major change in action potential, reduced ATP utilization or lower O_2 consumption. The actions of Ca^{++} antagonists are easily overcome by addition of Ca^{++} or by beta adrenergic agonists, which act on slow channels via cyclic AMP to increase the transmembrane Ca^{++} influx, thus promoting a positive inotropic and chronotropic effect. Cardiac pacemaker activity, according to Fleckenstein (1977), is also affected by Ca^{++}-antagonists. These drugs (verapamil, D600, nifedipine), seem to suppress SA or AV pacemaker activity in rabbits, guinea pigs and rats. Diminishing the Ca^{++} availability, they inhibit impulse production and propagation in the cardiac pacemaker tissue as well as cardiac automaticity. A negative inotropic effect in cat myocardium, as well as a reduction of slow inward current depending on frequency, was observed (Ehara & Doufman, 1978). In the rabbit left atrium and papillary muscle verapamil decreases the inotropic effect in a dose-related manner Herada *et al.* (1982).

It seems that verapamil induced heart failure can be abolished by histamine, thus leading to the conclusion that histamine may increase the influx of Ca^{++} ions into the myocardial cells or can promote the liberation of intracellular Ca^{++} ions, as observed in the guinea pig heart and lung preparations by Bernauer & Schanz (1974). They also observed that the positive chronotropic effect of histamine was blocked by verapamil.

It has been reported that histamine increases the Ca^{++} conductance across the cell membrane. Since the uptake of ^{45}Ca was increased due to the histamine effect on guinea pig ventricle, DeMello (1976), concluded that the histamine positive inotropic effect, greatly depends on the extracellular calcium concentrations. Levi *et al.* (1982), state that histamine acts either directly on the Ca^{++} channel to induce a positive influx of the Ca^{++} ion or consequently via increase of cAMP. Inoue (1979), reported that the involvement of a Ca^{++} current system on the guinea pig heart, following the H_2 receptor simulations with 4-methyl-histamine (H_2 agonist), was blocked by D600.

Therefore bearing in mind all these facts, the study was undertaken in our laboratory to investigate: 1. Histamine effect on the rate and tension of the right atrium, the paced right atrium, and the paced left atrium of the rat heart, using a technique in which drugs were infusing the tissue at constant rate for a given period of time. 2. To verify McNeill's (1980), hypothesis that

histamine in rat heart acts via the release of catecholamines. 3. To investigate the role Ca^{++} ion plays if any, in relation to the effect of histamine on the rat heart, by using the Ca^{++} antagonist verapamil.

2. METHODS AND EQUIPMENT

2.1 Dissection of the left and right atria

The animals used were Sprague-Dawley rats weighing from 200-400 gms. They were anaesthesized by ether, their chests were open along the midline, heart with lungs rapidly dissected out and placed into a beaker containing heparinized, cool Krebs solution. The heart was then transferred into a dissecting tray also filled with cool Krebs solution solution and pinned to the waxed bottom of the tray. Lungs and all connective tissue were removed, thus leaving both atria clean. A hook attached to a cotton thread was passed through each atrium and then both left and right atria were carefully divided from each other with scissors, along the midline. The left or right atrium was then hooked, with one end attached via cotton thread to a transducer and the other end fastened to the bottom of the infusion bath.

2.2 Dissection of the right atrial pacemaker

In order to destroy or remove the pacemaker so that the right atrium could be electrically paced, the following procedure was used: After detaching the right from the left atrium, the atrium was pinned to the bottom of the dish very carefully and then the posterior two thirds of the wall were dissected out using special dissecting scissors. Then a transverse cut was made, so that one third of the anterior wall was also removed.

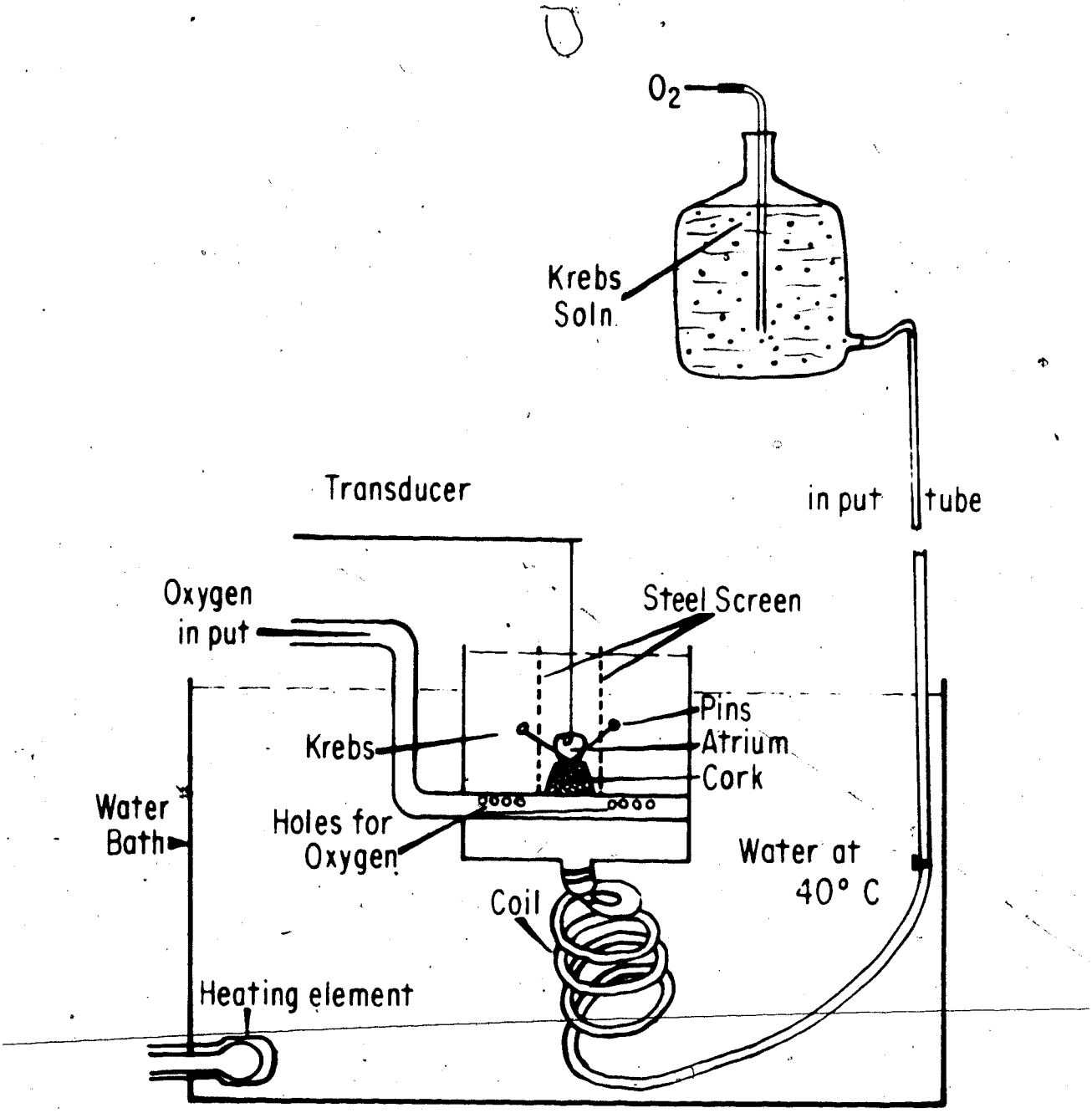
This type of dissection usually removed the pacemaker. If the dissection was not thorough, histamine, which has a positive chronotropic effect on the pacemaker, could excite the pacemaker node, or ectopic pacemaker. Such experiments were not used since the tension observed could not be measured.

2.3 Apparatus and tissue bath

The tissue bath is constructed as follows: It is constructed from plexy glass and has a capacity of 20 mls. It has two inputs; (1) an input tube through which Krebs solution is infused from a reservoir, placed one meter above the bath. (2) a tube which carries oxygen to the preparation. This oxygen tube has a cork mounted on its centre (see Fig. 1.), so that the atrium may be pinned in the bath. The bath is divided into three compartments by stainless steel screens. The screens protect the string which is attached to the transducer and the atrium, from the oxygen bubbles, which can cause interference to the transducer. However oxygen can still diffuse through the screens to the preparation.

The bath is then placed in a water bath with a constant temperature of 40° ± 0.5°C. The temperature of the preparation bath is kept constant at 37° ± 0.5°C. The solutions in the reservoir and the bath with the preparation were both equilibrated by continuous gassing with a mixture of 95% O_2 and 5% CO_2. This mixture also keeps the Krebs

Figure (1.) shows the tissue bath and apparatus which was used for this series of experiments.


O_2
Krebs Soln
Transducer
in put tube
Oxygen in put
Steel Screen
Pins
Atrium
Cork
Krebs
Water Bath
Holes for Oxygen
Water at 40° C
Coil
Heating element

solution at a pH of 7.4.

2.4 Solutions

Krebs solution was used throughout the experiments. The following concentration were made in distilled water in mM/l: NaCl 115; KCl 4.6; $CaCl_2$ 2.46; Mg SO_4 1.15; K_2PO_4 1.15; $NaHCO_3$ 2.41; glucose 5.45 gms, pH - 7.4. Krebs solution was made fresh each day and was kept in the refrigerator. It would usually infuse the heart tissue for about 60 minutes, until a constant tension and/or rate would be achieved and then the solution would be changed to the other solution, depending on the experiment. Flow rate of the solutions was 5 ml/min thus taking 3 min to replace the solutions in the bath.

Histamine Dihydrochloride (184 M.W.), was supplied by Sigma chemical company, in crystalline powder form. From it the stock solution of (1×10^{-3}M) was prepared freshly each day.

Verapamil (491 M.W.), supplied kindly by Searle Pharmaceuticals of Canada, was in powder form. The substance is light sensitive and not readily soluble in water. Verapamil (10^{-3}M) was used as a stock solution which was kept in the refrigerator.

Metoprolol (684.8 M.W.), kindly supplied by Ciba-Geigy Canada Ltd. was used in powder form, and stock solution of (10^{-3}M) was prepared.

Adrenaline Bitartarate (333.3 M.W.) supplied by Sigma chemical company was used to prepare the stock solution of (10^{-5}M).

2.5 Procedures and Recordings

Once the heart tissue was fastened and set up in the infusion bath, the preparation was allowed to equlibrate for 40-60 minues in the Krebs solution, until a steady rate and tension were obtained. Then the experimental procedures began.

The solutions were infused into the bath at a constant rate of 5 ml/min during which time the heart rate and tension were observed and recorded. A displacement transducer, designed in the workshop, was used to record changes in tension and rate. It operates on DC current with a 6V battery. A Beckman Type R 411 dynograph recorder was used, which recorded the responses in tension and rate. The responses were also transmitted from the Beckman recorder to the oscilloscope Type 549, where individual contractions as well as the rate could be observed. In turn this response was recorded by the computer TRS 80 where rate and tension were recorded every minute. The curves for both were plotted at the same time and the data was then stored. Computer recordings, were the average recordings per minute.

After 60 minutes of atrial infusion by Krebs solution the histamine (10^{-3}M) or verapamil, or both at the same time were then introduced by the same method of infusion for a

given period of time. The same recordings were done throughout this part of the experiment as already discussed above. The drugs were washed with Krebs solution by overflow method at the end of the given time period and again tension and rate changes were recorded. Histamine (10^{-3}M), was infused to the preparation for 30 minutes. The paced left and right atria were both electrically stimulated by two electrodes from a Grass SD9 stimulator at a voltage of about 2-4V, at a constant frequency of approximately 92 pulses/minute.

At the end of the experiment, the atrium was lightly blotted with a piece of tissue paper, and weighed. The calibration of tension was also made. Tension from the trace was measured in mm and converted into mgs/mg. wt. of the muscle. Rate was also noted from the computer sheet and recorded in table form. Recordings from the computer and from the dynamograph were stored in files. Calculations of half times for the tension and rate of rise or fall for histamine, verapamil and low Ca^{++} Krebs solutions, were done at the end of each experiment. Computer stored data was used to give the traces of single contractions for the atrium infused by Krebs solution, histamine, verapamil and low Ca^{++} Krebs solution.

Contraction time, time to maximum contraction, as well as relaxation time for histamine effect on the atrium, was observed and compared to Krebs solution, verapamil and low Ca^{++} Krebs solution.

2.6 Statistics

Statistical significance was determined by using the Student's t test. Differences were considered to be significant if $p < 0.05$.

3. RESULTS

3.1 Rate

The effects on the rate of the right atrium were observed with the following substances: histamine, metoprolol, verapamil and low Ca^{++}.

3.1.1 The effects of histamine (10^{-3}M)

The right atrium was first infused with Krebs solution for 60 minutes, until a stable rate was achieved. Then the infusion solution was changed to histamine (10^{-3}M). After a period of 30 minutes in histamine solution, the preparation was washed with normal Krebs solution.

Histamine (10^{-3}M) has a positive chronotropic effect on the right atrium. Table (1, Pg. 26) shows that the average basal rate in Krebs solution was 248 ± 21 beats/min and increases to 373 ± 27 beats/min in histamine. This increase was significant at $p < 0.05$. The average rate in histamine is 151 ± 16% of the average rate in Krebs solution; Krebs solution equals 100%. The average half time for maximum rate in histamine is 12.3 ± 5.7 minutes. The average rate after washing in Krebs solution was 69 ± 8% of the average rate in the presence of histamine. This change is significant at $p < 0.05$.

Fig. (2.A, Pg. 28) illustrates the computer output of the rate/min. It shows that after a period of 6 minutes in histamine, the rate begins to increase and reaches its

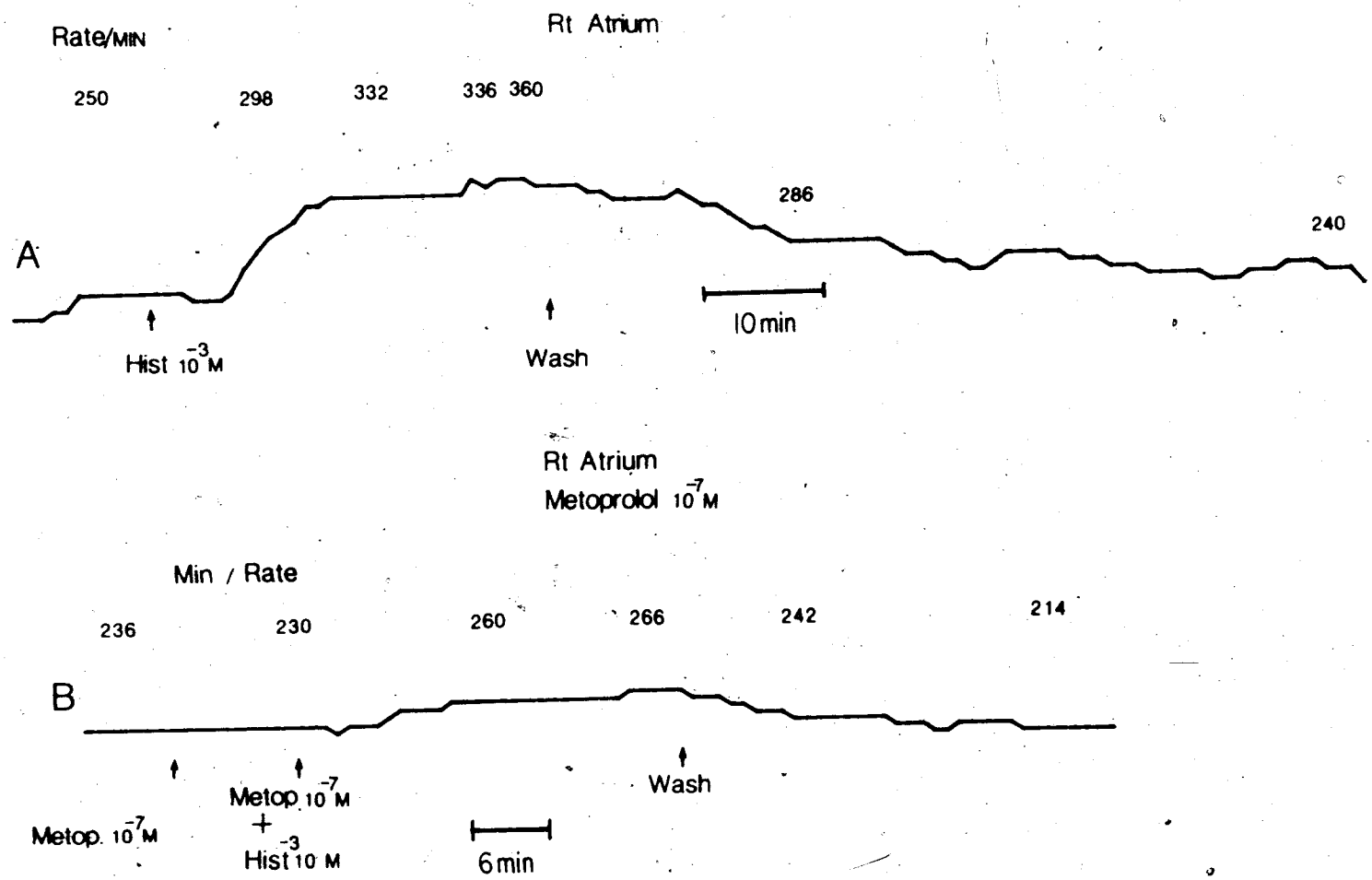
Table 1. Right atrium rate histamine 10^{-3}.

Number of Experiment	Krebs rate/min	histamine rate/min	% of normal histamine/Krebs	½ time for maximum (min)	Krebs rate/min	% Krebs/histamine
1	220	338	153%	6	238	70%
2	234	354	151%	8	226	63%
3	248	370	149%	8	264	71%
4	264	368	139%	11	264	71%
5	246	340	138%	13	288	84%
6	250	360	144%	9	242	72%
7	264	408	154%	8	240	58%
8	254	402	158%	10	284	70%
9	204	402	197%	14	224	55%
10	256	396	154%	16	262	66%
11	284	400	140%	26	310	77.5%
12	248	336	135%	19	252	75%
Mean ± S.D.	248 ± 21	373 ± 27	151 ± 16%	12.3 ± 5.7	258 ± 26	69 ± 8%

Figure (2.) This represents the computer output of the rate of the right atrium.

(A) Shows the effects of histamine (10^{-3}M).

(B) Shows the effects of metoprolol (10^{-7}M) plus histamine (10^{-3}M).


Rate/MIN
Rt Atrium
250
298
332
336 360
286
240
A
Hist 10⁻³ M
Wash
10 min
Rt Atrium
Metoprolol 10⁻⁷ M
Min / Rate
236
230
260
266
242
214
B
Metop. 10⁻⁷ M
Metop. 10⁻⁷ M
+
Hist 10⁻³ M
Wash
6 min

maximum after a period of 20 minutes. In this experiment the basal rate of 250 beats/min in Krebs solution, increased to a maximum of 360 beats/min in histamine. However, in some experiments the rate changed from i.e., 204 to 402 beats/min. On washing in Krebs solution, the rate returned close to its basal rate.

Fig. (3.a, Pg. 31) shows the rate in histamine relative to the rate in Krebs solution. All the points on the graph are on the left side of the median line, thus indicating a potentiating effect of histamine on the rate of the right atrium.

The histogram (Fig. 4, Pg. 33), illustrates the percentage difference of the average rate between Krebs solution and histamine. The average rate in Krebs solution is taken as 100%. It shows that the average rate in histamine is increased by 50% from Krebs solution.

Histamine also has a positive inotropic effect on the right atrium, but this will be discussed separately.

3.1.2 The effects of metoprolol (10^{-7}M) and histamine (10^{-3}M)

The right atrium was exposed to metoprolol (10^{-7}M) for 10 minutes. After that, histamine (10^{-3}M) with metoprolol (10^{-7}M), were added to the preparation.

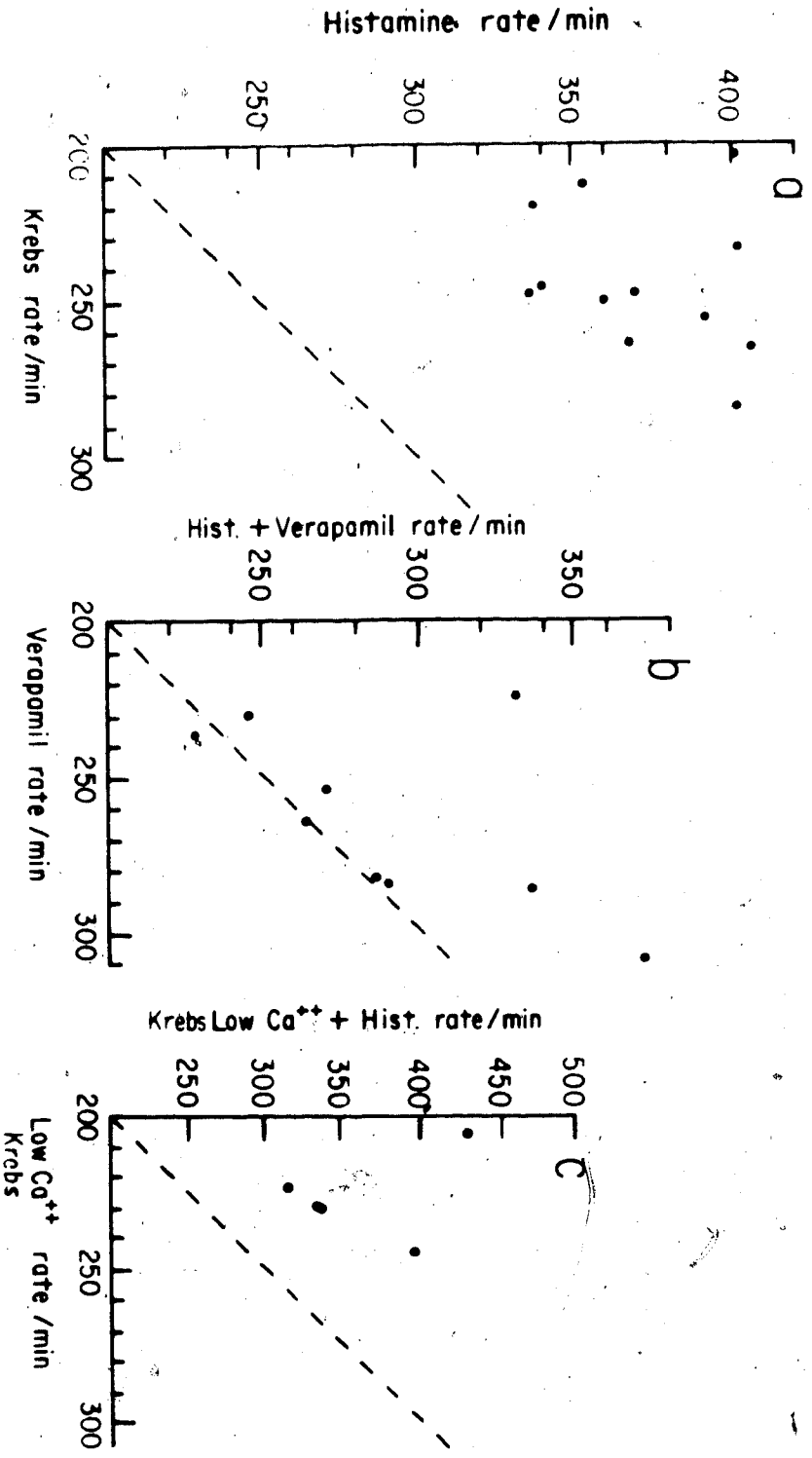
Table (2, Pg. 34) shows that the average rate of 235 ± 6 beats/min in Krebs solution. This rate was maintained the same when the solution was changed to metoprolol (10^{-7}M).

Fig. (3.)

(a) Represents the change in rate of the right atrium, in histamine (10^{-3}M), as compared to that in Krebs.

(b) Shows the rate of the right atrium in histamine (10^{-3}M) plus verapamil (10^{-6}M), as compared to that in verapamil (10^{-6}M).

(c) Represents the rate of the right atrium in histamine (10^{-3}M) and low Ca^{++} (0.35 mM) Krebs solution. Line through origin represents equal activity ratios.



Right Atrium Rate

Fig. (4.)

Illustrates the average rate of the right atrium ± SEM in Krebs solution and relative to that the average rate ± SEM in histamine (10^{-3}M), histamine (10^{-3}M) plus metoprolol (10^{-7}M), histamine (10^{-3}M) plus verapamil (10^{-8}M), histamine (10^{-3}M) plus low Ca^{++} Krebs solution (0.35 mM). The average rate in Krebs solution represents 100%.

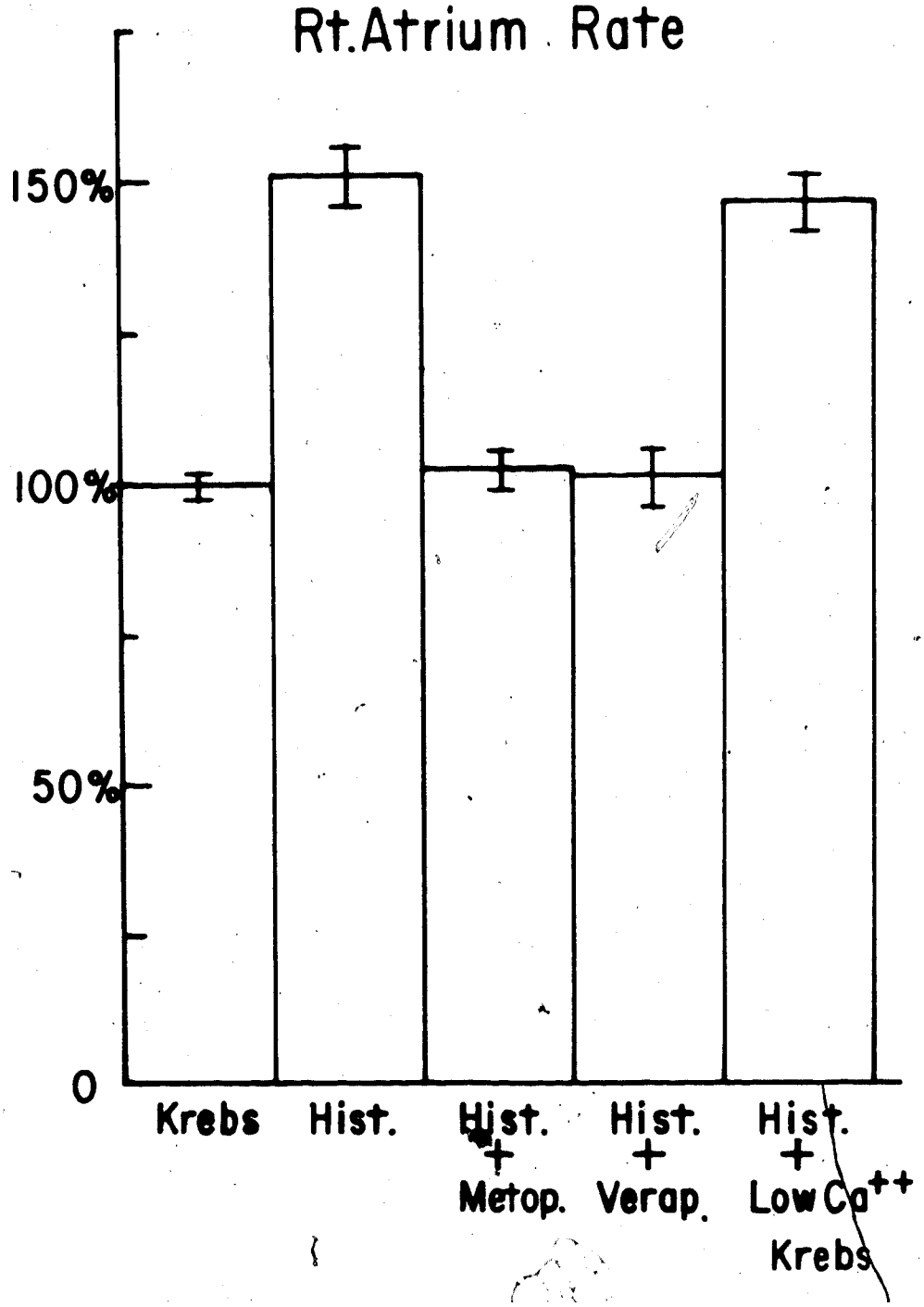

Rt.Atrium Rate
150%
100%
50%
0
Krebs
Hist.
Hist. + Metop.
Hist. + Verap.
Hist. + Low Ca++ Krebs

Table 2. Right atrium rate: metoprolol 10^{-7} + histamine 10^{-3}.

Number of	Krebs				histamine 10^{-3} + metop. 10^{-7}			Krebs	
Experiment	rate/min	rate/min	% of control metop./Krebs	½ time maximum (min)	rate/min	% of control histamine + metop./metop.	½ time maximum (min)	rate/min	% of control Krebs/histamine + metop.
1	240	240	100%	0	240	100%	0	246	102%
2	232	232	100%	2	224	96%	2	224	100%
3	240	240	100%	0	265	110%	2	212	80%
4	228	224	98%	1	234	104%	1	210	89%
Mean ± S.D.	235 ± 6	234 ± 7.6	99.5 ± 1%	0.75 ± 0.9	240.7 ± 17	102 ± 6%	1.2 ± 0.9	223 ± 16	93 ± 10%

metop. = metoprolol

The average rate in metoprolol was 234 ± 7.6 beats/min thus not being significantly different at $p < 0.05$ than in Krebs solution. In Table (2, Pg. 34), the average rate in histamine and metoprolol was 240.7 ± 17 beats/min. This is not significantly different at $p < 0.05$, from the average rate in metoprolol alone, which was (234 ± 7.6). The rate in histamine (10^{-3}M) plus metoprolol (10^{-7}M), expressed as the average precentage of metoprolol rate, was 102 ± 6%.

The half time for the rate to reach its maximum in histamine (10^{-3}M) with metoprolol (10^{-7}M), was 1.2 ± 0.9 minutes. The average rate after washing with Krebs solution, was 223 ± 16 beats/min, thus being lower than normal, but not significantly different at $p < 0.05$.

Fig. (2.B, Pg. 28) shows a slight increase in the rate, from 230 in metoprolol to 265 beats/min, in metoprolol plus histamine. After washing with Krebs solution the rate decreases to 214 beats/min.

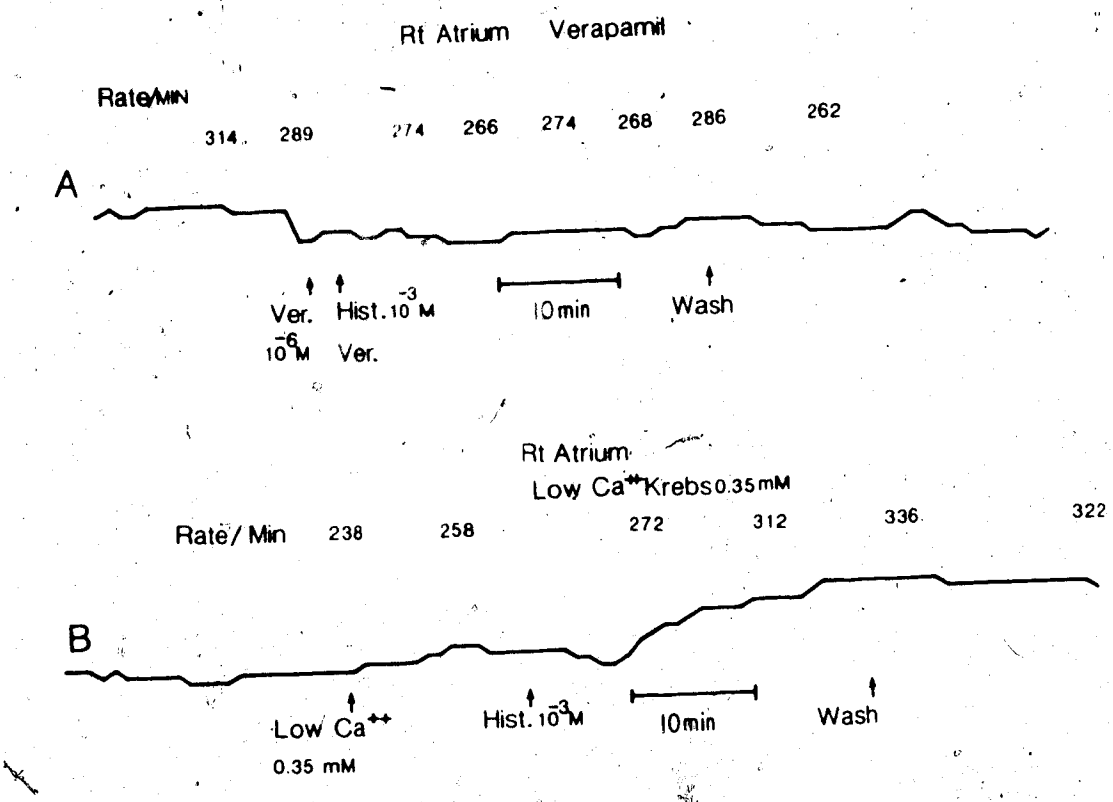
Fig. (4., Pg. 33) shows that there is only 2% difference between the rate in Krebs solution and the rate in histamine plus metoprolol. Our results therefore indicate, that metoprolol blocks the positive chronotropic effect of histamine on the right atrium. The positive inotropic effect of histamine on the right atrium was also blocked by metoprolol, but this will be discussed separately.

Fig. (5.)

This represents the computer output of the rate of the right atrium.

(A) shows the effects of verapamil (10^{-6}M) plus histamine (10^{-3}M).

(B) shows the effects of low Ca^{++} Krebs solution (0.35 mM) plus histamine (10^{-3}M).


Rt Atrium Verapamil
Rate/MIN
314 289 274 266 274 268 286 262
A
Ver. 10^{-6}M
Hist. 10^{-3}M
Ver.
10 min
Wash
Rt Atrium
Low Ca^{++} Krebs 0.35 mM
Rate/ Min 238 258 272 312 336 322
B
Low Ca^{++} 0.35 mM
Hist. 10^{-3}M
10 min
Wash

3.1.3 The effects of verapamil (10^{-6}M) and histamine (10^{-3}M

In our experiments we attempted to block the positive chronotropic effect of histamine (10^{-3}M), with the following concentrations of verapamil. Verapamil (2×10^{-6}M), (1.5×10^{-6}M), (1.4×10^{-6}M), (1.35×10^{-6}M) and (1×10^{-6}M) were used, of which verapamil (10^{-6}M) produced a blocking effect on the rate. The infusion time of verapamil was 2-3 min.

Table ((3., Pg. 39) represents the data from all nine experiments performed in this concentration of verapamil. The average rate in Krebs solution was 286 ± 17 beats/min. The average rate in verapamil is less, being 267 ± 25 beats/min and significantly different from the rate in Krebs solution at $p < 0.05$. The average rate in verapamil (10^{-6}M) is 92 ± 8% of the rate in Krebs solution solution. The half time for the decrease is 1.1 ± 0.3 min. However, the average maximum rate in verapamil plus histamine is 290 ± 46 beats/min. The difference in rate is not found to be significant at $p < 0.05$, from the rate in verapamil (10^{-6}M) alone. Expressed in percentage, rate in verapamil with histamine is 108 ± 17% of the rate in verapamil, or 101 ± 12% of the rate in Krebs solution. The half time to reach maximum rate in verapamil plus histamine, is 8.7 ± 9.7 min. In experiment number four, where there is an increase of rate from 224 beats/min in verapamil, to 326 in verapamil with histamine, the half time for maximum rate is 19 minutes, thus indicating that the response time was slow.

Table 3. Right atrium rate : verapamil 10^{-6}M + histamine 10^{-3}M.

Number of Experiment	Krebs	verapamil 10^{-6}M			verapamil 10^{-6}M + histamine 10^{-3}M				Krebs	
	rate/min	rate/min	% of normal verapamil/krebs	½ time (min)	rate/min	% of control verapamil + histamine/ verapamil	% of normal verapamil + histamine/krebs	½ time (min)	rate/min	% of control krebs/verapamil + histamine
1	304	309	97%	1	372	120%	122%	9	258	69%
2	288	264	91%	1	264	100%	91%	0	264	100%
3	272	282	103%	1	286	101%	105%	3	230	80%
4	296	224	75%	1	326	145%	110%	19	298	91%
5	312	284	91%	1	286	99%	91%	1	268	93%
6	260	250	96%	2	246	98%	94%	1	266	108%
7	280	256	91%	1	228	89%	81%	4	124	54%
8	268	254	94%	1	270	106%	100%	28	276	102%
9	298	286	95%	1	336	117%	112%	14	328	97%
Mean ± S.D.	286 ± 17	267 ± 25	92 ± 8%	1.1 ± 0.3	290 ± 46	108 ± 17%	101 ± 12%	8.7 ± 9.7	256 ± 57	88 ± 17%

The average rate upon washing with Krebs solution, was 256 ± 57 beats/min or 88 ± 17% of the average maximum rate in verapamil plus histamine.

Fig. (5.A, Pg. 37) shows the rate output from the computer. In this particular experiment, the basal rate in Krebs solution was 312 beats/min. In verapamil, the rate dropped almost immediately to 284 beats/min. The rate decreased further to 266 beats/min, in verapamil plus histamine solution. The maximum rate achieved was 286 beats/min. This effect occured after 25 minutes of histamine plus verapamil infusion. The tension was not completely blocked at this concentration, but again this will be discussed separately in detail. Washing the preparation in Krebs solution, decreased the rate in this experiment from 266 to 262 beats/min.

Histogram (Fig. 4, Pg. 33) also shows, that the average increase in rate in histamine plus verapamil is blocked in comparison to the average rate in histamine alone.

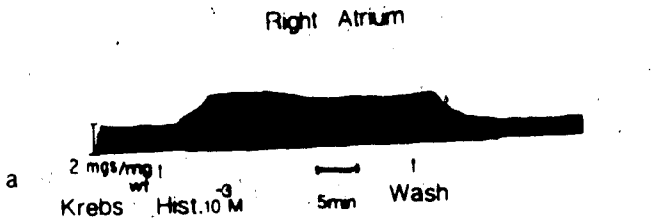
Fig. (3.b, Pg. 31) shows the rate in histamine (10^{-3}M) and verapamil (10^{-6}M), relative to the rate in verapamil (10^{-6}M). Six out of nine points show very slight change in rate, from verapamil to verapamil plus histamine. Three points indicate the increase in rate.

Fig. (5.)

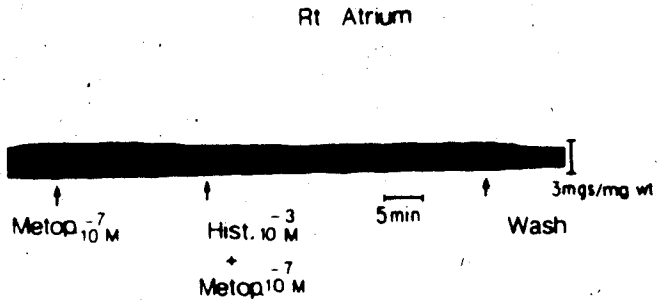
This represents the computer output of the rate of the right atrium.

(A) shows the effects of verapamil (10^{-6}M) plus histamine (10^{-3}M).

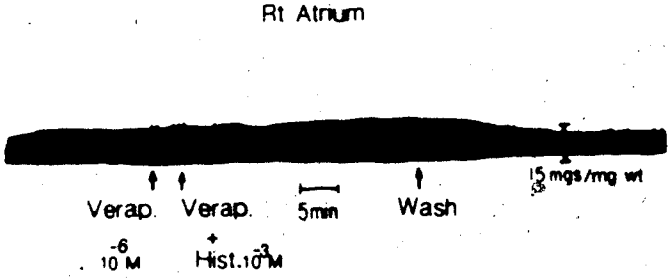
(B) shows the effects of low Ca^{++} Krebs solution (0.35 mM) plus histamine (10^{-3}M).


Right Atrium
2 mgs/mg wt
Krebs
Hist.10 -3 M
5min
Wash

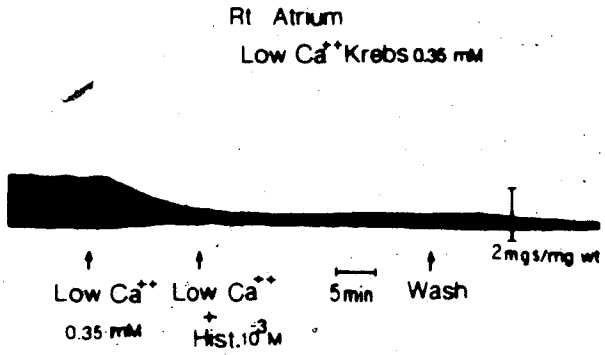
a


Rt Atrium
3mgs/mg wt
Metop.10 -7 M
Hist. 10 -3 M
5min
Wash
Metop.10 -7 M

b


Rt Atrium
5 mgs/mg wt
Verap. 10 -6 M
Verap.
Hist.10 -3 M
5min
Wash

c


Rt Atrium
Low Ca++ Krebs 0.35 mM
2mgs/mg wt
Low Ca++ 0.35 mM
Low Ca++
Hist.10 -3 M
5min
Wash

d

3.1.4 The effects of low Ca^{++} Krebs (0.35 mM) and histamine ($10^{-3}M$)

Lowering the concentration of Ca^{++} in Krebs solution to (1.2 mM) or (0.55 mM) did not alter the effects of histamine ($10^{-3}M$) on the right atrium rate or tension. The concentration of Ca^{++} was then lowered further to (0.35 mM) and this was found to be the lowest concentration in which the rate could be measured, because further decreases in the concentration of Ca^{++}, decreased the tension to a low value, which could not be measured.

In Table (4, Pg. 44), the results of individual experiments are represented. The average rate in Krebs solution, being 261 ± 34 beats/min, decreased slightly to 252 ± 28 in low Ca^{++} Krebs solution. This change was not found to be significant at $p < 0.05$. The average rate in low Ca^{++} Krebs solution was 97 ± 16% of the rate in Krebs solution. Average half time for this decrease was 3 ± 3.9 min. The average rate in low Ca^{++} Krebs solution plus histamine was 362 ± 49, that being 146 ± 33% of the rate in low Ca^{++} Krebs solution, or 139 ± 8% of the rate in Krebs solution. The increase in rate, from low Ca^{++} Krebs solution to low Ca^{++} plus histamine ($10^{-3}M$), was significantly different at $p < 0.05$. The average half time for the increase was 13 ± 1.3 minutes, which is only slightly longer than the half time for maximum increase, in histamine ($10^{-3}M$) alone. The average rate in Krebs solution wash maintained at higher value approximately 324 ± 44 beats/min,

Table 4. Right atrium rate: low Ca^{++} Krebs 0.35 mM + histamine 10^{-3}M rate/min.

Number of Experiments	Krebs rate/min	low Ca^{++} Krebs rate/min	% of normal lCa/Kr	½ time (min)	low Ca^{++} Krebs + histamine 10^{-3} rate/min	% of control Ca + Hist /lCa	% of normal lCa + hist /Kr	½ time (min)	low Ca^{++} Krebs rate/min	% of control lCa/lCa + hist
1	238	258	108%	1	336	130%	141%	12	318	94%
2	302	288	95%	2	396	137%	131%	14	344	87%
3	242	246	101%	1	314	127%	130%	13	292	92%
4	294	210	71%	10	432	205%	147%	12	390	90%
5	230	258	112%	1	336	130%	146%	15	280	83%
Mean ± S.D.	261 ± 34	252 ± 28	97 ± 16	3 ± 3.9	362 ± 49	146 ± 33%	139 ± 8%	135 ± 8%	324 ± 44	89 ± 4.3

lCa = Low Ca^{++}

Kr = Krebs

hist = Histamine

thus being 89 ± 4.3% of the rate in low Ca^{++} and histamine. This decrease in the rate was not found significant at $p < 0.05$.

Fig. (5.B, Pg. 37) shows the increase of the rate from 238 in Krebs solution, to 336 in histamine with low Ca^{++} Krebs solution.

The positive chronotropic effect of histamine in low Ca^{++} Krebs solution is also represented in Fig. (3.c, Pg. 31), relative to the rate in low Ca^{++} Krebs solution. The points on the graph are shifted to the left of the median line, indicating the potentiating effect of histamine (10^{-3}M) plus low Ca^{++} Krebs solution, on the rate. In Fig. (4., Pg. 33), the average rate in histamine and low Ca^{++} is presented as a percentage of the rate in Krebs solution. The average rate in Krebs solution is taken to be 100%. The increase in the average rate in low Ca^{++} and histamine from Krebs solution is slightly less than the increase in histamine. Thus lowering the [Ca^{++}] to (0.35 mM) produced little effect on the positive chronotropic effect of histamine.

3.2 Tension

The effects of the following substances on the tension of the right atrium, paced right atrium and the left atrium were observed: histamine (10^{-3}M), verapamil in different concentrations, metoprolol (10^{-7}M) and low Ca^{++} Krebs.

3.2.1 Right atrium

3.2.1.1 The Effect of histamine (10^{-3}M) on the right atrium

Table (5., Pg. 47) shows the tension in mgs/mg. wt. of atrial muscle. The average tension produced by the right atrium, in Krebs solution was 7.9 ± 7 mgs/mg. wt. Histamine increased the tension to an average of 16.4 ±14 mgs/mg. wt. which is 210 ± 111% of the average tension in Krebs solution, (Krebs solution tension taken as 100%). This was calculated to be a significant increase in the tension at $p < 0.05$. The average half time for the maximum tension to be achieved was 1.3 ± 0.9 minutes, which is much less than the average half time for the rate (Table 1., Pg. 26), to achieve its maximum. After histamine (10^{-3}M) infusion, washing with Krebs solution decreased the tension to values less than the basal level. The average tension after washing with Krebs solution for 20 minutes, was 6.2 ± 3 mgs/mg. wt. That was 42.8 ±14% of the maximum tension in histamine (10^{-3}M). This decrease in tension was also significantly different from the tension in histamine (10^{-3}M) at

Table 5. Right atrium tension: histamine 10^{-3}M

Number of Experiments	Krebs	histamine			Krebs	
	tension (mgs/mg. wt.)	tension (mgs/mg. wt.)	½ time (min)	% of normal histamine/krebs	tension (mgs/mg. wt.)	% of histamine krebs/histamine
1	8	12.9	0.6	161%	5.4	54%
2	12.8	17.7	0.7	138%	9.9	55%
3	22.6	33.25	0.8	147%	18.6	56%
4	17.2	52.8	2.9	306%	9.8	18%
5	2.7	4.9	0.4	180%	2.7	55%
6	1.6	3.6	0.6	225%	0.84	40%
7	4	6.5	1.2	162%	2.1	32%
8	8.4	13.1	0.9	155%	8.4	64%
9	3.4	10.7	2.6	314%	2.8	26%
10	1.8	8.8	2.4	488%	3.0	34%
11	5	15.0	1.6	300%	5.6	37%
Mean ± S.D.	7.9 · 7	16.4 · 14	1.3 · 0.9	210 · 111%	6.2	42.8 · 14%

$p < 0.05$.

This data also shows, that histamine sometimes causes a greater increase in the tension than the increase in the rate (Table 1., Pg. 26). Good examples are experiments no. 4,9,10,11, in which the tension increased to 488% of the tension in Krebs solution, while the maximal increase in rate (Table 1., Pg. 26) was 197% of the rate in Krebs solution.

In the right atrium, histamine's positive inotropic effect preceeds the positive chronotropic effect. Once the tension reaches its maximum, the rate then starts to rise to a maximum level. This may cause the slight drop in tension, as seen on the Fig. (5.A, Pg. 37).

Fig. (6.a, Pg. 42) shows the positive inotropic effect of histamine on the right atrium. The tension increased from 1.6 to 3.6 mgs/mg. wt. It reached its maximum after 5 minutes in histamine (10^{-3}M) infusion. This tension was maintained for seven minutes. However, it slightly decreased as the rate increased. Washing with Krebs solution, gradually decreased the tension to 0.84 mgs/mg. wt., which is less than the basal tension.

Both Fig. (7.a, Pg. 50) and Fig. (8., Pg. 52), show this increase in the tension during histamine (10^{-3}M) infusion. In Fig. (7.a, Pg. 50) the tension in histamine versus the tension in Krebs solution is represented, showing the shift of the points to the left of the graph, indicating an increase of the tension in

Fig. (7.)

(a) Represents the tension (mgs/mg. wt.) in histamine (10^{-3}M) in relation to the tension (mgs/mg. wt.) in Krebs solution, on the right atrium.

(b) Shows the tension (mgs/mg. wt.) of the right atrium in verapamil (10^{-6}M) and histamine (10^{-3}M) versus the tension (mgs/mg. wt.) of the right atrium in verapamil.

(c) Shows the tension (mgs/mg. wt.) of the right atrium in low Ca^{++} Krebs solution of the (0.35 mM) plus histamine 10^{-3}M versus the tension (mgs/mg. wt.) in low Ca^{++} Krebs (0.35 mM). Line through origin represents equal activity ratio.

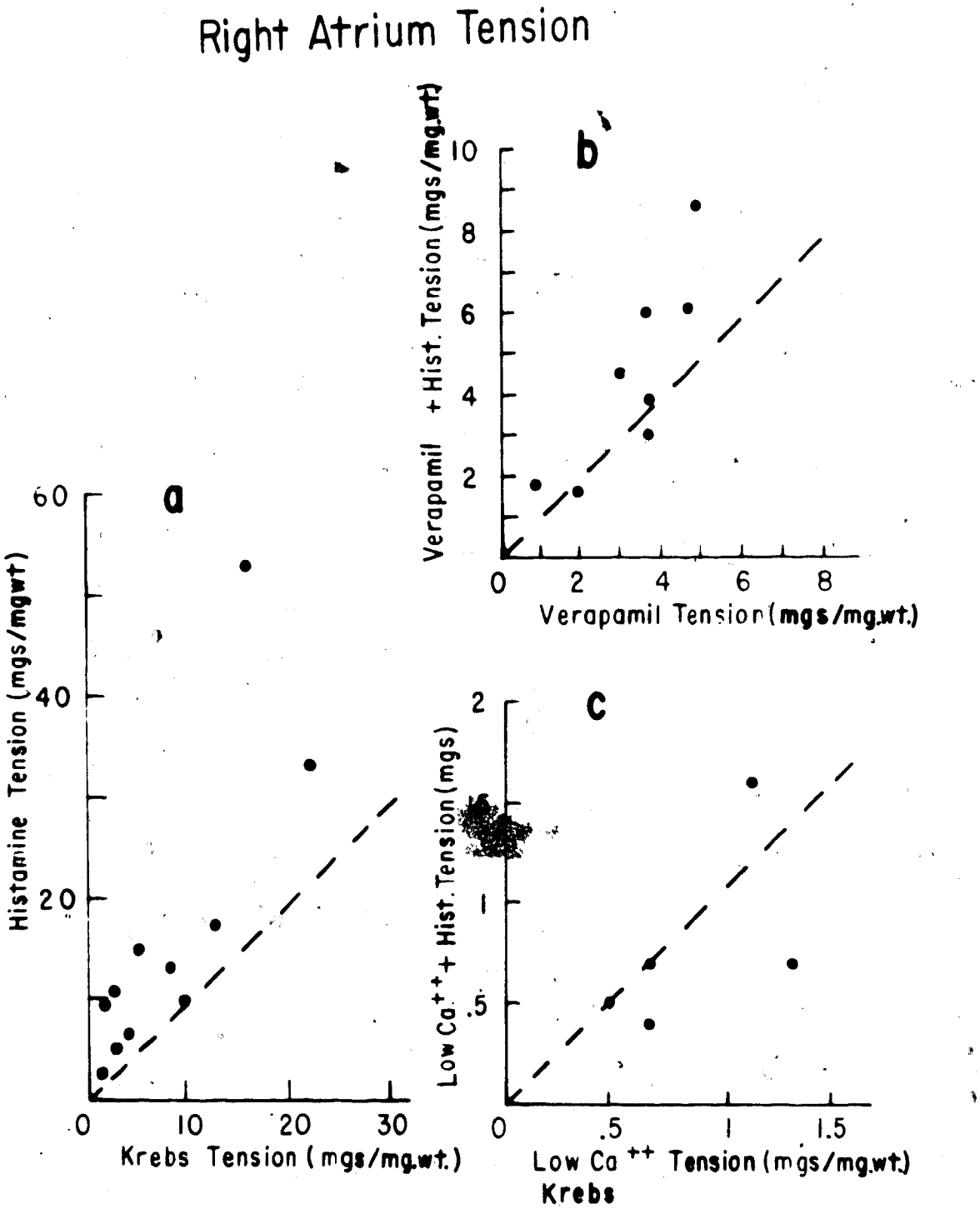
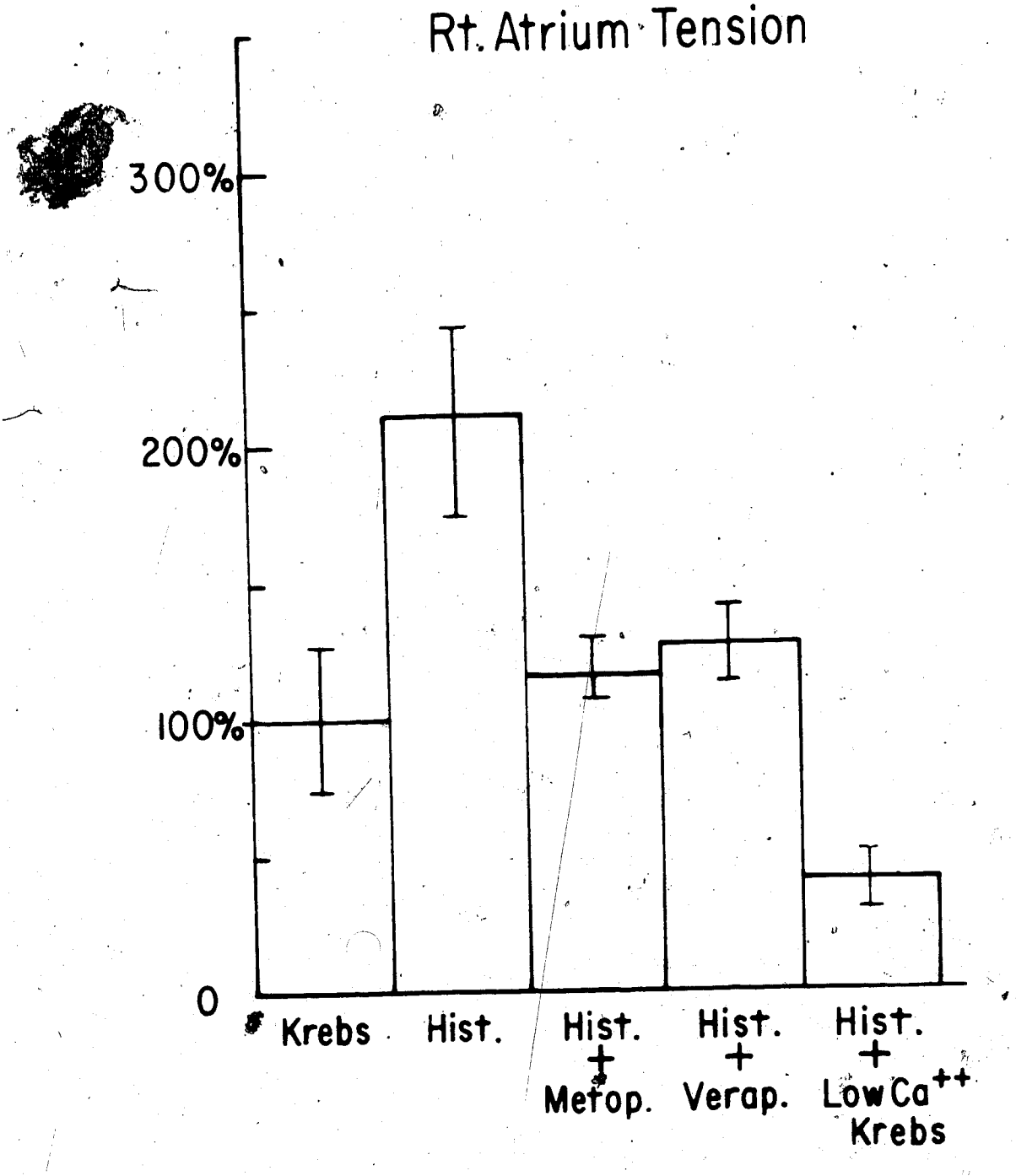

Right Atrium Tension
a
Histamine Tension (mgs/mgwt)
60
40
20
0
10
20
30
Krebs Tension (mgs/mg.wt.)
b
Verapamil + Hist. Tension (mgs/mg.wt)
10
8
6
4
2
0
2
4
6
8
Verapamil Tension (mgs/mg.wt.)
c
Low Ca++ + Hist. Tension (mgs)
2
1
.5
0
.5
1
1.5
Low Ca ++ Tension (mgs/mg.wt.)
Krebs

Fig. (8.)

Represents the histogram, showing the mean percentage difference ± SEM in the tension of the right atrium, in histamine (10^{-3}M), histamine (10^{-3}M) plus metoprolol (10^{-7}M), histamine (10^{-3}M) with verapamil (10^{-6}M), histamine (10^{-3}M) plus low Ca^{++} Krebs solution (0.35 mM), relative to the tension in Krebs solution, which is taken to be 100%.


Rt. Atrium Tension
300%
200%
100%
0
Krebs
Hist.
Hist. + Metop.
Hist. + Verap.
Hist. + Low Ca++ Krebs

histamine. Fig. (8, Pg. 52) shows the average tension in Krebs solution taken as 100%. The average tension of histamine and other substances were compared relative to the value of the tension in Krebs solution. The tension increase in histamine (10^{-3}M) is shown in this graph. It is 110 ± 111% greater than the average tension in Krebs solution.

3.2.1.2 The effects of metoprolol (10^{-7}M) plus histamine (10^{-3}M)

Metoprolol (10^{-7}M) was found to block the positive inotropic effect of histamine (10^{-3}M). As shown in Table (6., Pg. 54), the average tension in Krebs solution being 3.2 ± 1.2 mgs/mg. wt., slightly decreased to 2.7 ± 1.4 mgs/mg. wt. This decrease was not found to be significant at $p < 0.05$. The tension in metoprolol was in average of 82 ± 22% of the tension in Krebs solution. The tension in Krebs solution was taken to be 100%. The half time for this decrease was 0.6 ± 0.3 minutes. In histamine (10^{-3}M) plus metoprolol (10^{-7}M), the average tension was 312 ± 1.6 mgs/mg wt. This slight increase in the tension from the tension in metoprolol was found to be significant at $p < 0.05$. The average percentage of histamine (10^{-3}M), plus metoprolol (10^{-7}M) was 114 ± 16% of the tension in metoprolol (10^{-7}M), taking tension in metoprolol (10^{-7}M) as 100%. The average half time for the maximum change was 0.25 ± 0.26 minutes. Washing with Krebs solution, decreased the tension to an average of

Table 6. Right atrium tension, metoprolol (10^{-7}M) + histamine (10^{-3}M)

Number of Experiment	Krebs tension (mgs/mg. wt.)	metoprolol 10^{-7} tension (mgs/mg. wt.)	% of control metop./Krebs	½ time (min)	histamine 10^{-3} + metoprolol 10^{-7} tension (mgs/mg. wt.)	% of control hist + metop./metop.	½ time (min)	Krebs tension (mgs/mg. wt.)	% of control Kr/hist metop.
1	1.7	1.5	87%	0.6	1.6	107%	0.1	1.7	106%
2	4.1	2.28	55%	1.0	3.2	138%	0.6	1.6	52%
3	3.0	2.28	76%	0.5	2.28	100%	0	1.0	43%
4	4.3	4.7	110%	0.2	5.4	114%	0.3	1.9	35%
Mean SD	3.2 ± 1.2	2.7 ± 1.4	82 ± 22%	0.6 ± 0.3	3.12 ± 1.6	114 ± 16%	0.25 ± 0.26	1.55 ± 0.4	59 ± 32%

metop. = metoprolol

1.55 ± 0.4 mgs/mg. wt., which is 59 ± 32% of the average maximum tension achieved in metoprolol (10^{-7}M) plus histamine (10^{-3}M) infusion. This change was significant at $p < 0.05$. Fig. (6.b, Pg. 42) represents a trace of tension for metoprolol with histamine. The slight decrease in tension, from 3 mgs/mg. wt. to 2.28 mgs/mg. wt., occurs during the metoprolol infusion. However, metoprolol with histamine did not produce any increase in the tension. Washing with Krebs solution produces a gradual decrease in the tension, to less than the basal tension.

Fig. (8., Pg. 52) shows responses to metoprolol (10^{-7}M) plus histamine (10^{-3}M). The tension was expressed in the average percentage, relative to the average tension in Krebs solution, Krebs solution taken as 100%. According to the histogram, the average tension in histamine and metoprolol is 3% less than the tension in Krebs solution.

Our observations show that metoprolol (10^{-7}M) antagonizes the positive inotropic as well as the positive chronotropic effects of histamine (10^{-3}M), on the right atrium.

3.2.1.3 The effects of verapamil (10^{-6}M) plus histamine (10^{-3}M)

While the positive chronotropic effect of histamine (10^{-3}M) on the right atrium, was blocked by verapamil (10^{-6}M), the tension was not always blocked at this

concentration. Table (7., Pg. 57) shows that the average tension of 4.9 ± 2.7 mgs/mg. wt. in Krebs solution, decreased fractionally to 3.9 ± 2 mgs/mg. wt. in verapamil (10^{-6}M), thus being 94 ± 10% of the tension in Krebs solution. This decrease in tension was not considered significant at $p < 0.05$. The average half time for the decrease was 0.05 ± 0.08 minutes. Throughout the majority of the experiments, the tension did not decrease at all during the 2-3 minutes of the verapamil infusion. In six ~~out~~ of nine experiments, verapamil (10^{-6}M) did not block the histamine (10^{-3}M) positive inotropic effect on the right atria, while the positive chronotropic effects was blocked in all of the experiments (Table 3., Pg. 39). The average tension in verapamil (10^{-6}M) and histamine (10^{-3}M), during its 30 minutes infusion was found to be 5.5 ± 3.8 mgs/mg. wt. Even though this difference in tension, between verapamil (10^{-6}M) and verapamil (10^{-6}M) plus histamine (10^{-3}M) was found not to be significant at $p < 0.05$, the results showed, that the tension was not consistently blocked. Therefore this led us to use a higher concentration of verapamil to block the positive inotropic effect of histamine (10^{-3}M), which will be discussed separately.

The average of 5.5 ± 3.8 mgs/mg. wt. of tension in histamine (10^{-3}M) and verapamil (10^{-6}M), was 139 ± 43% of the average tension in verapamil, or 128 ± 34% of the

Table 7. Right atrium tension: verapamil (10^{-6}M) + histamine (10^{-3}M).

Number of Experiment	Krebs tension (mgs/mg. wt.)	verapamil (1.0×10^{-6}M) tension (mgs/mg. wt.)	verapamil (1.0×10^{-6}M) % of normal verapamil/krebs	verapamil (1.0×10^{-6}M) ½ time (min)	verapamil (10^{-6}M) + histamine (10^{-3}M) tension (mgs/mg. wt.)	% of control verapamil + histamine/ verapamil	% of normal verapamil + histamine/krebs	½ time (min)	Krebs tension (mgs/mg. wt.)	% of control krebs/verapamil + histamine
1	3.7	3.7	100%	0	3.0	81%	81%	0.2	1.5	49%
2	2.0	2.0	100%	0	1.6	80%	80%	0.2	1.4	87%
3	3.7	3.6	97%	≤0.05	6.0	166%	162%	0.5	2.7	46%
4	10.8	8.6	80%	0.2	14.0	163%	129%	0.3	5.4	38%
5	4.7	4.7	100%	0	6.1	130%	130%	0.6	2.8	46%
6	1.3	0.9	71%	0.2	1.8	200%	138%	0.5	0.8	46%
7	4.9	4.9	100%	0	8.6	176%	176%	1.6	3.9	46%
8	3.0	3.0	100%	0	4.5	150%	150%	0.3	2.5	55%
9	3.7	3.7	100%	0	3.9	105%	105%	0.05	3.2	84%
Mean ± S.D.	4.9 ± 2.7	3.9 ± 2	94 ± 10%	0.05 ± 0.08	5.5 ± 3.8	139 ± 43%	128 ± 34%	.0.47 ± 0.45	2.7 ± 1.4	55 ± 17.7

average tension in Krebs solution. The average half time for this change from verapamil to verapamil with histamine was 0.47 ± 0.45 min. A decrease in the tension followed washing of the preparation with Krebs solution. The tension decreased to an average of 2.7 ± 1.4 mgs/mg. wt., this decrease being significantly different at $p < 0.05$. This tension in Krebs solution was on an average of 55 ± 17.7% of the maximum tension in verapamil (10^{-6}M) plus histamine (10^{-3}M). Fig. (6.c, Pg. 42) respresents the experiment, in which one can observe the tendency for the tension in verapamil plus histamine to increase. In this experiment the tension did not change, being 4.7 mgs/mg. wt., which is the same as the tension in Krebs solution. In histamine plus verapamil the slight increase in tension begins after 15 min from the start of the infusion. The maximum tension was 6.1 mgs/mg. wt. The tension diminished gradually as the solution was changed to Krebs solution.

In Fig. (7b, Pg. 50), the tension in verapamil plus histamine versus the tension in verapamil is represented. Most of the points on the graph show a shift to the left side of the graph, indicating that verapamil (10^{-6}M) does not block the positive inotropic effect of histamine (10^{-3}M). In the histogram, Fig. (8., Pg. 52), the average percentage of verapamil and histamine relative to the tension in Krebs solution and to histamine average tension, can be observed. Krebs

solution is taken as 100%. The average increase in tension in verapamil (10^{-6}M) plus histamine (10^{-3}M) is less than an average response in histamine (10^{-3}M). However, comparing it to the tension in Krebs solution it is increased by 28%.

3.2.1.4 The effects of low Ca^{++} Krebs solution - (0.35 mM) plus histamine (10^{-3}M)

Lowering the [Ca^{++}] to 0.35 mM in Krebs solution, decreased the tension on the right atrium. Histamine (10^{-3}M), in low Ca^{++} Krebs solution does not show an increase in tension, while the increase in rate is observed, as discussed previously.

The average tension in Krebs solution, of 1.9 ± 0.3 mgs/mg. wt., decreased to 0.86 ± 0.3 mgs/mg. wt. at the end of 15 minutes infusion of low Ca^{++} Krebs solution (Table 8., Pg. 61). This was 44 ± 12% of the tension measured in the Krebs solution. The difference is significant at $p < 0.05$, with the average half time being 1.9 ± 0.5 min. When histamine (10^{-3}M) and low Ca^{++} Krebs, were infusing the preparation for 30 minutes, the average tension was calculated to be 0.8 ± 0.5 mgs/mg. wt., this being of no significant difference at $p < 0.05$. The average tension in low Ca^{++} Krebs solution plus histamine (10^{-3}M) was 93 ± 36% of the average tension in low Ca^{++}, Krebs solution or 41 ± 22% of the tension in Krebs solution. The average half time of 0.5 ± 0.4 minutes, was calculated for this slight change to occur, from low Ca^{++} Krebs solution to low Ca^{++} Krebs solution with histamine. Washing the preparation with low Ca^{++} Krebs solution, decreased the tension further to an average of 0.4 ± 0.2, this being 52 ± 12% of the tension in low Ca^{++} Krebs solution plus histamine. This

Table 8. Right atrium tension low Ca^{++} Krebs (0.35 mM) + histamine (10^{-3}M)

Number of Experiment	Krebs tension (mgs/mg. wt.)	low Ca^{++} Krebs tension (mgs/mg wt.)	low Ca^{++} Krebs % of normal low Ca^{++}/Krebs	low Ca^{++} Krebs ½ time (min)	low Ca^{++} Krebs + Histamine (10^{-3}M) tension (mgs/mg. wt.)	low Ca^{++} Krebs + Histamine (10^{-3}M) % of control low Ca^{++} + histamine/Low Ca^{++}	low Ca^{++} Krebs + Histamine (10^{-3}M) % of normal low Ca^{++} + histamine/Krebs	low Ca^{++} Krebs + Histamine (10^{-3}M) ½ time (min)	low Ca^{++} Krebs tension (mgs/mg. wt.)	low Ca^{++} Krebs % of control low Ca/Low Ca^{++} + histamine
1	1.4	0.5	35%	2.1	0.5	100%	36%	0.1	0.2	42%
2	2.1	1.3	61%	1.0	0.7	56%	33%	0.7	0.4	57%
3	2.1	0.7	32%	2.2	0.7	100%	33%	0	0.35	50%
4	1.8	0.7	39%	2.3	0.4	61%	22%	0.6	0.28	71%
5	2.0	1.1	53%	1.9	1.6	146%	80%	1.1	0.7	43%
Mean ± S.D.	1.9 ± 0.3	0.86 ± 0.3	44 ± 12%	1.9 ± 0.5	0.8 ± 0.5	93 ± 36%	41 ± 22%	0.5 ± 0.4	0.4 ± 0.2	52 ± 12%

difference is not significant at $p < 0.05$. When the preparation was washed with Krebs solution the tension returned to basal level.

Fig. (6.d, Pg. 42) represents a record from one of the experiments. This shows the decrease in tension from 2.1 mgs/mg. wt. in Krebs solution, to 0.7 mgs/mg. wt. in lower [Ca^{++}]. This low tension is not increased when histamine with low Ca^{++} Krebs was infused into the bath, thus indicating that the diminished [Ca^{++}], antagonized the histamine effect on the tension. The rate diminished only slightly (Fig. 5.b, Pg. 37) Fig. (7.c, Pg. 50) shows, that the tension in low Ca^{++} Krebs solution with histamine, in comparison to low Ca^{++} Krebs solution either maintains the same or decreases. Fig. (8., Pg. 52) also shows, that the average tension in low Ca^{++} Krebs solution with histamine is 60% lower than the average tension in Krebs solution and 88% lower than the average tension in verapamil plus histamine.

3.2.2 Paced right atrium

The right atrium was dissected, the pacemaker being removed so that the effects of the following substances, on the tension alone could be observed: histamine (10^{-3}M), verapamil (10^{-5}M) and low Ca^{++} Krebs solution (0.22 mM).

3.2.2.1 The effects of histamine (10^{-3}M)

Histamine (10^{-3}M) has a positive inotropic effect on the paced right atrium. Table (9., Pg. 63) shows,

Table 9. Paced right atrium tension: histamine (10^{-3}M).

Number of Experment	Krebs tension (mgs/mg. wt.)	histamine tension (mgs/mg. wt.)	histamine % of control histamine/Krebs	time (min)	Krebs tension (mgs/mg. wt.)	Krebs % of control Krebs/histamine
1	2.8	7.1	253%	1.4	1.8	25%
2	5.4	10.8	200%	1.2	2.25	20.8%
3	10.5	35.6	339%	2.4	8.4	23%
4	20.6	53.9	258%	2	15.8	29%
5	21.4	35.7	166%	0.8	14.2	40%
6	8.7	15.0	172%	1	4.9	33%
7	26.6	48.8	183%	1	15.5	31%
8	14.0	26.5	189%	0.8	9.3	35%
9	14.7	26.6	181%	1.7	7.7	28%
Mean ± S.D.	13.8 ± 7.8	28.8 ± 16.3	215% ± 57	1.4 ± 0.6	8.8 ± 5.4	29.4% ± 6%

that the average tension in Krebs solution of 13.8 ± 7.8 mgs/mg. wt., increased to an average of 28.8 ± 16.3 mgs/mg. wt. in histamine (10^{-3}M). That was calculated to be of significant difference at $p < 0.05$. This is an average of 215 ± 57% of the average tension in Krebs solution. The average half time for this increase was 1.4 ± 0.6 minutes, while the average half time of the right atrium with intact pacemaker was 1.3 ± 0.9 min (Table 5., Pg. 47). The average tension in Krebs solution was 8.8 ± 5.4 mgs/mg. wt., this being 29.4 ± 6% of the average maximum tension in histamine. This difference is significant at $p < 0.05$.

Fig. (9.a, Pg. 66) is an example of the tension response from one of the experiments. As histamine began to infuse the paced right atrium, a latency period of seven minutes was observed before the tension began to increase. In this particular experiment, the tension increased after a period of 10 minutes, from 5.4 mgs/mg. wt. in Krebs solution, to 10.8 mgs/mg. wt. in histamine, which is an increase of 100%. This tension returned to less than a basal value, in this particular case to 2.25 mgs/mg. wt., when washed with Krebs solution. This is a significant decrease at $p < 0.05$.

Fig. (10.a, Pg. 68) represents the tension in histamine (10^{-3}M), relative to the tension in Krebs solution. All the points on the graph show an increase in the tension in histamine.

Fig. (9.)

Those traces show:

(a) Effects of histamine (10^{-3}M), on the tension of the paced right atrium.

(b) Effects of verapamil (10^{-5}M) plus histamine (10^{-3}M), on the tension of the paced right atrium.

(c) Effects of low Ca^{++} Krebs solution plus histamine (10^{-3}M) on the tension of the paced right atrium.

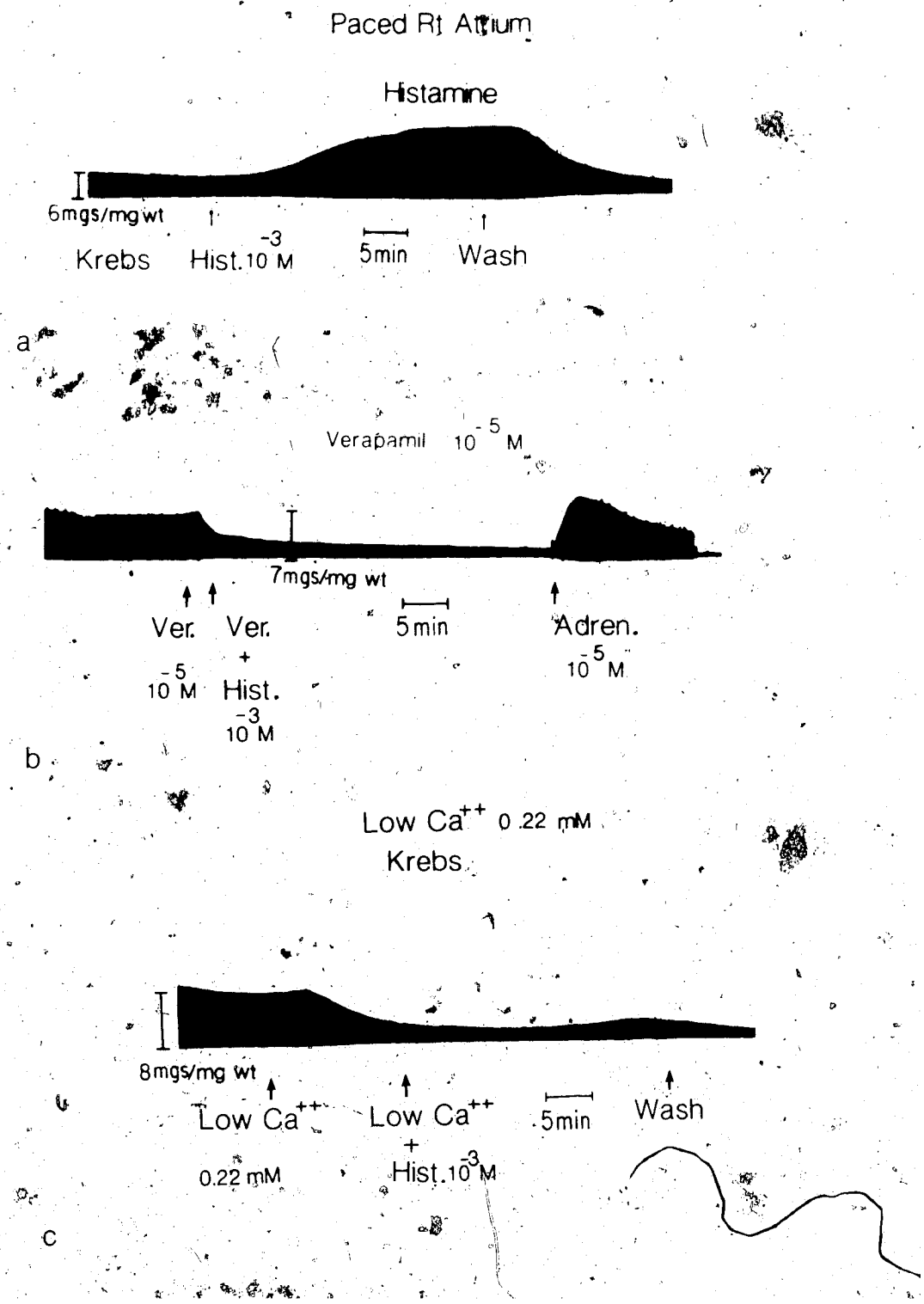
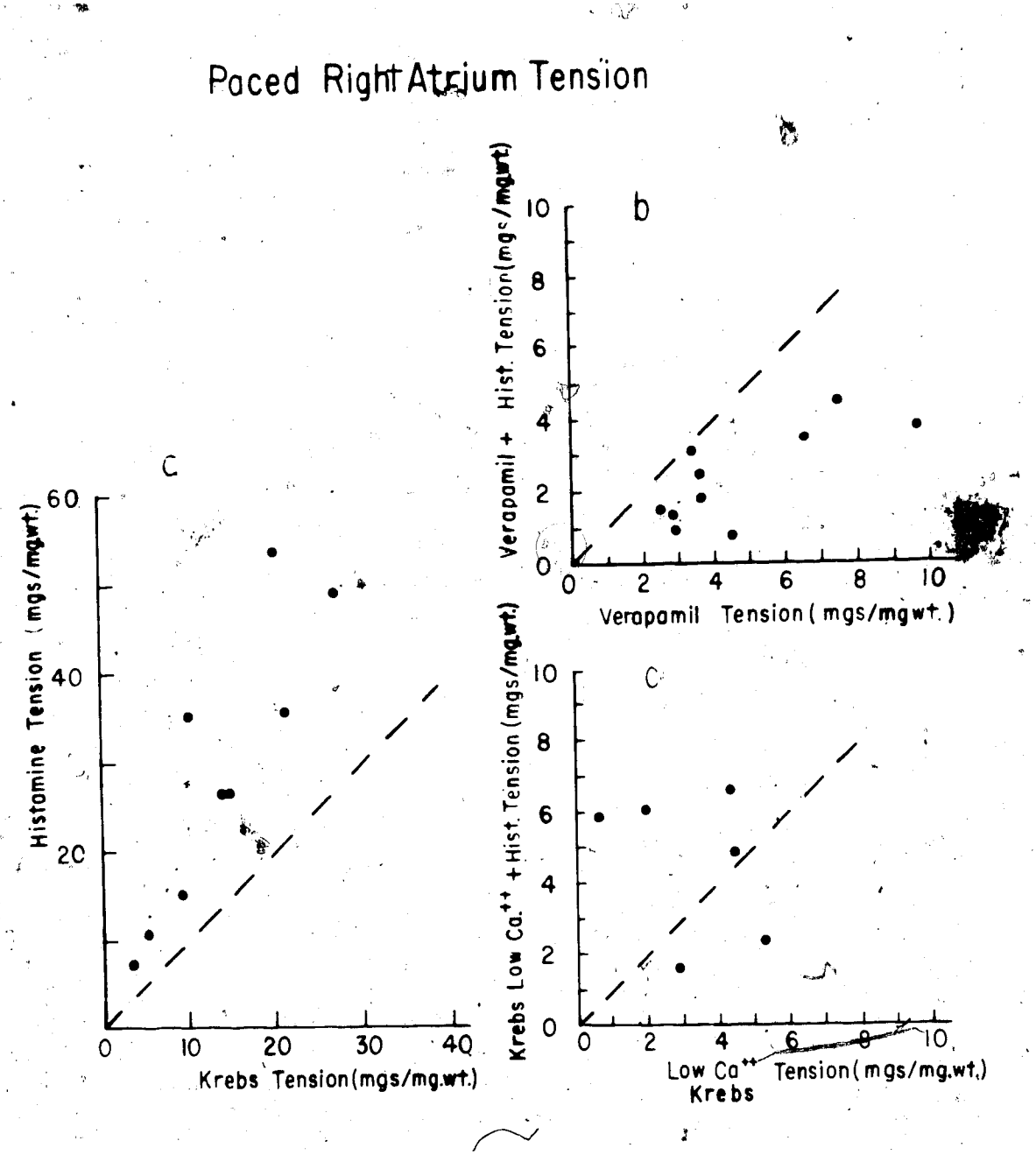

Paced Rt Atrium
Histamine
6mgs/mg wt
Krebs
Hist. 10^{-3} M
5min
Wash
a
Verapamil 10^{-5} M
7mgs/mg wt
Ver. 10^{-5} M
Ver. + Hist. 10^{-3} M
5min
Adren. 10^{-5} M
b
Low Ca^{++} 0.22 mM
Krebs
8mgs/mg wt
Low Ca^{++} 0.22 mM
Low Ca^{++} + Hist. 10^{-3} M
5min
Wash
c

Fig. (10.) represents:

(a) The tension of the paced right atrium paced in histamine (10^{-3}M), in relation to the tension in Krebs.

(b) The tension in verapamil (10^{-5}M) plus histamine (10^{-3}M), in relation to the tension in verapamil (10^{-5}M), on the paced right atrium.

(c) The tension in low Ca^{++} Krebs (0.22 mM) plus histamine (10^{-3}M), relative to the tension in low Ca^{++} Krebs solution (0.22 mM), on the paced right atrium. Line through origin represents equal activity ratios.


Paced Right Atrium Tension
b
Verapamil + Hist. Tension (mgs/mg.wt.)
Verapamil Tension (mgs/mg wt.)
C
Histamine Tension (mgs/mg.wt.)
Krebs Tension (mgs/mg.wt.)
C
Krebs Low Ca++ + Hist. Tension (mgs/mg.wt.)
Low Ca++ Krebs Tension (mgs/mg.wt.)

In the histogram (Fig. 11., Pg. 71), the average tension in Krebs solution is taken at 100%. Histamine, verapamil with histamine, and histamine in low Ca^{++} Krebs solution are represented relative to the tension in Krebs solution. Histamine (10^{-3}M) caused the tension to increase 115% from the average tension in Krebs solution.

3.2.2.2 The effects of verapamil (10^{-5}M) plus histamine (10^{-3}M)

The effects of verapamil (10^{-5}M) and histamine (10^{-3}M) on the tension of the paced right atrium were observed. After unsuccessful trials to completely block positive inotropic effect of histamine with verapamil (1×10^{-6}M), (1.5×10^{-6}M) and (1.8×10^{-6}M), verapamil (10^{-5}M) was used next, but with the short infusion time of two min. The mean tension in Krebs solution, was 10 ± 10 mgs/mg. wt. as shown in the Table (10., Pg. 7[illegible] This tension decreased to an average of 4.7 ± 2.4 in verapamil (10^{-5}M) infusion. This decrease in tension was significant at $p < 0.05$. At the end of this short time of verapamil (10^{-5}M) infusion, the tension decreased to 81 ± 48% of the average tension in Krebs solution, taking the tension in Krebs solution as 100%. The average half time for this decrease in tension was 0.7 ± 0.5 minutes. Histamine (10^{-3}M) plus verapamil (10^{-5}M), decreased the tension further, so that the average tension was 2.4 ± 1.3 mgs/mg. wt., which is 52.5 ± 20.1%

Fig. (11.)

This represents the average tension ± SEM in Krebs solution (taken as 100%) and relative to it the average tension in histamine (10^{-3}M), histamine (10^{-3}M) plus verapamil (10^{-5}M) and histamine (10^{-3}M) in low Ca^{++} Krebs solution (0.22 mM), on the paced right atrium.

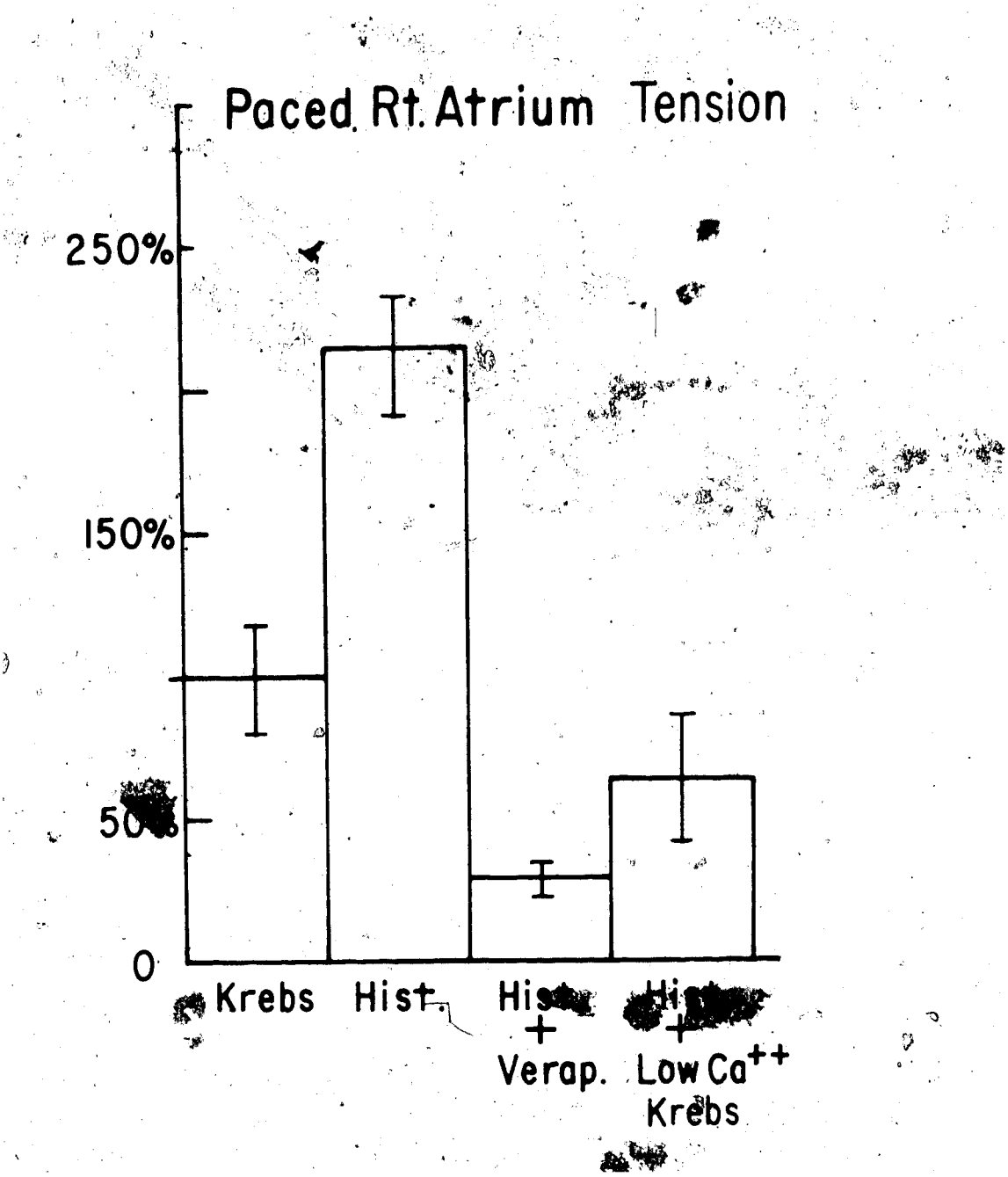

Paced Rt. Atrium Tension
250%
150%
50%
0
Krebs
Hist.
His
+
Verap.
His
+
Low Ca++
Krebs

Table 10. Paced right atrium tension: verapamil (10^{-5}M) + histamine (10^{-3}M).

Number of Experiment	Krebs tension (mgs/mg. wt.)	verapamil (10^{-5}M) tension (mgs/mg. wt.)	verapamil (10^{-5}M) % of control verapamil/krebs	verapamil (10^{-5}M) ½ time (min)	histamine (10^{-3}M) + verapamil (10^{-5}M) tension (mgs/mg. wt.)	histamine (10^{-3}M) + verapamil (10^{-5}M) % of control histamine + verapamil/verap.	histamine (10^{-3}M) + verapamil (10^{-5}M) % of control Histamine + verapamil/krebs	histamine (10^{-3}M) + verapamil (10^{-5}M) ½ time (min)	Adrenaline tension (mgs/mg. wt.)
1	14.4	9.6	66%	0.5	3.8	40%	26%	0.6	10.8
2	6.9	3.6	52%	1.0	1.8	50%	12%	0.6	12.6
3	4.4	3.4	78%	0.3	3.1	92%	70%	0.4	12.6
4	3.7	2.5	66%	0.4	1.5	61%	40%	0.7	
5	3.1	2.9	93%	0.1	0.96	33%	30%	1.0	3.29
6	6.2	3.6	58%	0.6	2.5	70%	40%	1.0	8.7
7	39	7.5	21%	2	4.5	60%	11%	0.1	
8	7.2	2.8	40%	0.9	1.4	51%	19%	0.3	
9	5.5	4.5	82%	0.3	0.7	15%	13%	1.0	6.5
10	10.0	6.5	65%	0.7	3.5	53%	35%	0.6	
Mean S.D.	10 ± 10	4.7 ± 2.4	81 ± 48%	0.7 ± 0.5	2.4 ± 1.3	52.5 ± 20%	29.6 ± 18%	0.6 ± 0.3	8.3 ± 3.6

verap. = Verapamil

of the average tension in verapamil (10^{-5}M) alone, or 29.6 ± 18% of the average tension in Krebs solution. This change in tension was found to be significant at $p < 0.05$ with the average half time of 0.6 ± 0.3 min.

In some experiments, adrenaline (10^{-5}M) was injected into the bath at the end of the experiment. The increase in tension was very large. As shown in Table (10., Pg. 72), the average tension in adrenaline was 8.3 ± 3.6 mgs/mg. wt. for five experiments. This was a four fold increase from the tension after verapamil plus histamine.

Fig. (9.b, Pg. 66) is a typical record for a experiment with verapamil (10^{-5}M). The tension of 6.9 mgs/mg. wt. started to decrease almost immediately as verapamil (10^{-5}M) was infused in the bath. The tension decreased to a value of 3.6 mgs/mg. wt., after two minutes. Infusion of the paced right atrium, with histamine (10^{-3}M) plus verapamil (10^{-5}M), did not increase the tension. Instead, the tension decreased even further to a value of 1.8 mgs/mg. wt. Adrenaline (10^{-5}M) increased the tension five times, after its injection into the preparation bath, thus reversing verapamil's negative inotropic effect.

Fig. (10.b, Pg. 68) shows all the points representing the tension of the paced right atrium in verapamil (10^{-5}M) with histamine (10^{-6}M), relative to the tension in verapamil (10^{-5}M). The points are below

the median line, to the right. This represents the supression of the tension in verapamil plus histamine.

In Fig. (11., Pg. 71), the tension in histamine (10^{-3}M) and verapamil (10^{-5}M) is presented in relation to the percentage of the tension in Krebs solution. Krebs solution is taken as 100%. The average tension in verapamil plus histamine is 70% less than the average tension in Krebs solution, and 185% less than the average maximum response in histamine (10^{-3}M) alone.

3.2.2.3 The effects of low Ca^{++} Krebs solution (0.22 mM)

The effects of low Ca^{++} Krebs solution of (0.22 mM) plus histamine (10^{-3}M) was observed on the tension of the paced right atrium. Lowering the concentration of Ca^{++} to (1.9 mM) and (0.5 mM), did not alter the positive inotropic reponse of histamine (10^{-3}M), except for the latency period of the response which was was prolonged.

Table (11., Pg. 75), shows that the tension decreased from an average of 9.3 ± 3.3 mgs/mg. wt. in Krebs solution to an average of 3.3 ± 1.7 mgs/mg. wt. in low Ca^{++} Krebs solution, this being significantly different at $p < 0.05$. The average half time for the decrease in tension was 1.7 ± 0.6 minutes. Tension in low Ca^{++} Krebs solution with histamine (10^{-3}M), was 4.7 ± 2 mgs/mg. wt., this being 40% higher than the average tension in low Ca^{++} Krebs solution. This is not found to be significantly different at $p < 0.05$. Comparing the

Table 11. Paced right atrium tension low Ca^{++} Krebs (0.22 mM) + histamine (10^{-3}M).

Number of Experiment	Krebs tension (mgs/mg. wt.)	low Ca^{++} Krebs (0.22 mM) tension (mgs/mg. wt.)	low Ca^{++} Krebs (0.22 mM) % of normal lCa/Krebs	low Ca^{++} Krebs (0.22 mM) ½ time (min)	low Ca (0.22 mM) + histamine 10^{-3} M tension (mgs/mg. wt.)	low Ca (0.22 mM) + histamine 10^{-3} M % of control lCa + h/lCa	low Ca (0.22 mM) + histamine 10^{-3} M % of normal lCa + h/Krebs	low Ca (0.22 mM) + histamine 10^{-3} M ½ time (min)	low Ca^{++} Krebs (0.22 mM) tension (mgs/mg. wt.)	low Ca^{++} Krebs (0.22 mM) % of control lCa/h + lCa
1	11.9	5.3	45%	1.1	2.4	45%	20%	0.5	2.4	100%
2	7.7	2.0	26%	2.5	6.1	305%	79%	1.8	1.1	18%
3	14.0	5.0	35%	1.4	5.5	110%	39%	0.1	2.0	36%
4	3.8	0.6	17%	2.5	5.9	980%	155%	4.2	0	0
5	7.9	2.9	37%	1.2	1.6	57%	20%	0.3	0.83	29%
6	11	4.4	40%	1.8	6.6	150%	60%	0.1	1.1	16%
Mean ± S.D.	9.3 ± 3.3	3.3 ± 1.7	33.3 ± 10%	1.7 ± 0.6	4.7 ± 2		62 ± 50%	1.4 ± 1.7	1.3 ± 0.8	31 ± 35%

lCa = Low Ca^{++}

h = Histamine

average percentage of the tension in low Ca^{++} Krebs solution with histamine ($10^{-3}M$), with the average tension in Krebs solution taken as 100%, it was found that it was 62 ± 50%. When washed with low Ca^{++} Krebs solution the tension significantly decreased to an average of 1.3 ± 0.8, or 31% of the tension in low Ca^{++} with histamine. Fig. (9.c, Pg. 66) is an example of an experiment in 0.22 mM concentration of Ca^{++}. In this particular case, the tension of 8 mgs/mg. wt. decreased by two thirds in low Ca^{++} Krebs solution. The slight increase in tension was observed after 25 minutes in histamine and low Ca^{++} Krebs solution.

Fig. (10.c, Pg. 68) shows that in relation to the tension in low Ca^{++} Krebs solution, the tension in low Ca^{++} Krebs solution with histamine ($10^{-3}M$) increased, the points being mostly on the left side of the median line.

In Fig. (11., Pg. 71) the decrease in the average tension in low Ca^{++} Krebs solution (0.22 mM) and histamine ($10^{-3}M$) is shown relative to the Krebs solution and to the average histamine ($10^{-3}M$) response. However, the average tension in low Ca^{++} Krebs solution with histamine increased, relative to the tension in histamine ($10^{-3}M$) with verapamil ($10^{-5}M$).

3.2.3 Left atrium

On the paced left atrium, the effects on the tension of histamine (10^{-3}M), metoprolol (10^{-7}M) plus histamine (10^{-3}M), verapamil (1.5×10^{-6}M) plus histamine (10^{-3}M) and low Ca^{++} Krebs plus histamine (10^{-3}M), were observed.

3.2.3.1 The effect of histamine (10^{-3}M)

Table (12. [illegible]78) represents the data for the experiments performed on the left atrium with histamine (10^{-3}M). The increase in the tension produced by histamine can be seen in this table. The average tension in Krebs solution being 15 ± 11 mgs/mg. wt., increased significantly at $p < 0.05$ to an average of 28 ± 20 mgs/mg. wt. This is 190 ± 64% of the tension in the Krebs solution. The average half time or maximum response is 1.3 ± 0.9 mgs/mg. wt., which is the same as the average half time for maximum response in the right atrium (1.3 mgs/mg. wt.) and paced right atrium (1.4 mgs/mg. wt.). The tension after washing in Krebs solution, decreased to an average of 10 ± 8, being 39 ± 10% of the maximum tension in histamine (10^{-3}M), and significantly different than histamine (10^{-3}M) average tension, at $p < 0.05$.

The example of the effect of histamine (10^{-3}M) on the left atrium tension is shown in Fig. (12.a, Pg. 80) After a period of seven minutes, the tension started to increase from 32 mgs/mg. wt. in Krebs solution, to reach its maximum of 65 mgs/mg. wt., after 20 minutes of

Table 12. Left atrium tension: histamine 10^{-3}M

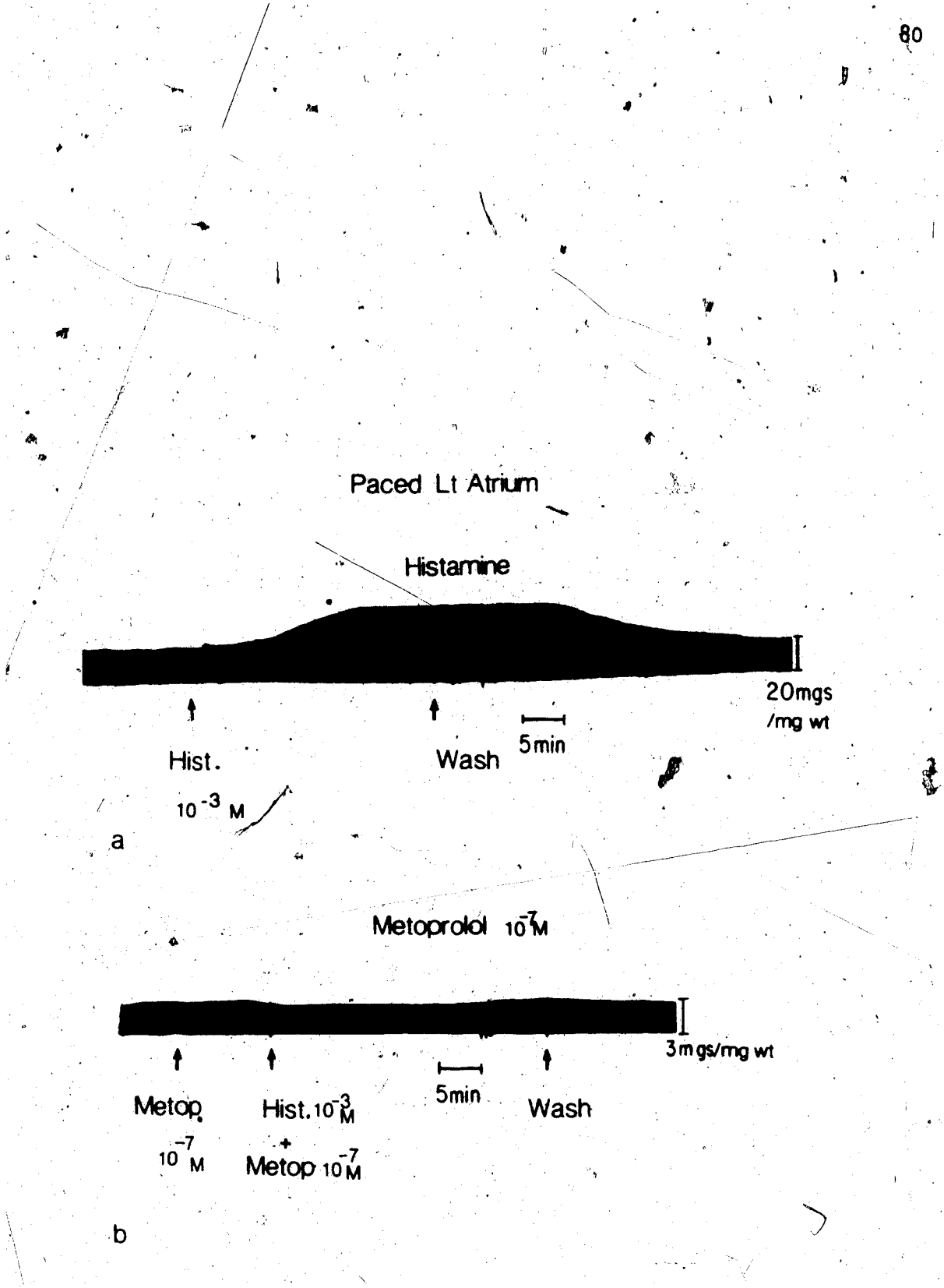
Number of Experiment	Krebs tension (mgs/mg. wt.)	histamine tension (mgs/mg. wt.	½ time (min)	% of control histamine/Krebs	Krebs tension (mgs/mg. wt.)	% of control Krebs/histamine
1	3.3	6.7	2	204%	2.1	31%
2	33	41.2	0.5	124%	21.4	52%
3	8.7	15.0	1	172%	4.9	33%
4	26.6	48.8	1	183%	15.5	31%
5	14.8	50.2	3	353%	12.5	24%
6	3.7	5.4	0.4	146%	3.2	60%
7	10.0	21.4	2.2	214%	8.0	37%
8	34.6	64.9	2	205%	26.6	41%
9	9.5	17.1	0.8	180%	5.7	33%
10	10.1	13.9	0.3	137%	6.2	44%
Mean S.D.	15 ± 11	28 ± 20	1.3 ± 0.9	190 ± 64%	10.6 ± 8	39 ± 10%

Fig. (12.)

The tracings show:

(a) The effect of histamine (10^{-3}M) on the tension of the left atrium.

(b) The effect of metoprolol (10^{-7}M) plus histamine (10^{-3}M) on the left atrium tension.


Paced Lt Atrium
Histamine
20mgs
/mg wt
Hist.
10 -3 M
Wash
5min
a
Metoprolol 10-7M
3mgs/mg wt
Metop.
10 -7 M
Hist. 10-3M
+
Metop 10-7M
5min
Wash
b

histamine. This maximum tension was maintained while histamine was being infused. After washing the atrium with Krebs solution, the tension gradually decreased to the basal value.

In Fig. (13.a, Pg. 84), the potentiating effects of histamine on the left atrium tension can be observed, since all of the points, except one, fall to the left of the median line.

In Fig. (14., Pg. 86) the positive inotropic effect of histamine (10^{-3}M) is shown relative to the average tension in Krebs solution, where average tension in Krebs is taken as 100%. It can be observed that the tension in histamine shows an increase of 90% from the tension in Krebs solution.

Fig. (15., Pg. 88) shows the computer output of an individual contraction in Krebs solution and in histamine (10^{-3}M). Certain parameters observed in Krebs solution and in histamine for the single contraction are shown in Table (13., Pg. 82).

This data shows that histamine (10^{-3}M), decreases the latency period, contraction time as well as relaxation time and total contraction time. The peak tension as well as the rate of contraction and relaxation are increased approximately two fold from Krebs solution.

Table 13. Left atrium - single contraction analysis for histamine (10^{-3}M) expressed as % of control (Krebs).

	% histamine 10^{-3}M/control (Krebs)
latency period	89%
contraction time	93%
relaxation time	91%
total twitch time	88%
peak tension	270%
rate of contraction/msec	321%
rate of relaxation/msec	325%

Fig. (13.)

(a) The tension of the left atrium in histamine (10^{-3}M) in relation to Krebs.

(b) The tension of histamine (10^{-3}M) plus verapamil (1.5×10^{-6}M) on the left atrium in relation to the tension in verapamil (1.5×10^{-6}M).

(c) The tension of histamine (10^{-3}M) plus low Ca^{++} Krebs solution on the left atrium relative to the tension in low Ca^{++} Krebs solution (0.22 mM). The line through origin represents equal activity ratios.

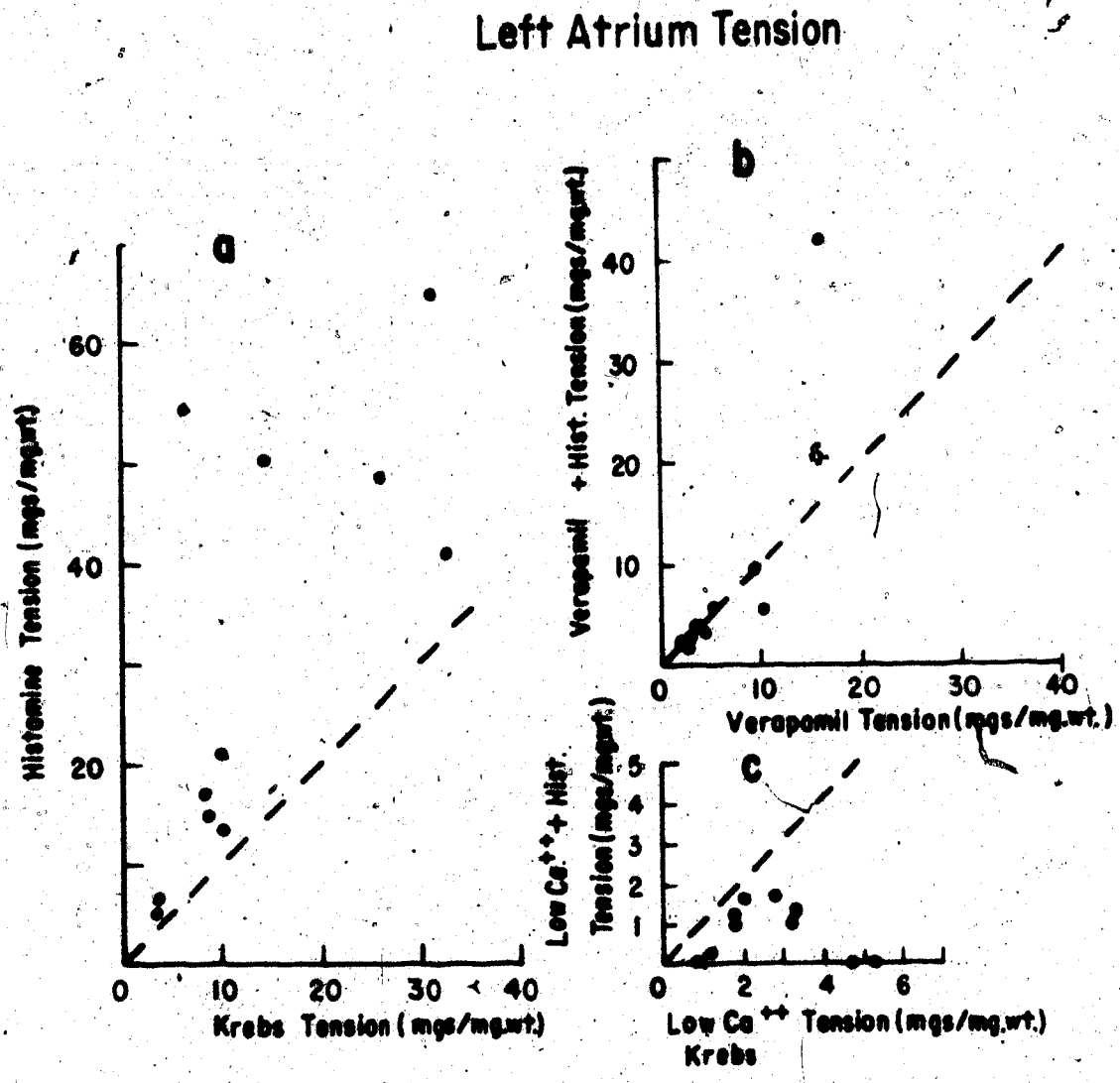

Left Atrium Tension
a
Histamine Tension (mgs/mg.wt.)
Krebs Tension (mgs/mg.wt.)
b
Verapamil + Hist. Tension (mgs/mg.wt.)
Verapamil Tension (mgs/mg.wt.)
c
Low Ca++ + Hist. Tension (mgs/mg.wt.)
Low Ca++ Tension (mgs/mg.wt.)
Krebs

Fig. (14.) is a histogram showing the average tension of the left atrium in Krebs ± SEM (taken as 100%) and relative to it the average tension ± SEM in histamine (10^{-3}M), histamine (10^{-3}M) plus metoprolol (10^{-7}M), histamine (10^{-3}M) plus verapamil (1.5×10^{-6}M) and histamine (10^{-3}M) plus low Ca^{++} Krebs solution (0.22 mM).

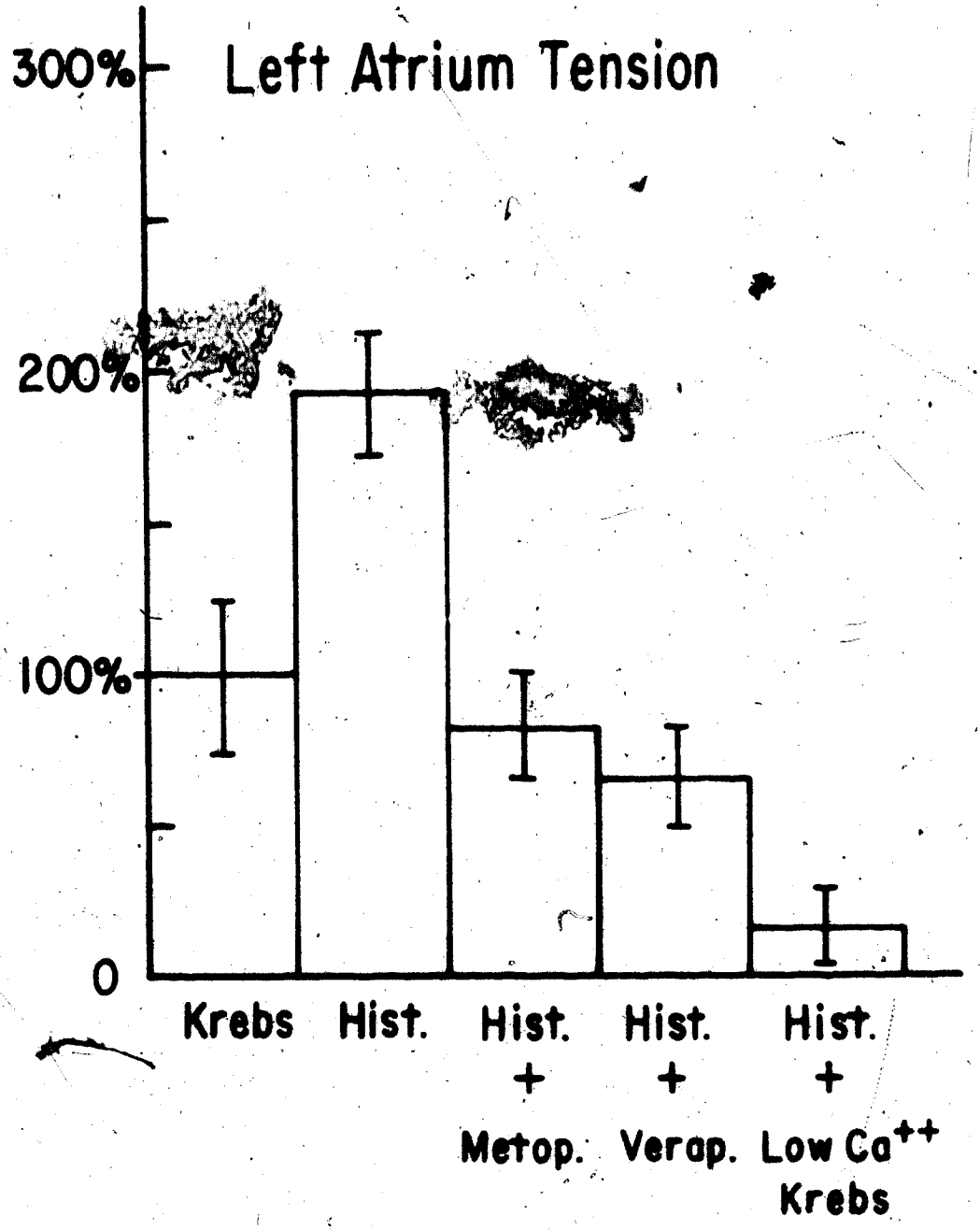

Left Atrium Tension
300%
200%
100%
0
Krebs
Hist.
Hist. + Metop.
Hist. + Verap.
Hist. + Low Ca++ Krebs

Fig. (15.) shows the computer output of the individual contractions per msec of the left atrium in Krebs solution (solid line) and histamine 10^{-3}M infusion (dotted line).

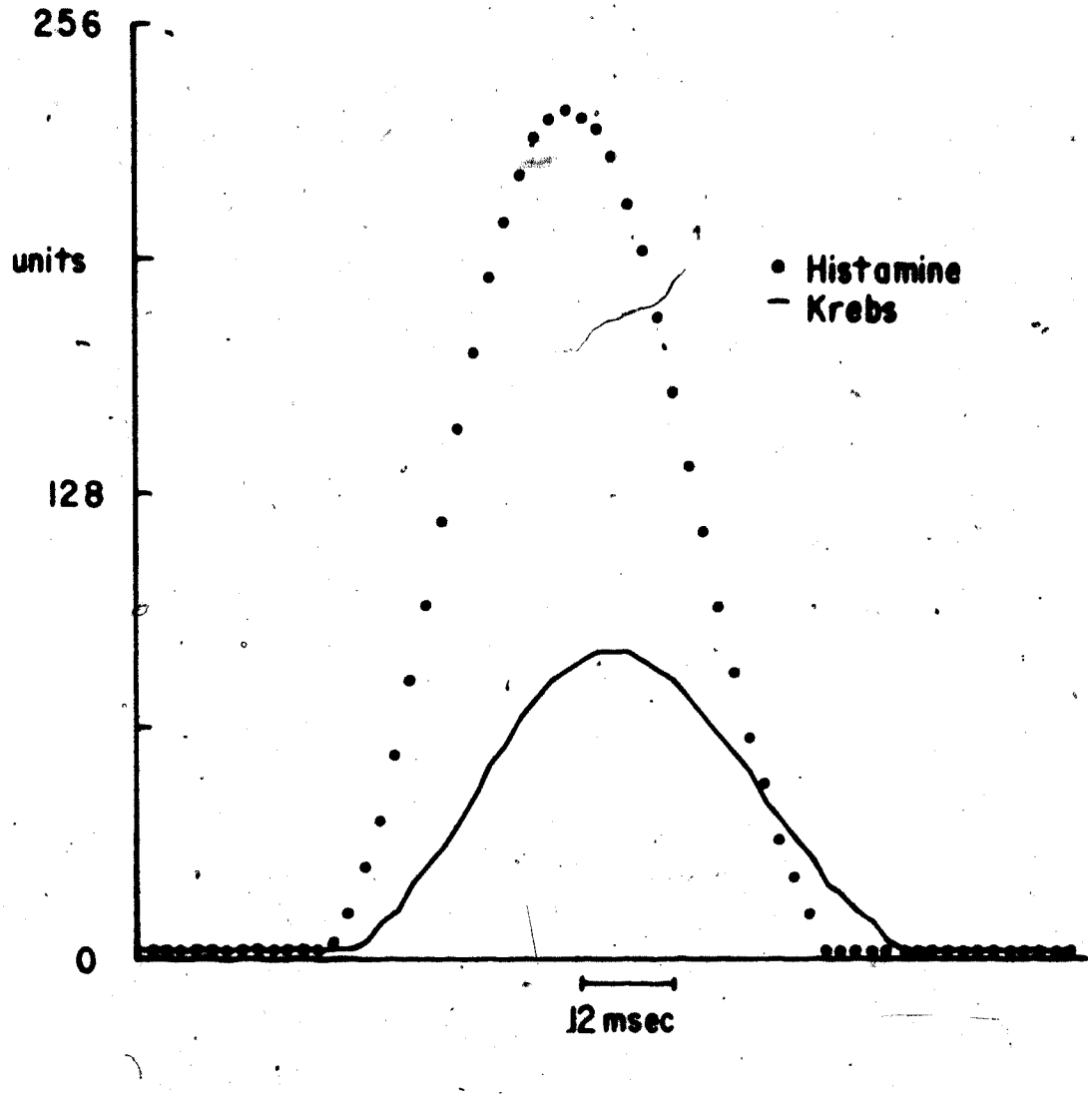

256
units
128
0
• Histamine
— Krebs
12 msec

Table 14. Left atrium - metoprolol (10^{-7}M) + histamine (10^{-3}M)

Number of Experiment	Krebs tension (mgs/mg. wt.)	metoprolol (10^{-7}M) tension (mgs/mg. wt.)	% of control metop./Krebs	½ time (min)	histamine (10^{-3}) + metoprolol (10^{-7}) tension (mgs/mg. wt.)	% of control histamine + metop./metop.	½ time (min) (max)	Krebs tension (mgs/mg. wt.)	% of control Krebs/histamine + metop.
1	8.5	6.8	80%	0.4	7.5	108%	0.1	5.6	77%
2	5.9	6.2	105%	0.1	4.9	79%	0.5	4.9	100%
3	7.6	6.9	91%	0.2	3.4	50%	1.0	2.4	71%
4	8.3	8.6	104%	0.1	8.4	101%	0.1	7.8	94%
Mean ± S.D.	7.5 ± 1.2	7 ± 1.1	95 ± 11%	0.2 ± 0.1	6.2 ± 2.5	86 ± 27%	0.4 ± 0.4	5 ± 2	83 ± 12%

metop. = metoprolol

3.2.3.2 The effects of metoprolol (10^{-7}M) plus histamine (10^{-3}M)

Metoprolol (10^{-7}M), blocked the positive inotropic effect of histamine (10^{-3}M), on the left atrium in all experiments. Table (14., Pg. 89) shows, that the average tension of 7.5 ± 1.2 mgs/mg. wt. in Krebs solution solution decreased slightly to an average of 7 ± 1.1 mgs/mg. wt., this being 95 ± 11% of the average tension in Krebs solution. The difference was not significant at $p < 0.05$. The average half time for the decrease was 0.2 ± 0.1 min. Histamine plus metoprolol did not significantly decrease the mean tension at $p < 0.05$. The average tension was 6.2 ± 2.5 mgs/mg. wt. in histamine plus metoprolol. This was found to be 86 ± 27% of the average tension in metoprolol. The average half time for this value was 0.4 ± 0.4 min. Washing with Krebs solution, usually diminished the tension further to an average of 5 ± 2 mgs/mg. wt., this being 83 ± 12% of the tension in histamine plus metoprolol. The tension in Krebs solution wash, was also 44% less than the basal tension.

Fig. (12.b, Pg. 80) represents one of such experiments in which the tension response can be observed. In this particular experiment metoprolol (10^{-7}M) did not decrease the tension markedly. Histamine (10^{-3}M) with metoprolol (10^{-7}M) decreased the tension fractionally. At the end of 30 minutes of infusion, the

tension increased slightly, reaching the value of 8.4 mgs/mg. wt. as it was in Krebs solution.

The average tension in histamine (10^{-3}M) plus metoprolol (10^{-7}M) is shown in Fig. (14., Pg. 86), relative to the average tension in Krebs solution, Krebs solution taken as 100%. This shows the decrease in the average tension of histamine plus metoprolol from the average tension in Krebs solution and from histamine (10^{-3}M).

3.2.3.3 The effects of verapamil (1.5×10^{-6}M) plus histamine (10^{-3}M)

Various concentrations of verapamil were used in the experiments, to block the histamine (10^{-3}M) positive inotropic effect on the left atrium, Verapamil (1×10^{-6}M), (1.4×10^{-6}M) were used, but none blocked consistently the effect of histamine on the tension. However verapamil (1.5×10^{-6}M) antagonized the positive inotropic effect of histamine, in the majority of the experiments.

Table (15., Pg. 92) shows nine experiments, of which only one demonstrates the positive inotropic effect of histamine in the presence of verapamil. The average tension of 10 ± 7.6 mgs/mg. wt. in Krebs solution, decreased slightly to an average of 6 ± 4.7 mgs/mg. wt., in verapamil infusion of 15 minutes. This was not a significant difference at $p < 0.05$. The average tension in verapamil decreased to 60 ± 12% of

Table 15. Left atrium tension, histamine 10^{-3}M + verapamil 1.5×10^{-6}M.

Number of Experiment	Krebs tension (mgs/mg. wt.)	verapamil (1.5×10^{-6}M), histamine (10^{-3}M) + verapamil (1.5×10^{-6}M) tension (mgs/mg. wt.)	% of control verapamil/Krebs	½ time (min)	tension (mgs/mg. wt.)	% of control histamine + verap./verap.	% of normal histamine + verap./Krebs	½ time (min)	Krebs tension (mgs/mg. wt.)	% of control Krebs/histamine + verapamil
1	24.7	16	65%	0.8	42.5	265%	172%	2.9	7.3	17%
2	8.3	5.3	64%	0.5	5.9	111%	71%	0.1	2.4	40%
3	5.4	2.8	53%	0.8	2.8	100%	51%	0	1.6	56%
4	20.0	8.6	43%	1.3	9.5	111%	47%	0.1	2.6	27%
5	5.8	3.4	58%	0.5	2.9	85%	50%	0.1	1.9	66%
6	4.0	2.1	53%	0.6	2.1	100%	53%	0	1.5	78%
7	3.6	2.2	61%	0.5	2.3	104%	63%	0.05	1.4	60%
8	12.6	10,5	83%	0.2	5.2	50%	41%	0.4	4.2	80%
9	5.5	4.1	75%	0.4	3.1	75%	56%	0.3	2.4	77%
Mean ± S.D.	10 ± 7.6	6 ± 4.7	60 ± 12%	0.6 ± 0.3	8.5 ± 1.3	111 ± 60%	85 ± 40%	0.4 ± 0.9	2.8 ± 1.9	33 ± 22%

verap. = Verapamil

the tension in Krebs solution with the average half time of 0.6 ± 0.3 minutes. Histamine (10^{-3}M) plus verapamil (1.5×10^{-6}M), slightly increased the tension to an average, of 8.5 ± 1.3 mgs/mg. wt., which is 111 ± 60% of the average tension in verapamil or 85 ± 40% of the tension in Krebs solution. This difference in the average tension from verapamil was not found to be significant at $p < 0.05$. The average tension in Krebs solution wash was 2.8 ± 1.9 mgs/mg. wt., this being 33 ± 22% of the tension in histamine with verapamil and not significant at $p < 0.05$. Fig. (16.a, Pg. 95) shows the effects of verapamil (1.5×10^{-6}M) and histamine (10^{-3}M) on the left atrium tension. Tension decreased in verapamil, by one third. During histamine plus verapamil infusion, the tension did not change. However after 15 minutes of infusion it increased slightly by 0.6 mgs/mg. wt., from the tension in verapamil. Washing with Krebs solution, decreased the tension further to one third of the basal tension in Krebs solution.

In Fig. (13.b, Pg. 84) one can observe that the points, representing the tension in verapamil (1.5×10^{-6}M) with histamine (10^{-3}M) relative to verapamil (1.5×10^{-6}M), are placed along the mid-line of 45° angle. This shows that the tension was neither potentiated nor suppressed by verapamil (1.5×10^{-6}M) plus histamine (10^{-3}M).

Fig. (16.)

These tracings represent:

(a) The tension in verapamil (1.5×10^{-6}M) plus histamine (10^{-3}M), on the left atrium.

(b) The tension in low Ca^{++} Krebs solution (0.22 mM) with histamine (10^{-3}M), on the left atrium.

(c) The tension in low Ca^{++} Krebs solution (0.55 mM) with histamine (10^{-3}M), on the left atrium.

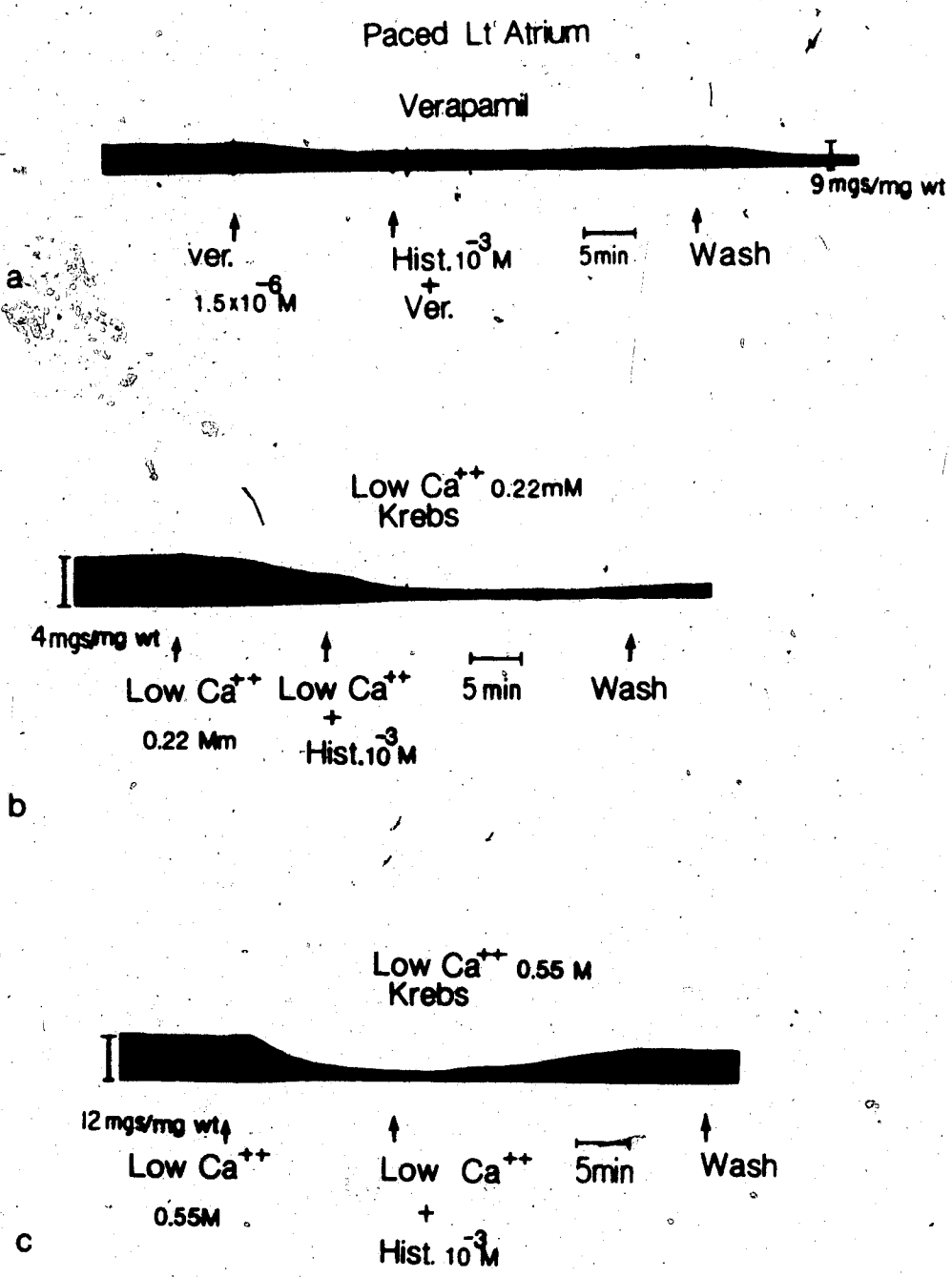

Paced Lt' Atrium
Verapamil
9 mgs/mg wt
ver.
1.5x10⁻⁶M
Hist.10⁻³M
+
Ver.
5min
Wash
a
Low Ca⁺⁺ 0.22mM
Krebs
4mgs/mg wt
Low Ca⁺⁺
0.22 Mm
Low Ca⁺⁺
+
Hist.10⁻³M
5 min
Wash
b
Low Ca⁺⁺ 0.55 M
Krebs
12 mgs/mg wt
Low Ca⁺⁺
0.55M
Low Ca⁺⁺
+
Hist. 10⁻³M
5min
Wash
c

Fig. (14., Pg. 86) shows that verapamil (1.5×10^{-6}M) with histamine (10^{-3}M) decreases the average tension by 35% from the average tension in Krebs solution. The difference between the average tension in histamine, compared to an average tension in histamine with verapamil, indicates that verapamil at this concentration, in most of the cases, blocks the positive inotropic effect of histamine.

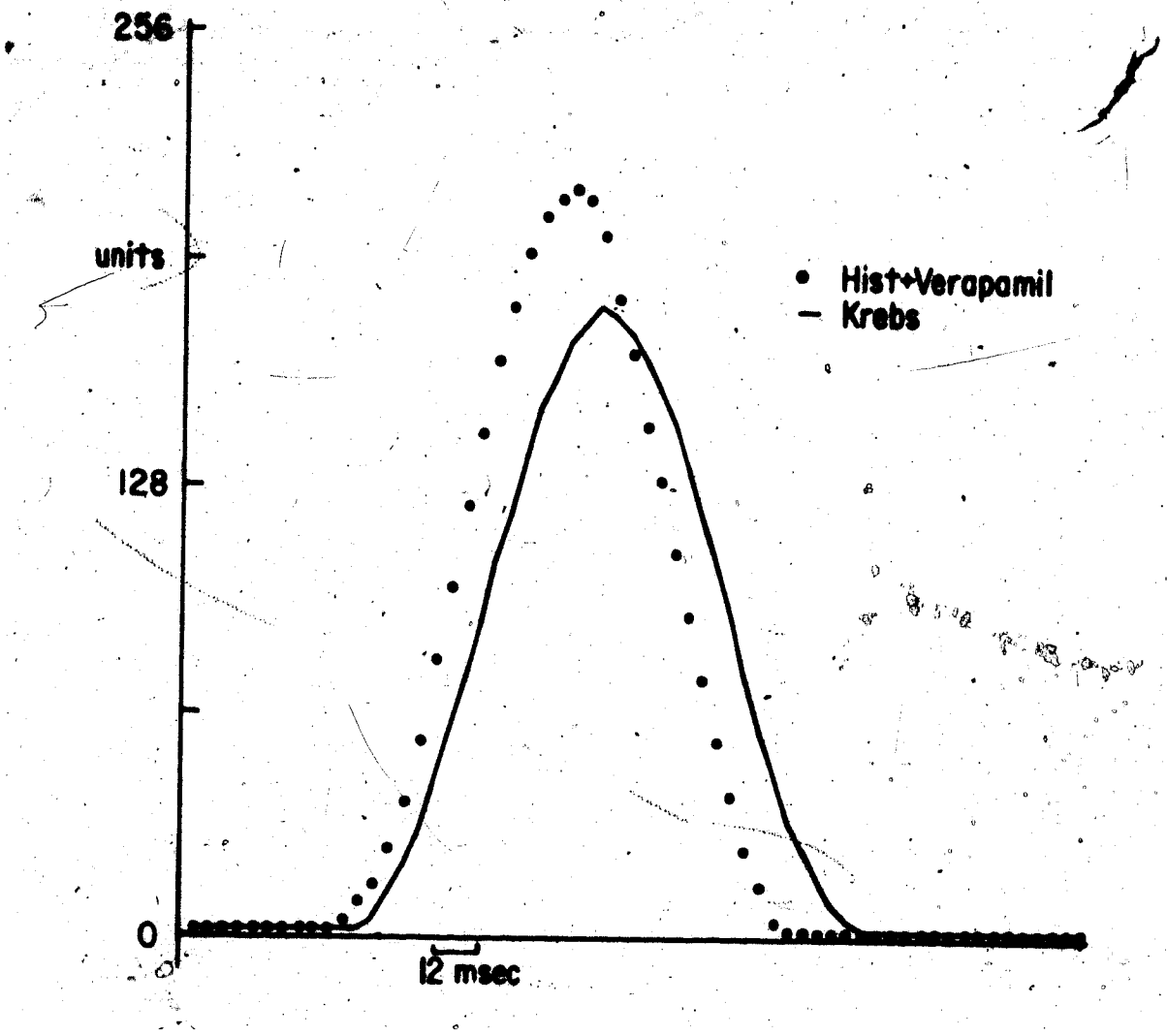
Fig. (17., Pg. 99) is a computer output of two single contractions. One is in Krebs solution, which is represented by the solid line. The second contraction, in verapamil plus histamine, is represented by the dotted line. Data for this computer output is represented in Table (16., Pg. 97).

It can be observed that contraction time is longer in histamine (10^{-3}M) plus verapamil (1.5×10^{-6}M) than in Krebs solution. However, the relaxation time is shorter. The total contraction time in histamine plus verapamil is longer than in Krebs solution. The peak tension and the rate of contraction is not increased greatly in histamine plus verapamil, relative to Krebs solution. The rate of relaxation is the same as in Krebs solution. Comparing these values with the values for histamine alone in Table (13., Pg. 82), it can be observed that verapamil does suppress the positive inotropic effect of histamine on the left atrium.

Table 16. Left atrium - single contraction analysis for histamine (10^{-3}M) + verapamil (1.5×10^{-6}M) expressed as % of control (Krebs).

	% histamine 10^{-3} + verapamil 1.5×10^{-6}M/control (Krebs)
latency period	81%
contraction time	107%
relaxation time	82%
total twitch time	116%
peak tension	119%
rate of contraction/msec	122%
rate of relaxation/msec	100%

Fig. (17.) is a computer output of two single contractions. One is in Krebs solution, which is represented by the solid line. The second contraction in verapamil plus histamine is represented by the dotted line. Data for this computer output is represented in Table (16., Pg. 97).


256
units
128
0
12 msec
• Hist+Verapamil
— Krebs

3.2.3.4 The effects of low Ca^{++} Krebs (0.22 mM) plus histamine (10^{-3}M)

The effects of lowering the [Ca^{++}] to (1.2mM), (0.5 mM) and (0.22 mM), were observed on the paced left atrium. Concentrations of (1.2 mM) and (0.5 mM) did not antagonize effects of histamine (10^{-3}M), on the left atrium tension.

In Table (17., Pg. 101) the effects of low Ca^{++} Krebs solution of (0.22 mM) with histamine (10^{-3}M) can be observed. Lowering the [Ca^{++}] to one eighth of its normal concentration, diminished the tension from an average of 5.8 ± 2.6 mgs/mg. wt. in Krebs solution, to an average of 2.7 ± 1.4 mgs/mg. wt. in low Ca^{++} Krebs solution (0.22 mM). The decrease is considered significant at $p < 0.05$. Therefore this indicates that the tension in low Ca^{++} decreased to 45 ± 10% of the tension in Krebs solution. The average half time for this decrease was 1.7 ± 0.6 min. The positive inotropic effect of histamine was blocked with low Ca^{++} Krebs solution (0.22 mM). Thirty minutes of infusion with histamine plus low Ca^{++} Krebs solution, decreased the tension further to an average of 0.84 ± 0.7 mgs/mg. wt., this being 36.8 ± 30% of the tension in low Ca^{++} Krebs solution or 15 ± 14% of the tension in Krebs solution. This change was found to be significant at $p < 0.05$. The average half time for this decrease in tension was 0.7 ± 0.4 minutes.

Table 17. Left atrium tension low Ca^{++} Krebs (0.22 mM) + histamine (10^{-3}M).

Number of Experiment	Krebs tension (mgs/mg. wt.)	low Ca^{++} Krebs (0.22 mM) tension (mgs/mg. wt.)	low Ca^{++} Krebs (0.22 mM) % of normal ICa Krebs/Krebs	low Ca^{++} Krebs (0.22 mM) ½ time (min)	low Ca^{++} Krebs (0.22 mM) + histamine (10^{-3}M) tension (mgs/mg. wt.)	low Ca^{++} Krebs (0.22 mM) + histamine (10^{-3}M) % of control ICa Krebs + hist./ICa Krebs	low Ca^{++} Krebs (0.22 mM) + histamine (10^{-3}M) % of normal ICa Krebs + hist. /Krebs.	½ time for change (min)
1	8.2	5.2	63%	0.9	0	0%	0%	1.4
2	4.5	2.0	44%	1.7	1.6	80%	35%	0.2
3	2.3	1.2	52%	1	0.3	25%	13%	0.6
4	4.5	1.8	40%	2.3	1.3	72%	28%	0.4
5	4	1.8	45%	1.4	1.0	55%	25%	0.5
6	4.8	2.8	58%	1.2	1.8	64%	37%	0.6
7	8.5	3.3	[illegible]	2.5	1.4	42%	16%	0.6
8	3.5	0.9	[illegible]	2.6	0	0%	0%	0.6
9	8	[illegible]	[illegible]	2.0	1	30%	12.5%	1
10	10	[illegible]	47%	1.4	0	0%	0%	1.2
Mean ± S.D.	5.8 ± 2.6	2.7 ± 1.4	[illegible] 10%	1.7 ± 0.6	0.84 ± 0.7	36.8 ± 30%	15 ± 14%	0.7 ± 0.4

ICa = Low Ca^{++}

hist. = Histamine

Fig. (16.b, Pg. 95) is an example of the tension record from one of the experiments, in low Ca^{++} Krebs (0.22 mM) with histamine (10^{-3}M). It shows the decrease in the tension in low Ca^{++} Krebs solution. Histamine plus low Ca^{++} does not show any response in tension, but rather a decrease in tension to a lesser value (1.3 mgs/mg. wt.), which is one fifth of the tension in Krebs solution (4 mgs/mg. wt.). Fig. (16.c, Pg. 95) represents the tension response of the left atrium in low Ca^{++} Krebs solution of (0.55 mM). The tension decreased in low Ca^{++} Krebs solution alone. Histamine (10^{-3}M) in low Ca^{++} Krebs solution caused an increase in the tension. This response in tension was less than the normal histamine (10^{-3}M) response. Latency period for a response to histamine was longer and the rate of increase in tension was slower. The maximum tension was achieved, after 25 minutes in histamine plus low Ca^{++} Krebs solution. The time to reach the maximum tension, compared to the normal histamine response, as shown in Fig. (9.a, Pg. 66) was slower by 3-5 minutes.

Fig. (13.c, Pg. 83) shows the tension in low Ca^{++} Krebs solution (0.22 mM), with histamine (10^{-3}M), relative to the tension in low Ca^{++} Krebs solution (0.22 mM). The points on the graph are all shifted to the right of the median line, this indicating the supression of the tension in low Ca^{++} Krebs solution plus histamine.

Fig. (14., Pg. 86) also shows the average tension in low Ca^{++} Krebs solution (0.22 mM) and histamine (10^{-3}M) with verapamil (1.5×10^{-8}M). The average tension in the low Ca^{++} Krebs with histamine solution is markedly less, compared to the average tension in Krebs solution or histamine.

Computer output of the single contraction of the left atrium is presented in Fig. (18., Pg. 105). Solid line represents the single contraction in msec in Krebs solution, while dotted line is a single contraction in msec, in low Ca^{++} Krebs solution (0.55 mM). Table (18., Pg. 106) shows various parameters involved in the contraction. Analysis of single contraction in low Ca^{++} Krebs solution (0.55 mM) is represented as a percentage of the control (Krebs solution).

Lowering the $[Ca^{++}]$ of Krebs solution to (0.55 mM), increased slightly the relaxation time, while the peak tension, rate of contraction as well as the rate of relaxation decreased by two thirds, compared to the same in Krebs solution. Contraction time in low Ca^{++} Krebs solution, is also less then the contraction time in Krebs solution, since the peak tension is lower in low Ca^{++} Krebs solution than in Krebs solution. This data indicates, that the lowering $[Ca^{++}]$ to one quarter of its normal concentration decreases the force of the single contraction.

Fig. (18.) represents the computer output of the single contraction curve per msec, of the left atrium in Krebs and low Ca^{++} (0.55 mM) Krebs.

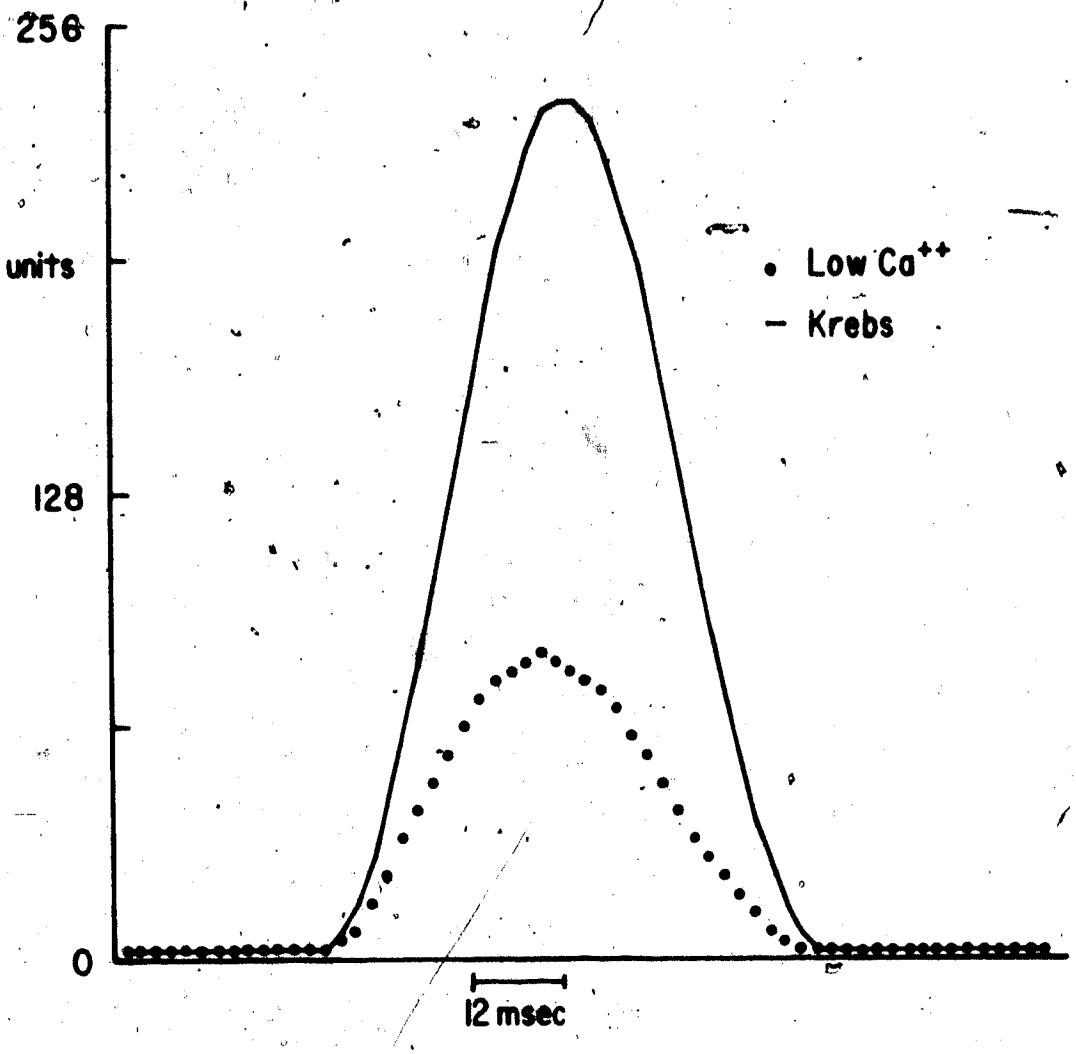

256
units
128
0
• Low Ca++
– Krebs
12 msec

Table 18. Left atrium - single contraction analysis for low Ca^{++} Krebs 0.55 mM expressed as % of control (Krebs).

	% low Ca^{++} Krebs (0.55 mM)/control
latency period	100%
contraction time	88%
relaxation time	106%
total twitch time	97%
peak tension	35%
rate of contraction/msec	38%
rate of relaxation/msec	33.3%

4. DISCUSSION

Our observations showed that histamine (10^{-3}M) has a positive chronotropic and inotropic effect on the rat's right and left atria. It appears that the rate and force of contraction are interrelated. Thus, in the right atrium there is at first an increase in the tension with the slight increase in the rate. Once the tension reaches its maximum, the rate then begins to rise, reaching higher values. This can sometimes cause a slight decrease in the tension. On the rat left atrium and the paced right atrium the positive inotropic effect often produces a two fold increase from the basal tension.

Metoprolol was shown to block the positive chronotropic and inotropic effect of histamine (10^{-3}M) on the right and left atria. Verapamil (1×10^{-6}M) antagonized the positive chronotropic effect on the right atrium. However the positive inotropic effect of histamine (10^{-3}M) on the paced right atrium, was blocked by verapamil (1×10^{-5}M). The tension increase due to histamine (10^{-3}M) on the left atrium was antagonized by verapamil (1.5×10^{-6}M).

Data presented also shows, that the tension in the left and right atria is affected by lowering the $[Ca^{++}]$. Decreasing the $[Ca^{++}]$ to one eighth of its normal concentration, antagonized the positive inotropic effect of histamine on the right and left atria, this causing the tension to decrease to lower values, while the positive chronotropic effect on the right atrium was not found to be

very sensitive to the lower Ca^{++} concentrations.

Our results are not in agreement with the findings of Bartlet (1963), in which he observed the depressant effect of histamine on the Langendorff prefused rat heart. One of the reasons for his findings may be due to the difference in the technique used to administer the histamine, this being by injection of histamine into the perfusion fluid, thus limiting the time for histamine to have an effect on the heart. However, the concentrations of histamine used by Bartlet (1963), were also much lower and this could also account for such finidings. Dai (1976), also found that histamine produced a transient decrease in the rate and amplitude of contractions, on the Langendorff perfused rat heart. The concentrations of histamine that he used in his experiments were also low, thus the highest dose being ($4 \times 10^{-7}M$), which could have been the reason for the differences between our results and his.

Satayavivad (1977), reported that rat atria responds best to $ED_{50} = (1.65 \pm 0.68 \times 10^{-3}M)$. This correlates well with our findings since we have used histamine ($10^{-3}M$) to produce a marked response in the tension and rate of the left and right atria. Therefore, one can agree with the report from Korosec and Erjavec (1978), who observed this low sensitivity of the rat to histamine. In contrast to the rat heart, guinea pig heart is much more sensitive to histamine and it can respond to the concentration of histamine ($10^{-6}M$), Verma and McNeill (1976).

Laher and McNeill (1980c), found that at high doses (10^{-3}M), (10^{-2}M), histamine produces increase in both the rate and the force of contraction, which agrees with our findings. By administering histamine to the preparation via injection, their average rate increased from 260 ± 6 beats/min in control, to 286 ± 8 beats/min in histamine. Our results show an increase in the rate from an average of 248 ± 21 from Krebs solution to 373 ± 27 beats/min in histamine which is a significant difference at $p < 0.05$. This difference in the average increase in the rate, between our findings and McNeill's, was due to the greater time over which histamine was allowed to act. In our experiments, the infusion of histamine into the bath at a constant rate for a period of 30 minutes, allowed us to observe the specific pattern of the previously discussed effect of histamine on the tension and rate increase on the right atrium. Laher & McNeill (1980c), measured their responses three minutes following histamine injection. This did not allow them to observe the maximum response of histamine on the rate, which according to our results, occurs after about 20 minutes of histamine infusion. Therefore, Laher and McNeill (1980c), observed only the initial increase in the rate, which would correspond with our experiments to be between 280-295 beats/min. It follows that they could not observe the slight decrease in the tension when the rate exceeded a certain value (approximately 360 beats/min). Their increase in tension of 80 ± 12% due to the effect of histamine on the

left atrium, corresponds to our average increase of about 90%.

Computer analysis of the single contraction of the left atrium, showed that histamine decreases time to peak tension as well as relaxation time, while it increases the rate of contraction, the rate of relaxation and the height of the peak tension. This effect of histamine on the rat left atrium is similar to the finding of DeMello (1976), who observed the same effect of histamine, but on the guinea pig ventricular muscle.

Suggestion that the effect of histamine on the rat heart may not be due to stimulation of H_1- or H_2-receptors, first came from Dai (1976). Laher and McNeill (1980c), sugested that in the rat, histamine in large doses causes an indirect stimulation of β-adrenoceptors, in the right and left atrium, by the release of endogenous cateholamines, which would mediate the response. They used propranolol and reserpine pretreatment to effectively block the histamine effect on the right atrium rate and left atrium tension. To verify this hypothesis, we used β-adrenoceptor antagonist metoprolol, which antagonized the positive inotropic and chronotropic effect of histamine on the right and left atria, in all our experiments. Our findings in which β blocker metoprolol antagonized the actions of histamine on the tension and rate, support the idea that histamine could mediate its effects via cateholamine release in the rat heart.

In our experiments verapamil also blocked the effects of histamine on the right atrium rate and the right and left atrium tension. Bernauer and Schanz (1974), found similarly that in guinea pig heart, histamine can abolish verapamil produced myocardial insufficiency. Also that the positive chronotropic effect of histamine was blocked by verapamil. Those findings correspond well to our findings, since we observed that the pacemaker is the most sensitive to verapamil, this being inhibited by verapamil (10^{-8}M). The tension was inhibited at higher concentrations of verapamil. In our experiments, the rate was maintained the same as the basal rate, except in a few experiments where it caused a slight insignificant decrease or increase. Fleckenstein (1977), also discussed the sensitivity of supraventricular pacemakers to Ca^{++} ions defficiency and Ca^{++} slow-channel blockers, such as verapamil. He stated that myocardial contractility is much more susceptible to the variations in external Ca^{++} supply than the skeletal-muscle, since its intracellular stores are of limited capacity. Greater sensitivity of the rate to Ca^{++} ion influx impairment is understandable, bearing in mind that the action potentials in SA and AV nodes are different than the other tissue of the heart, such as atria or ventricles. It is well known that the action potentials of the SA and AV nodes are characterized by the slow upstroke and the lack of the plateau phase. The slow upstroke in the nodal tissue is mainly carried out by the Ca^{++} ions current, while the rapid

influx of Na^{+} ions, responsible for the rapid depolarization phase in other cardiac tissue, is absent. Thus antagonizing the influx of Ca^{++} into the nodal cells with verapamil, will decrease the rate of diastolic depolarization and will cause an increase in membrane treshold potential, making it difficult for the pacemaker tissue to fire. However, verapamil also has a direct depressing effect on the cardiac automaticity, therefore slowing the rate (Fleckenstein 1977). The negative inotropic effect of verapamil on the left and right atria observed in our experiments, support the findings of Nayler (1972), on the dog heart, Herada et al (1981), on the rabbit myocardium and Kohlhardt (1977), on the cat ventricular tissue.

The positive chronotropic and inotropic effects of histamine on the heart antagonized by verapamil, suggest that histamine somehow increases the influx of Ca^{++} ions into the cell, to induce a response. This would be in agreement with the findings of Inoue et al. (1979), who found that D-600, a methoxy derivative of verapamil and also an antagonist of the Ca^{++} current system, blocks the restorative effect of 4-methyl-histamine on the guinea pig cardiac muscle, suggesting the involvement of the Ca^{++} current in the H_2-receptor activation. Bernauer and Schanz (1974), also suggest the dependence of histamine on the Ca^{++} ions for its actions, by either promoting the influx of Ca^{++} ions from the intracellular binding sites or from the extracellular space. DeMello (1976), reports that the Ca^{++}

ion influx into the cell is increased by histamine during the plateau phase of the action potentials, thus increasing the peak tension. This would also agree with our findings that histamine increases the peak tension of the single contraction. In our experiments the peak tension was decreased when histamine with verapamil infused the left atrium. Single contraction analysis shows, that verapamil decreases the usual 170% increase of the peak tension from Krebs solution to histamine, to only 19% of an increase from Krebs solution to histamine with verapamil. Time to reach its peak, total contraction time, rate of contraction and relaxation were also decreased in histamine with verapamil solution. Therefore this finding would support DeMello (1976), in the idea that histamine increases efflux of Ca^{++} ions, since our experiments showed that blocking this Ca^{++} ion efflux with verapamil, decreased the positive inotropic effect of histamine.

Our observation shows that the negative chronotropic and inotropic effect of verapamil can be reversed by adrenaline, correlates well with Fleckenstein (1977), who states that verapamil and β-adrenergic catecholamines operate in opposite manners, both on the slow Ca^{++} channels. He states that β-adrenergic catecholamines could increase the number of Ca^{++} ions accumulating binding sites via cyclic AMP. This would also agree with the report of Shinebourne and White (1970), who reported the effects of catecholamines-induced increase in cyclic AMP and hence

increased permeability of the cell to Ca^{++} ions. Furthermore, Karliner et al. (1982), showed that verapamil is a competitive antagonist of myocardial α-adrenergic and muscarinic but not of β-adrenergic receptors.

The interesting finding in our experiments is that the effect of histamine on the tension of the paced right atrium, was blocked at higher concentrations (10^{-5}M) of verapamil than on the left atrium, which was blocked with verapamil concentration of (1.5×10^{-6}M). One can only postulate that either the left atrium is more sensitive to the Ca^{++} ion changes then the paced right atrium, or that the action of histamine on the Ca^{++} ion influx is greater in the paced right atrium than on the left atrium, thus a higher concentration of verapamil would be required to block the Ca^{++}-slow channel current and consequently the increase in tension. Another interesting observation was that lowering the [Ca^{++}] from 2.5 mM in Krebs solution to 0.35 mM did not cause a further increase, in the rate caused by histamine. This would indicate that there is either enough external Ca^{++} ions available for histamine to have an effect on the rate or that histamine mobilizes the internal stores of Ca^{++} ion. However the force of contraction on the paced left atrium and right atrium, was diminished markedly when [Ca^{++}] in the Krebs solution with histamine, was decreased from 2.5 mM to 0.22 mM. Fleckenstein (1977), reported that contractility is reversibly lost upon Ca^{++} withdrawal, because Ca^{++} ions not only trigger the contractile proteins

but control the mechanical tension output by the amount of the ATP split. His statement that Ca^{++} withdrawal does not neccessarily abolish the bioelectric processes of ventricular excitation, would be similar to our findings that lowering $[Ca^{++}]$ does not alter the rate. Single contraction analysis in low Ca^{++} Krebs solution (0.55 mM) in comparison to the contraction in normal Krebs solution shows that the peak tension, rate of contraction and rate of relaxation are decreased by approximately 60%, indicating a suppressed force of contraction.

In conclusion, our results show that our technique of infusing histamine to the preparation at a constant rate, has some advantages over the injection technique used by Laher & McNeill (1980c), because it allows the greater increase in rate to be observed, as well as its relation to the increase in tension. Furthermore, it seems that histamine mediates its positive inotropic and chronotropic effects on the rat heart via catecholamine release and Ca^{++} ion current enhancement, as well as that there is a difference in sensitivity between the right and left atria to verapamil. It would be useful to investigate further the mechanism of histamine actions, which are still not clear. Further work would be necessary to speculate more on the mechanism of the action of histamine on the rat heart. Some preliminary work showed that the ventricular muscle in the rat does not respond to histamine (10^{-3}M). It would be of interest to study histamine effects, if any, on the

ventricles using our infusion technique. One could also compare the action potentials and the changes, if any, in the actions of histamine versus histamine plus verapamil on the rat atrium, to observe the effect of verapamil on the action potentials. It would be of interest to perform an assay for catecholamines and also for cAMP to see whether and to which extent they are related to the effects of histamine in the rat. The effect of D-600, which is twice as potent as verapamil, could also be used. One of the interesting experiments that could be done would be to deplete the internal stores of Ca^{++} with EDTA and then observe whether any changes in the effects of histamine and verapamil occur. This could also clarify the role of internal Ca^{++} stores, if any, on histamine action.

To conclude, one must be intrigued by the fact that histamine has been on the scene now, for almost a century and still many of its functions and mechanisms of actions are not yet clear.

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