CARDIAC ADRENERGIC MECHANISMS IN CORONARY DRUG RESPONSES - H. L. GARVEY

CARDIAC ADRENERGIC MECHANISMS IN CORONARY DRUG RESPONSES

by

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TABLE OF CONTENTS

INDEX OF TABLES

INDEX OF FIGURES

INTRODUCTION

Experimental animal studies on the effects of drugs on the coronary circulation have usually been employed to provide quantitative and qualitative data for clinical trials of this group of agents in humans. Species variations in the responses to these drugs have been largely overlooked. The concensus of opinion is that sympathetic accelerator nerve stimulation, or intracoronary administration of catecholamines (noradrenaline and adrenaline) induces coronary vasodilatation (Anrep, 1926; Gregg, 1946; Gregg, 1950; Wegria, 1951) in all species. Although there is considerable disagreement as to whether this dilatation is a direct effect or is secondary to metabolic activities of the myocardium, the view expressed in most physiology texts is that noradrenaline or adrenaline has a direct vasodilating effect on the vessels of the heart.

Barbour and Prince (1915) obtained evidence that a decrease in coronary flow was the constant response to adrenaline in freshly isolated perfused hearts of monkeys. Since then, several workers have reported species variations in responses of the coronary circulation to catecholamines (Drury and Smith, 1924; Katz et al., 1948). However, in most of these reports, the results were hardly sufficient to allow adequate conclusions to be drawn. Several investigators have presented data to support the view that observed decreases in coronary flow in response to the catecholamines are

most likely due to mechanical impedance of coronary flow, resulting from the associated myocardial stimulation induced by these agents (Ahlquist, 1948; Lu and Melville, 1951).

In view of the earlier experiments of Barbour and Prince (1915), and the conflicting reports on the responses of the coronary circulation to catecholamines in other species, it was of interest to undertake a systematic investigation of the effects of adrenergic, as well as adrenergic-blocking agents, on the coronary flow and heart contractions, as recorded simultaneously in isolated hearts of monkeys. It was also felt that the effects of these agents on the coronary circulation in this species, might be more like their effects in man than the effects observed in other species.

It has been reported that blockade of adrenergic beta receptor mechanisms in the heart by the drug pronethalol, is of some clinical importance in the therapy of cardiac ischemic diseases (Dornhurst and Robinson, 1962). It has also been reported that glyceryl trinitrate and trolnitrate possess cardiac adrenergic blocking actions in experimental animals (Gillis, 1965). Hence, it was of interest to study the role of adrenergic mechanisms in the actions of nitroglycerin and trolnitrate on the coronary circulation.

Anginal pain is believed to be due to coronary arteriolar constriction or vasospasm, and nitroglycerin when administered sublingually is reported to be the most effective agent in relieving this pain, on the assumption that it produces a coronary dilator effect. Gillis (1965) using anesthetized open-chest dogs, failed to observe a coronary dilator effect by sublingual nitroglycerin administration;

and, even with the intravenous route, only a transient dilator response could be evoked. On the basis of these findings, it seemed unlikely that such a weak and transient dilator effect of nitroglycerin (even in large doses) could be effective in antagonizing vasospasm, if angina is due to spasm of the coronary vessels. To investigate this problem more thoroughly, it was considered worthwhile to study the effects of nitroglycerin when administered sublingually during experimental spasm of the coronary vascular bed induced by vasopressin.

II. HISTORICAL REVIEW

A. SPECIES VARIATIONS IN CARDIAC ADRENERGIC RESPONSES

In the response to adrenergic and many other agents, species differences exist. While various investigators had hitherto demonstrated coronary dilatation in the response to catecholamines, Wiggers (1909) was the first to report a coronary vasoconstrictor action of adrenaline. Isolated hearts were perfused with a stream of normal saline regularly interrupted by stopcocks, and activated by a cam and motor device. As the coronary vessels were supplied by a rhythmically interrupted stream of fluid, any oscillatory changes could be recorded kymographically after being magnified by a membrane manometer. Using this procedure it was demonstrated that adrenaline (0.1 to 3.0 mg - Parke Davis and Company) when added to the perfusion fluid, was capable of constricting the coronary vessels in the isolated hearts of dogs, cats and rabbits. The simultaneous effect on the heart of this agent was eliminated since perfusion with normal non-oxygenated saline brought the hearts to a complete standstill and following adrenaline administration, there was no evidence of cardiac stimulation. The exact physiological state of such preparations is open to some question.

Brodie and Cullis (1911) studied the actions of various substances upon the heart beat and upon the rate of flow of perfusion fluid through the coronary vessels of isolated mammalian hearts (no species given). These isolated hearts were perfused with oxygenated saline

and the fluid escaping from the coronary vessels was measured with a volume recorder connected to a funnel. The apparatus was calibrated so that a definite height in the recorder indicated a definite flow. Following administration of 100 µg of adrenaline chloride (1:100,000) a preliminary coronary constriction was observed followed by marked coronary dilatation. Because of the importance of this observation, in respect to the question of a sympathetic vasoconstrictor nerve supply to these vessels, the presence of which had previously been denied; the actions of this agent was further investigated on isolated rabbit hearts, using the same procedure. It was observed that following injections of adrenaline chloride (1 µg), a 50% decrease in coronary flow occurred. This vasoconstriction, however, was soon converted to a vasodilatation, and with larger doses (10 µg) the primary response was vasodilatation. On the basis of these observations, it was concluded that adrenaline in small doses (not enough to alter the heart action) may cause true coronary constriction and that the decrease in flow with large doses may be due to the accelerator effect and increased cardiac muscle contractility impeding flow through the coronary vessels. The coronary vessels were, therefore, believed to possess constrictor and dilator nerve supplies of sympathetic origin, although this was not directly established.

Cow (1911) using a 50 ml capacity constant temperature bath with oxygenated saline and recording isotonically changes in tension, noted

that isolated strips taken from different sections of the pulmonary artery of sheep, oxen, goats, rabbits and humans (as soon as possible after death), varied in their responses to adrenaline chloride (0.001%). However, in this study, pure vasodilatation was demonstrated for the coronary arteries (larger portion). Cow inferred from these observations that different portions of the same artery may react differently to this agent.

A species variation was clearly demonstrated by Barbour (1912), who observed relaxation in isolated coronary strips obtained from dogs, cats, rabbits, oxen, sheep and pigs, but contraction in human coronary strips. He employed Meyers (1906) method by which arterial strips usually of about 2 cm long were suspended in a 50 ml bath (filled with oxygenated Ringer solution), and maintained under tension to remove the tonus. Doses (625 µg) of synthetic adrenaline (1:1,000 suprarenin - Parke Davis and Company) when added to the bath produced relaxation in the arteries of all species studied, except in the human coronary arteries where contraction was the constant response. It was concluded that unlike in other animals, the human coronary vessels were presumably supplied with vasoconstrictor nerves of sympathetic origin. This method, however, investigated the responses of the larger arteries only, and did not take into account the remaining portion of the coronary arterial system. No report of the state of the human coronary arteries was made and serum was also added to this preparation presumably, to prevent oxidation of adrenaline.

In spite of these observations, the concensus of opinion was that adrenaline and noradrenaline when administered intravenously produced

augmentation of coronary flow. Morawitz and Zahn (1912) confirmed the finding of Maass (1899) that accelerating nerve stimulation in anesthetized cats caused an increase of coronary flow, which Maass thought, indicated the presence of sympathetic coronary dilator nerve fibres in this species. They inserted a catheter through the right auricle into the coronary sinus of hirudinized cats and dogs. Coronary outflow was recorded with a volume recorder. The coronary blood thus collected was returned through a slow feeding burette into the jugular vein. Adrenaline chloride (0.3 mg/kg) was administered intravenously. The effect on the coronary outflow was shown to be one of extreme augmentation. It was suggested that the observed effect might be either due to an actively stimulated vasodilator mechanism, or an increase in metabolites. In fact, based on this observation, angina pectoris was treated with adrenaline chloride by Budingen (1914) but no positive results were obtained (reported by Morawitz and Zahn, 1914). This led to the speculation that anginal attacks may not be due to spasm of the coronary vessels.

In all previously reported investigations concerning the coronary dilatory action of adrenaline, only intact animals were used. Such investigation did not take into account indirect factors e.g. blood pressure changes which might influence coronary flow. Barbour and Prince (1915) using freshly isolated hearts of monkeys demonstrated pure coronary vasoconstriction in response to adrenaline chloride. A method was selected which excluded the indirect factors which may

have been encountered by Morawitz and Zahn (1912). This was the perfusion method of Rosenhein (1908). By this procedure, the perfusion fluid flowed through the coronary vessels by hydrostatic pressure; was collected, oxygenated, and recirculated through the system. Coronary outflow was recorded with a simple (Codon 1913) volume recorder. No recording of heart contraction amplitude was made.

Monkeys of the species Macacus rhesus were used and normal rabbits used as the control animals. The animals were decapitated and the blood collected into a vessel containing 0.02 to 0.04 gm of hirudin dissolved in 50 ml of Locke solution. The blood mixture was filtered and diluted when necessary to make about 150 ml, so that the blood constituted one-third to one-half of the total mixture. After the animals were exsanguinated, the hearts were immediately excised and connected to a curved aortic cannula of the perfusing system, so that the apex was positioned upward (to prevent accumulation of blood in the right auricle and ventricle). After thorough irrigation of the coronary vessels with Locke solution, the blood mixture was transferred to the reservoir of the apparatus. Perfusion was carried out at pressures of 50, 75 and 100 mmHg and at a temperature of 38°C. The perfusion fluid volume varied in the series of experiments by about 2 ml. Injections were made obliquely through the rubber tubing at a point a few centimeters above the aortic cannula. To avoid changes in perfusion pressures, this was done slowly and with the needle directed against the current of the perfusion

fluid. In the control animals (rabbits) all doses of adrenaline chloride (0.1 to 0.25 mg) employed, produced an increase in coronary flow, while in the hearts of monkeys coronary constriction was the constant response to all doses, (0.025, 0.1, 0.25, 0.5, 1 and 2 mg) and at all pressures employed. This observation was constant in 14 experiments made on the hearts of 3 monkeys and was present in both active and quiescent hearts. In fact, it was even more marked in the latter.

These results, in conjunction with the earlier findings of Barbour (1912) concerning strips of human coronary arteries, suggested that the constricting influence of adrenaline upon the coronary circulation in man while having a possible bearing upon the therapy of anginal attacks, might also prove to be in some ways related to the etiology of these attacks. This suggestion was supported by earlier reports of an increased discharge of adrenaline into the circulation during certain emotional states, and anginal attacks were often associated with excitement.

The observations of Barbour and Prince (1915) are, however, subject to several criticisms particularly in the fact that there was a constant re-circulation of perfusing fluid containing various metabolites which may have affected the responses of the preparation. Also, the method of recording coronary outflow hardly permitted accurate qualitative or quantitative assessments of the results obtained.

Savodsky (1921), Anichov (1923) and Sechenov (1923) perfusing the isolated quiescent heart of humans (removed as soon as possible

after death) reported that the coronary blood vessels of the newborn and young children dilated on perfusion with adrenaline while in most adults hearts, the coronary flow was diminished. No registration of the state of the cardiac muscle was, however, made (quoted by Anrep 1926). This would tend to imply an age difference in the response to adrenaline.

Drury and Smith (1924), who had previously observed coronary dilatation in the hearts of other animals, reported direct vasoconstriction in the heart of the tortoise Testudo graeca. The heart was exposed by cutting a circular hole in the carapace and the animals artificially respired. The exposed coronary vessels were observed directly through a microscope. Adrenaline chloride (1:1,000 and 1:100,000) was applied directly to the artery and the changes in diameter observed. In 11 out of 13 animals, within 2 minutes of application, the coronary arteries were definitely constricted and blood flow was reversed in these. Often large portions of the artery under observation disappeared, its course being indicated only by sections in which corpuscles were trapped. Intense vasoconstriction was present for over 90 minutes and was usually more pronounced in the smaller arteries. Microscopic examination of the main branches showed no significant changes in diameter.

Katz et al. (1938) reported a species difference in the response of the coronary circulation to adrenaline in the isolated hearts of cats and dogs. A modified Langedorff procedure was employed, and

defibrinated heparinized blood used as the perfusing fluid. Glucose (3 mg) and calcium gluconate (10%) were added to the solution to increase the usefulness of the preparation. Prior to the experiments, several dogs or cats were completely exsanguinated and the blood after being defibrinated was passed through the lungs of one of the dead animals (presumably to remove vasoconstrictor substances). In making the isolated heart preparation, a physiological "heart lung preparation" was first established. A cannula was then inserted into the central end of the aorta, and connected to an inflow reservoir maintained at a pressure of 100 mmHg. A second cannula was introduced into the right ventricle and drained into an aerating reservoir during intervals between blood flow measurements. The heart was then thrown into fibrillation by a strong faradic current from an inductorium and the left ventricle drained by an apical incision as soon as fibrillation occurred. The coronary vessels were perfused through the aortic cannula and the blood draining into the right heart, including that from the coronary sinus, was measured in a graduate with a stopwatch. In 12 experiments, using the isolated hearts of cats, 23 injections of adrenaline chloride (1:100,000 to 1:5,000) were made. Using doses ranging from 1 ml of 1/10,000 to 3 ml of 1/5,000, it was observed that repeated injections (regardless of dosage and concentration) produced consistent results. Four preparations showed a pure vasoconstriction (28%) and 8, pure vasodilatation (60%). In the isolated hearts of dogs on the other hand, the primary response was vasodilatation (72%) and this

vasodilatation response occurred either alone or preceded by a transitory vasoconstriction (9 injections of 1 ml of 1/100,000 to 1/1,000 in 6 preparations).

These observations were correlated with those of previous authors concerning the responses to adrenaline of isolated coronary strips, and perfused coronary vessels with the heart in fibrillation or at a standstill. It was concluded that when administered, adrenaline had 2 opposite coronary vasomotor effects depending on various conditions. These depended on the species of the animal and the nature of the perfusing fluid. The initial decrease in coronary flow in the dog preparations, was believed to be due to an action on sympathetic vasoconstrictor nerve endings, since this was abolished by dioxane derivatives. They further concluded that the experiments provided indirect evidence in support of the hypothesis that a differential distribution of sympathetic vasoconstrictor and vasodilator nerve fibres occurred in cats and dogs.

Marsh et al. (1948) failed to confirm any coronary vasoconstriction in response to adrenaline in the isolated perfused hearts of cats and rabbits. Using a modified Langendorff procedure with Ringer-Locke solution, it was observed that both adrenaline and noradrenaline (100 ug of free base/litre of perfusing fluid) produced cardioacceleration and an increase in coronary flow.

Relatively recent studies concerning the effects of noradrenaline and adrenaline on the coronary circulation of dogs, have called for a

re-examination of points which appeared then to be well established. All previous investigators had confirmed marked coronary dilatation in this species of animals. However, Berne (1958) and Garcia-Ramon (1950, cited by Charlier 1961) have reported that intracoronary injections of noradrenaline and adrenaline in intact anesthetized dogs, produced a consistent reduction in coronary flow. This initial reduction was always followed by a prolonged rise. From these observations they concluded that these agents cause primarily vasoconstriction of the coronary vessels in dogs. The secondary increase in coronary flow was attributed to stimulation of myocardial metabolism by the hypoxia produced in the cardiac muscles.

Lu and Melville (1951) suggested that an observed decrease in coronary flow was not necessarily an evidence of vasoconstriction of the coronary circulation in response to adrenaline i.e. a species difference, but may have been a resultant effect of intense myocardial stimulation which impeded coronary flow. Using the isolated hearts of rabbits perfused with Locke solution, it was shown that small doses of adrenaline (0.6 µg) produced transient decreases in coronary flow and this was more pronounced than when larger doses (0.3 µg) were employed. Since the coronary constriction was always associated with some cardiac acceleration and stimulation, they suggested that the marked stimulation was largely responsible for mechanically impeding flow through the coronary vessels.

In summary, the experimental studies cited, indicate that a species difference exists in the response of the coronary circulation to noradrenaline and adrenaline. However, in most of these studies, there was one or other factors which could have affected the observed species difference in responses to these catecholamines. In the perfusion of the isolated hearts, there was a re-circulation of the perfusion fluid (most often a composite mixture of blood and Locke or Ringer solution) and so the possibility of metabolites, or a deterioration of this fluid, affecting the observed responses cannot be ruled out. The observations of Drury and Smith (1924) and Katz et al. (1938), are most demonstrative of a species difference, since in the first of these reports, direct observations were made and in the second, a difference was observed between animals of the same species under identical conditions. In view of the older experiments of Barbour and Prince (1915), Savodsky (1921), Anichov (1923) and Sechenov (1923) it would be of interest to determine whether a species difference in the response of the coronary circulation to catecholamines does exist and particularly in the hearts of monkeys (Macacus rhesus) since these animals are considered closest to man of all the animals used in current experimental research.

B. ADRENERGIC RECEPTOR BLOCKADE ON THE CORONARY CIRCULATION

The concept of a receptive mechanism was introduced by Langley (1905) to explain the action of curare on skeletal muscles, and Dale (1906) was the first to make use of the receptor concept in connection with the sympathetic nervous system. He recognized that what he called the sympathetic myoneural junction could also be called the "receptive mechanism for adrenaline" and this concept was used to explain the fact that the ergot alkaloids prevented only the motor (excitatory) actions of adrenaline but had no effect on the inhibitory actions of this agent.

The work of Ahlquist (1948) supported a concept of two types of adrenotropic receptors, but claimed that these could not be classified simply as excitatory or inhibitory since each type may have either action depending on where it was found. Studies were made on intact or isolated tissue or organs of dogs, cats, rabbits and rats. Based on the relative responsiveness to a series of racemic sympathomimetic amines most closely related structurally to adrenaline, he postulated an alpha, and a beta receptor. Alpha receptors were postulated to be associated with most of the excitatory functions, such as vasoconstriction, while beta receptors mediated vasodilatation and the cardiac excitatory effects of positive chronotropism and inotropism. This, however, was not demonstrated for the coronary vascular bed.

Powell and Slater (1958) found that a dichloro analogue of isoproterenol blocked the inhibitory but not the excitatory actions

of sympathomimetic amines. This investigation was made on (a) blood pressures of cats anesthetized with chloralose, (b) uterine motility of female cats, recorded by an attached gravity lever, (c) femoral arterial blood flow recorded with a rotameter in dogs anesthetized with sodium phenobarbital (150 mg/kg), (d) isolated frogs hearts, (e) isolated intestine of rabbits and isolated uterus from rats suspended in a constant temperature bath containing Tyrode solution, and (f) guinea pig tracheal chains suspended in a muscle chamber. In the experiments on cats, 5 mg/kg of dichloroisoproterenol or (DCI) (Compound 20522) blocked only the secondary depressor effects of adrenaline but had no effects on the pressor response to noradrenaline and adrenaline. From these and other experiments, it was observed that this agent selectively blocked some inhibitory effects of adrenaline and noradrenaline. The depressor actions of isoproterenol were completely abolished. This blocking action was observed to be a competitive one since large doses of adrenaline could overcome the blockade. They concluded that DCI was combining with certain adrenergic inhibitory receptor sites without itself causing much physiological effects, and yet was competing for these active site with physiologically active amines.

The observations of Powell and Slater (1958) were confirmed by Moran and Perkins (1958) who suggested that in view of the lack of response to exogenous catecholamines DCI blocked beta adrenergic receptor sites. This agent was investigated using (a) vagatomized

pentobarbitalized dogs; (blood pressure and contractile force recorded) and (b) isolated perfused rabbits hearts using a modified Langendorff procedure. It was observed that DCI (1-4 mg/kg) selectively blocked the cardiac positive inotropic and chronotropic effects of adrenergic stimuli, in dogs with intact circulatory systems, and in isolated hearts of rabbits. After large doses of DCI (7-15 mg/kg) depression of contractile force was frequently observed in response to sympathomimetic amines (0.5-1.0 µg/kg), and sympathetic nerve stimulation. No inhibition of the positive inotropic effects of digitoxin, theophylline or calcium chloride were observed, which demonstrated the specificity of the cardiac adrenergic blockade. In the isolated rabbits hearts, DCI had qualitatively the same blocking actions. On the basis of these observations, it was concluded that the adrenergic receptors of mammalian hearts are functionally homologous to the adrenergic inhibitory receptors of other tissues i.e. that the receptors subserving cardio-acceleration and augmentation of cardiac contractile force are of the beta type.

Hashimoto et al. (1960) reported data obtained on isolated fibrillating hearts of dogs to indicate the existence of sympathetic vasoconstrictor, parasympathetic vasodilator but an absence of pure sympathetic vasodilator receptors in the coronary vessels wall. A modified Langendorff preparation was used, with a donor dog to perfuse the isolated fibrillating heart. Coronary flow was measured using a bubble flowmeter and arteriovenous oxygen difference was measured using

the Van Slyke apparatus. Treatment with DCI increased coronary flow by 13% and oxygen consumption by 34% above pre-treatment control values. Prior to DCI treatment, administrations of adrenaline and noradrenaline (0.5 µg) had increased coronary flow and oxygen consumption, but when these were administered after DCI (1 mg/ml) coronary flow decreased slightly (1-15%) and recovered to the initial level but never increased. Isoprenaline (0.1 µg/ml) was ineffective after DCI treatment, even when large doses were administered. In DCI pre-treated animals, myocardial oxygen consumption was little affected by the sympathomimetic amines. They concluded that since DCI completely blocked the coronary vasodilatation and myocardial metabolic effects of adrenaline, noradrenaline and isoprenaline, the indication was that these amines cause coronary vasodilatation through a myocardial metabolic mechanism and not through any direct vasodilator mechanism.

The effects of the alpha receptor blocking agent phenoxybenzamine was also investigated by these workers. Control hearts treated with this agent (1 mg/ml) showed an increase (11%) in coronary flow and also an increase in oxygen consumption (42%) over the non-treated steady state controls. Administrations of adrenaline and noradrenaline (1 µg) resulted in a greater increase of coronary flow per unit change of oxygen consumption than in the untreated animals. However, phenoxybenzamine caused no significant change in the ratio of coronary flow to myocardial oxygen consumption following administrations of isoprenaline (0.5 µg). From these results they concluded that sympathetic

vasoconstrictor receptors existed in the coronary vessel wall. The presence of sympathetic vasodilator receptors could not be postulated since blockade of the coronary flow changes seen in control untreated animals might be due to either a blockade of myocardial metabolic mechanisms or to a direct vasodilator effect.

Donald et al. (1954), using (1- 2- napthyl - 2-isopropyl)amino ethanol hydrochloride (pronethalol also known as Nethalide or Alderlin) demonstrated an effective blockade of beta receptors in the cardiovascular system of the dog. They studied the effect of this agent on cardiac output, heart rate, stroke volume and systemic arterial pressure, using intact dogs, atropinized dogs, and dogs with chronic cardiac denervations. Preliminary studies were made on the ability of pronethalol to block the accelerator effect of isoproterenol. After administration of pronethalol (1 mg/kg min infused for 6 minutes), the accelerator effects of isoproterenol (20 µg infused for half an hour) were abolished. It was shown that pronethalol could attentuate the actions of sympathetic nerves on the heart. In dogs with chronic cardiac denervation, injections of the catecholamines, noradrenaline and adrenaline (0.5-1 µg) induced 35% and 87% increase respectively in heart rate. Pre-treatment with pronethalol abolished the 87% increase caused by noradrenaline and reduced to 8% the heart rate increase caused by adrenaline. An effective blockade of peripheral beta receptors as evidenced by a marked increase in systemic vascular resistance in response to adrenaline, a known dilator of peripheral vascular blood vessels, was also observed. They

concluded that the observed peripheral blockade was consistent with the concept of a blockade of beta receptors in the blood vessels of the muscles so that the constrictor (alpha) actions of adrenaline predominated.

Chiong et al. (1965) confirmed the beta receptor blocking actions of pronethalol. This blocking agent had previously been reported to be free of the initial sympathomimetic stimulation seen after the administration of other beta-adrenergic blocking agents such as DCI (Black and Stephenson 1962). Experiments were performed on pentobarbitalized dogs and recordings were made of systemic arterial and right ventricular pressures. Oxygen consumption, arteriovenous oxygen difference, cardiac output (Fick method) heart rate, stroke volume, stroke work of left ventricle, total peripheral resistance and arterial haematocrit were also measured. The most significant cardiovascular effects of pronethalol were observed within 15 minutes after administration (2.5 mg/kg iv) and indicated that this agent was not devoid of an initial sympathomimetic stimulation, as postulated by Black and Stephenson (1962). The cardiac responses to adrenaline after pronethalol pre-treatment agreed quite well with the receptor theory proposed by Ahlquist (1948, 1962) since pronethalol abolished the adrenaline-induced rises in cardiac output, heart rate, stroke volume and right ventricular systolic pressure, as well as the smaller increases in stroke work. The metabolic effects of adrenaline (Ahlquist 1962; Pilkington et al. 1963; Froberg and Oro 1963) were also abolished by pronethalol.

The experiments of Hashimoto et al. (1960) indicated the presence of sympathetic (alpha) vasoconstrictors in the coronary vessels walls. The existence of beta-adrenergic receptors in the coronary vascular bed was demonstrated in dogs by Klocke et al. (1964), measuring coronary blood flow and coronary venous pO_2 simultaneously. When isoprenaline (0.1-0.3 µg/kg/min) was infused, a rise in coronary pO_2 always accompanied the increase in coronary blood flow, in spite of the tachycardia and a fall, or no change in arterial pressure. This response suggested a direct vasodilatory effect of isoprenaline, although a metabolically or mechanically induced increase in coronary flow could not be excluded. Accordingly, in 13 isolated dogs heart preparations, potassium arrest was induced to prevent the myocardial stimulating effects of isoprenaline. In this case, a decrease of coronary vascular resistance (4-46%) was the constant response to administrations of isoprenaline while myocardial VO_2 showed no consistent changes. It was concluded that isoprenaline stimulated beta receptors to cause direct dilatation of the coronary vascular bed. This was further substantiated in the arrested hearts by experiments in which coronary venous pO_2 was measured continuously, and by studies in which the vasodilatory effects of isoprenaline were blocked by pronethalol.

Zuberbuhler and Bohr (1964) also demonstrated the existence of beta receptors in isolated coronary vessels of dogs. Helical strips of dog coronary "resistance" vessels (2,00 -500 µ diameter) were mounted in physiological salt solution and their contractions recorded isometrically.

Addition of catecholamines (noradrenaline and adrenaline, 0.3-1.0 µg/litre) induced relaxation. This relaxation was reversibly blocked by pronethalol (0.5 mg/litre). The catecholamines sometimes produced a slight contraction.

Bohr (1966) studied the role of adrenergic receptors in isolated strips of coronary arteries from dogs, rabbits, monkeys and humans. Using helical strips of these arteries mounted for tension recording in a muscle bath, it was found that smooth muscles from arteries of less than 500 µ in diameter relaxed in response to adrenaline, noradrenaline and isoproterenol. The relative potency of the 3 agents was 1:10:30. The observation that noradrenaline was much more potent than adrenaline was not in accord with the structure-activity generalization, that increased size of the substitution on the amino group of the catecholamine increases the beta receptors activity. He concluded that beta receptor sites must differ sufficiently so that the relative effectiveness of different catecholamines may vary greatly. Beta receptor blocking agents (propranolol and pronethalol) were effective in blocking the beta receptor activation caused by these catecholamines. Coronary arteries of 1 mm or greater in diameter showed both alpha and beta receptor activity and on these vessels, adrenaline was 10 times as potent as noradrenaline in producing alpha receptor activity. Responses to stimulation with increasing concentrations of adrenaline showed first, at low concentrations only constrictor activity, then, with higher concentrations, an initial constriction followed by beta activity relaxation.

He further demonstrated that in the presence of normal concentrations of calcium (1.6 mM), the intense contractions of smooth muscles from vessels of less than 500 μ in diameter, induced by 50 mM KCl could not be relaxed even by high concentrations of catecholamines (no dosage given). If, however, the calcium concentration was reduced to 0.1 mM then the catecholamines were capable of producing relaxation. He concluded that one link in the chain of events initiated by beta receptor activation is a decrease in the concentration of ionized calcium in the environment of the basic contractile machine (actomyosin-ATP). It was also postulated that the possible determinants of the predominance of alpha or beta effect on vascular smooth muscle may lie in the antagonistic actions initiated by these 2 receptors on the intracellular concentration of free ionized calcium.

In summary then, the data presented indicate quite clearly the presence of both alpha and beta receptors on the coronary vessel walls. However, while most of these experimental studies have proven conclusively the presence of sympathetic vasoconstriction (alpha) receptors the evidence in favour of sympathetic vasodilator receptors (beta) are relatively inconclusive. The fact that increases in coronary flow occur after the increase in oxygen consumption has been manifested, suggests that coronary vasodilatation is dependent on this metabolic effect. However, the rate of coronary flow also increases during hypoxia and the possibility of a direct stimulation of beta vascular receptor occurring later in time cannot be ruled out.

C. OBSERVATIONS ON THE ANTAGONISM BETWEEN NITRITES CORONARY DILATION AND VASOPRESSIN CORONARY CONSTRICTION

It is well known that coronary vasodilator agents will antagonize the actions on the coronary vessels of vasoconstrictor substances. Since angina pectoris is postulated to be due to temporary spasm of the coronary vessels, it is believed that the prevention of spasm is directly related to the vasodilator actions of these drugs. All experimental evidence indicates that the nitrites including nitroglycerin increase coronary flow in isolated perfused hearts of all species of animals studied (Wegria 1951; Charlier 1961). However, studies on the intact animals have failed to confirm vasodilation as the primary mechanism of action of nitrites.

Essex et al. (1936) studied the effect of nitrites on the coronary circulation of intact anesthetized dogs. Using the Rein thermostromuhr, changes in coronary flow were recorded in the circumflex branch of the left coronary artery. Repeated intravenous injections of nitroglycerin (0.22 mg) produced a temporary increase in coronary flow of about 5 minutes duration. The magnitude of this increase was about twice the control value. When the nasal passages of the animal were cocainized to prevent reflex stimulation, inhalation of amyl nitrite produced an increase in coronary flow of a similar magnitude as nitroglycerin. Although inhalation of the drug was maintained, the coronary flow declined after 2 minutes to a value of 20 to 30% above the control. The control coronary flow was restored within 5 minutes after inhalation was stopped. A second

administration of amy nitrite produced only 23% increase in flow. It was concluded that both nitroglycerin and amyl nitrite are capable of producing a temporary increase in coronary flow and tolerance may occur on repeated administration of amyl nitrite.

Essex et al. (1940), using the thermostromuhr technique, described the comparative effects on the coronary flow of nitroglycerin when administered intravenously and sublingually in trained unanesthetized dogs. Doses of 0.56 to 1.3 mg of nitroglycerin caused an increase in flow in both right and left coronary arteries. The augmentation in the right coronary artery varied from 30% to 100%, while that in the left from 35% to 72%. The effect lasted from 1 to 3 minutes. Similar doses given by mouth produced 70% increase in flow and even after 15 minutes this was still above control values.

Boyer and Green (1940) demonstrated an increase in coronary flow in response to nitroglycerin or sodium nitrite in anesthetized dogs. Using an orifice-meter, it was demonstrated that the intravenous or intracoronary injections of nitroglycerin or sodium nitrite (0.3 to 100 mg) increased coronary flow, at the same time as the blood pressure and cardiac work were decreased by these "nitrites".

Eckenhoff and Hafkenschield (1947), using the bubble flowmeter failed to confirm consistent coronary dilatation with nitroglycerin or amyl nitrite. In closed chest spontaneously breathing dogs, anesthetized with pentobarbital sodium (30 mg/kg), neither nitroglycerin nor amyl nitrite led to consistent changes in coronary blood flow. Intra-arterially (coronary) nitroglycerin (0.002-0.02 mg) increased coronary flow initially but this increase was soon converted to a decrease, as

peripheral dilatation occurred. Administrations intramuscularly, intravenously, subcutaneously and sublingually (0.5-0.6 mg) all gave inconsistent results. Amyl nitrite was more effective as a coronary vasodilator but again the results were inconsistent. In several instances, striking increases in coronary flow were observed in spite of considerable decreases in blood pressures. They concluded that the failure of nitroglycerin and amyl nitrite to show consistent coronary vasodilator actions did not provide sufficient evidence to conclude that no therapeutically useful effects of these agents were present. They also postulated that these nitrites could improve the nutrition of cardiac muscle cells without an observed increase in coronary flow.

Recent studies of Bergamaschi and Glasser (1963) reported that nitroglycerin, in increasing doses, will augment coronary flow. Using dogs under thiopentone - chloralose anesthesia, change in coronary flow were recorded in the anterior descending branch of the left coronary artery using the Rein thermostromuhr, and the Shipley-Wilson rotameter. When administered intravenously, nitroglycerin (1.0 to 100 µg/kg) augmented coronary blood flow but there was not a consistent dose response relationship, and the degree of response to the same dose varied from animal to animal. When administered by intracoronary injections, the results were the same. At a dose of 1.0 µg/kg, coronary blood flow increased 12%; at 2 µg, 23%; at 5 µg, 62%; and at 10 µg, only 22%.

In view of the fact that no unified theory of action of the nitrite could be achieved, Marchetti et al. (1964) reported investigation aimed at discriminating between direct and indirect actions of nitroglycerin on the coronary circulation. In pentobarbitalized dogs with right ventricular bypass, changes in coronary flow, cardiac output, coronary arteriovenous oxygen difference and arterial pressure were recorded following single injections of nitroglycerin (0.2 mg/kg) and continuous infusions (0.1 mg/kg/min). It was observed that intravenous administration of this agent consistently increased the coronary blood flow but that this effect was clearly evident only when the arterial pressure and cardiac frequency were kept constant. By keeping the arterial pressure in the aortic bulb at its normal value, nitroglycerin caused a marked increase in coronary flow which indicated a considerable vasodilating action of the drug on the coronary vascular bed. An increase in coronary flow was less easily detected when the hypotensive effects of nitroglycerin on the general circulation were allowed to occur, since the hypotension opposed the increase in coronary flow. They concluded that the mechanism of action of nitroglycerin on the coronary circulation of the dog was primarily, a lowering of the arterial blood pressure and reducing the oxygen consumption of the myocardium; the latter as a consequence of the reduced cardiac work, rather than by direct action. They further concluded that unlike other hypotensive agents, nitroglycerin does not reduce the coronary blood flow as it does the arterial pressure because it dilated the coronary vessels concurrently.

Scriabine and McShane (1965) reported a comparative study on the mechanism of antianginal actions of nitroglycerin and trolnitrate phosphate in isolated aortic strips from rabbits and in intact dogs. Their investigation was based on the hypothesis that nitroglycerin stops an anginal attack by a peripheral vasodilator action resulting in a reduction of cardiac work and myocardial metabolic demands with the maintenance of, rather than an increase in coronary blood flow. To study in vitro, the peripheral vascular activity of trolnitrate and nitroglycerin, isolated aortic strips were suspended in a bath containing 25 ml oxygenated Krebs-Henseleit (1932) solution at 37.5°C. After stabilization, spasmogens (adrenaline or angiotensinamide, 5×10^{-8} gm/ml) were added prior to the administration of the antagonists, trolnitrate and nitroglycerin. It was observed that nitroglycerin (1×10^{-6} gm/ml when left in contact with the tissue for 30 seconds, produced complete blockade of adrenaline-induced contractions of the aortic strips. Trolnitrate at the same concentrations significantly reduced (70%) the adrenaline-induced contractions also. Nitroglycerin was determined to be 35 times more potent than trolnitrate in reducing adrenaline-induced contractions. These 2 agents also reduced angiotensinamide-induced contractions and on a weight basis nitroglycerin was found to be 11 times more potent in doing so.

To study the in vivo effects of both drugs on the coronary and peripheral circulation, pentobarbitalized open-chest dogs were used

and coronary flow was measured with a Shipley-Wilson flowmeter. Doses, as low as 5 µg/kg intravenously, decreased significantly mean aortic blood pressure, coronary perfusion pressure and coronary vascular resistance. Increasing the dose (80 µg/kg) cardiac output, arteriocoronary sinus oxygen concentration differences, and left ventricular oxygen uptake were also significantly lowered. Onset of action was immediate and maximum effect was observed within 1 minute after administration, but within 5 minutes there was a substantial recovery of all parameters. Trolnitrate was similarly effective but at a higher level of dosage (320-1280 µg/kg/i.v.). Maximum effect was reached in 4 to 10 minutes and the duration of action lasted for over 20 minutes. They concluded that their investigations justified the hypothesis postulated; i.e. that the mechanism of action of nitrites involved peripheral vasodilatation.

Studies concerning the effects of the nitrites on the coronary circulation in man are quite conflicting. Levy et al. (1940), using the anoxemia-tolerance test, showed that in patients with coronary sclerosis, nitroglycerin delayed the appearance of anginal pain and diminished the electrocardiographic signs, pathognomic of cardiac anoxia. Nitroglycerin (0.6 mg, 1/100 gr hypodermic tablets) and erythrol tetranitrate (30.0 mg) were given orally after control electrocardiogram and blood pressure recordings were taken. With the induction of anoxemia, nitroglycerin caused a prolongation of 51% in the time of appearance of pain. Deviations of the R-ST junctions observed in the control ECG were diminished by 47%. There

was no consistent action on the blood pressure. With erythrol tetranitrate, there was no prolongation in the time of appearance of the pain, and no consistent effect on the blood pressure, but R-ST deviations were diminished by 26%. They concluded that nitroglycerin dilated the coronary arteries of patients with coronary sclerosis, and its usefulness in relieving anginal attacks was due to a direct coronary dilator action and not by virtue of the fact that it lowered systemic blood pressure. Erythrol tetranitrate, although it caused slight coronary dilatation, was not effective in raising the threshold for pain, hence they concluded this agent was of limited value in coronary insufficiency.

Brachfield et al. (1959) presented evidence to indicate that although coronary vasodilatation occurs in man, it appears to be secondary to changes in myocardial oxygen requirements. In 10 normal (or nearly normal) subjects, catherization studies were made in coronary and systemic circulatory dynamics and myocardial metabolism before, and after the administration of nitroglycerin. Following administration of nitroglycerin (0.6 mg), myocardial oxygen consumption increased, and coronary flow also increased (probably directly related to an increased oxygen demand). Cardiac work was unchanged and myocardial efficiency decreased.

A similar study was conducted by Gorlin et al. (1959) on 17 patients, 7 with coronary artery diseases and 10 with increased left ventricular work load (14 had angina). Sublingual nitroglycerin (0.6 mg) resulted in decreased cardiac output, blood pressure and left ventricular load.

When compared with normal subjects, these patients while at rest, showed an increased coronary blood flow and myocardial oxygen consumption with a slight decrease in myocardial efficiency. Following nitroglycerin administration (0.6 mg), coronary vascular resistance remained essentially fixed, coronary flow and oxygen consumption were unchanged or actually decreased and efficiency fell. It was concluded that prior exhaustion of the available vasodilator capacity of the heart either by intrinsic coronary arterial obstruction, or through haemodynamic overloading of the left ventricle by diseases had occurred to prevent a vasodilatory response.

In summary, the data presented indicate that there is still at present no unified theory concerning the mechanism of action of the nitrites. All studies on isolated tissue or organs have confirmed a vasodilator property, but reports on intact animal studies and in humans have produced only conflicting results. Since the discovery of nitroglycerin and the observation that it increases coronary blood flow, there have been a constant search to find new and better agents to relieve myocardial ischemia, the majority of which are coronary vasodilators. Most of these have proven ineffective and clinical trials have given overwhelming evidence that the mere vasodilator effect of a drug is not an absolute criteria for its usefulness in cardiac ischemic diseases. So far, objective proof of a relaxation of smooth muscle walls is lacking although arteriography have confirmed the smooth muscle relaxant property of nitroglycerin. The findings of Brachfield et al. (1959) suggest vasodilatation coincident with, if

not responsible for an increase in oxygen needs of the heart and not by relaxing arterioles per se. Pure vasodilatation in which myocardial oxygen is unaltered has not yet been demonstrated in man by current methods. However, in clinical trials, the relief of pain and improvement of the electrocardiogram are readily achieved by this agent.

The experimental production of temporary spasm of the cardiac vessels provide a reliable experimental model to study the effects of coronary dilators and the possible involvement of adrenergic mechanisms in the observed antagonistic responses. Oliver and Schaefer (1895) in the course of their investigations of the effects of extracts of various glands on the blood pressure of dogs anesthetized with chloroform, described the vasoconstrictor actions of an extract of the posterior pituitary gland. This action was attributed to peripheral vasoconstriction of the smooth muscles of the arterioles. This pressor response, along with the phenomenon of tachyphylaxis and cardiac slowing was confirmed by Schaefer and Vincent (1899). Extracts prepared from the pituitary gland of the ox was injected in the external jugular vein of morphinized curarized cats, anesthetized with chloroform or ether. It was observed that intravenous injection of the dried extract (1 part to 4 parts of water) produced a rise in blood pressure, and after atropinization this rise was even greater being accompanied by very little slowing of the pulse as observed in animals not atropinized. A second dose administered soon after the first produced little or no effect on the blood pressure. Evidence in favour of arteriolar vasoconstriction was also presented by Pal (1909) who demonstrated that the addition of preparation of dried

pituitary extract to a bath of known capacity in which arterial strips were suspended, produced contractions only. Krogh (1925), in studying the anatomy and physiology of capillaries, reported capillary constriction in the interdigitary membrane of frogs legs when a solution of pituitary extract was applied.

The first indication of the involvement of the coronary circulation in the response to extracts of the posterior pituitary gland, was reported by Kolls and Geiling (1925). Intravenous injections of 0.25 to 1 ml of pituitary liquid (Armours) in ether-anesthetized dogs resulted in prompt cardial dilatation (fluoroscopic and roentgenographic observations). Simultaneous with this dilatation, there was a rise of mean arterial pressure due chiefly to an increase in the diastolic pressure, indicating increased peripheral resistance. There was also a decrease in pulse pressure, with marked slowing of the heart rate, and a decrease in the minute volume output of the heart (as determined by the Fick Principle). It was concluded that the peripheral circulatory changes were largely due to intense peripheral constriction of arterioles and capillaries, and verified this by direct observation of blood vessels of the ear, and veins of the tongue which showed intense constriction and congestion. Slowing of the heart occurred after removal of the vagal influence by atropinization and this they concluded was due to a direct action in the heart or secondary to a diminished coronary blood flow. Metabolic determinations indicated a 50% reduction in cardiac oxygen consumption and diminution of cardiac output.

Based on the hypothesis that the primary action of pituitary extract when injected, was on the myocardium, Resnick and Geiling (1925) examined the electrocardiogram of unanesthetized dogs following injections of the extract. They observed an increase in heart rate with disappearance of the respiratory arrhythmia. Coupled beats, marked degree of S-A block, exaggerated conduction and an increase in the amplitude of the T wave occurred soon after injection. The conclusion was that the primary effect of the extract was myocardial depression and that the coronary circulation was involved. However, their observations did not permit conclusions as to whether or not the depression was secondary to the coronary spasm which occurred. The marked similarity between the effect of the extract on the electrocardiogram, and anoxemia of the heart, as reported earlier by Gilbert and Greene (1922), was also reported by these investigators.

Rössler (1930) demonstrated that the primary constriction produced by pituitary extract was on the coronary vessels. Using the heart-lung preparations, it was observed that injection of 0.1 to 0.3 ml of the extract (Pitressin) caused strong or complete coronary constrictions with resultant myocardial damage due to a lack of coronary blood supply. This impairment was confined to the left ventricle but later extended to the right ventricle with subsequent fibrillation. When the preparation was pre-treated with papaverine hydrochloride (30 mg) and adrenaline (0.1 mg) coronary constriction was prevented.

Goldenberg and Rothberger (1932) claimed that apart from a reflex vagal action, pituitary extract (Pitressin) led only to coronary constriction with its resultant myccardial damage. Dietrich (1933) correlated more closely and directly the effects of coronary constriction upon the electrocardiographic changes observed after injection of the extract (0.3 ml). Using chloralosed dogs and recording coronary flow with the Rein thermostromuhr, it was observed that concomitant with a decrease in coronary flow characteristic electrocardiographic (ECG) changes occurred. The T wave heightened during the immediate reduction in coronary flow, and with pronounced constriction, the ST segment increased in amplitude to be even higher than the QRS complex. With milder constriction, it was observed that the T wave may be reversed and variations in position occurred during restoration of the coronary flow. They claimed that the electrocardiographic abnormalities were not due to the reduction in coronary flow per se but to the resultant myocardial hypoxia. In fact, this was demonstrated by inducing similar changes in dogs by the inhalation of 6% oxygen and 94% nitrogen.

These observations on the action of pituitary extract indicated that a reduction in coronary blood flow may play a dominant role in the production of cardiac ischemic changes. It was also indicative that the primary action of the extract was on the coronary vascular bed leading to the observed electrocardiographic changes which bore some resemblance to the ECG pattern of patients with angina pectoris.

However, no attempts were made to determine the exact mechanism of action of the extract, hence the concensus of opinion was that pituitary extract acted by a direct musculotropic or other unknown mechanisms to constrict the coronary vessels.

The observations of Rössler (1930) that papaverine and adrenaline could antagonize the coronary constriction of pituitary extract was further investigated by Melville and Stehle (1931). Their hypothesis was that the observed greater pressor responses to pituitary extract after adrenaline pre-treatment, as reported by earlier workers, were due to elimination of the coronary constriction induced by the extract. Using (a) chloretone anesthetized dogs (b) "artificial hearts" (c) heart-lung preparations, it was observed that pre-treatment with ephedrine (0.1 mg/kg) and adrenaline (0.15 mg infused) greatly augmented the pressor actions of pituitary extract on the blood pressure in the anesthetized dogs. The usually observed diphasic action of the extract, attributed primarily to coronary constriction, was also abolished. The artificial heart then was used to prove conclusively that the effects of the extract observed after ephedrine and adrenaline pre-treatment, were not of peripheral origin. Using this preparation, neither ephedrine nor adrenaline were observed to have any effects upon the responses to pituitary extract.

Melville (1933 a) continued these observations of the antagonism to pituitary extracts by coronary dilators on the normal unanesthetized dogs. Under local anesthesia (procaine hydrochloride 0.5%), the femoral arteries and veins of dogs were cannulated and sodium citrate

administered as an anticoagulant. Administration of pituitary extract (0.1 mg/kg) caused a fall in blood pressure (130 to 44 mm Hg) within 1 minute, with subsequent return to control levels but no pressor effects. He concluded that the severe cardiac action may have obscured the peripheral effects of the extract. Pre-treatment with ephedrine (2 mg/kg) prevented the fall in blood pressure and doses of over 2 mg/kg of ephedrine potentiated the pressor effect of the extract so much so that the pressor effect was evident for over 30 minutes. When adrenaline was mixed with the extract, a pure pressor response was observed. He inferred that the interaction between coronary constriction and coronary dilatation was a quantitative matter and the observed effect on the blood pressure was the resultant of the predominating action.

Melville (1933 b) next investigated the influences of other coronary dilator agents on the blood pressure response to pituitary extract. In normal unanesthetized dogs, sodium nitrite 10 mg/kg/min was infused for 5 minutes or amyl nitrite (inhalation) administered for 4 minutes. In either case, at a sign of definite nitrite action 1 mg/kg of pituitary extract was given. The depressor effect of the extract was completely abolished after pre-treatment with either nitrite. It was concluded that the nitrite was effective in antagonizing the coronary vasoconstriction produced by pituitary extract. Histamine dihydrochloride (0.5 mg/kg/min) was also shown to be effective in antagonizing the coronary vasoconstriction.

Based on a coronary dilator, coronary constrictor antagonism between pituitary extract (vasopressin) and the nitrites, several investigators

attempted to use vasopressin spasm as a test for coronary insufficiency (Ruskin 1947) and as an experimental model for the evaluation of coronary vasodilator drugs. Varma (1961) made an attempt to evaluate coronary dilator agents against vasopressin induced cardiovascular changes in pentobarbitalized rabbits. The effects of 4 I.U./kg of vasopressin(repeated injections)on the ECG changes and arterial blood pressure were studied. It was observed that the response to vasopressin varied markedly in different animals both in duration and intensity. Further, no particular changes in the ECG were observed consistently in all animals and the response to a second dose could not be duplicated in the same animal. However, he felt that if the antagonism between vasopressin and the coronary vasodilator agents was marked, it could be observed. The method was nevertheless found unsatisfactory for comparative evaluation of drugs against coronary insufficiency, firstly because signs of coronary insufficiency in the form of ST-T depression were not consistently observed and secondly, because the degree of antagonism by different coronary dilator drugs to the vasopressin-induced changes, could not be ascertained with accuracy. Consequently, this investigation was abandoned.

Kareva (1963) using vasopressin-induced vasoconstriction as a model of experimental spasm of the cardiac vessels, investigated the prophylactic and therapeutic effects of various agents in preventing vasopressin-induced spasm. In acute experiments on cats anesthetized with urethane and chloralose, the resistance in the system of the circumflex branch of the left coronary artery was recorded by the method

of resistography. The principle of this method involved artificial stabilization of the blood flow in the vessels. This was achieved by perfusion with the animals own blood or by means of a special pump. Under these conditions, the haemodynamic effects of changes in the blood pressure were eliminated and the pressure in the coronary arteries was determined entirely by the minute volume of blood supplied by the pump.

Vasopressin (Pituitrin) in a dose of 1.0 I.U./kg increased the resistance of the coronary vessels by $44 \pm 7.3\%$. This increase was maximal at the time of the most marked ECG changes which developed immediately after the injection of the drug and indicated disturbance of the coronary blood supply. The ST segment in most cases formed the ascending line of a giant T wave or sometimes fell below the isoelectric line in which case the T wave was negative or diphasic. Arrhythmias were frequently observed. Nicotine 50 µg/kg was also used in the experimental production of spasm in this study. To study the prophylactic action of coronary dilator drugs, these agents were injected 2 minutes before vasopressin or nicotine. Nitroglycerin (nitroglycerol 300 µg/kg) while it completely eliminated nicotine spasm, was ineffective in relieving the vasopressin spasm of the coronary vessels.

To study the therapeutic effects of these agents against spasm of the coronary vessels, they were injected 5 to 10 seconds after injection of nicotine or vasopressin, since the period of development

of both forms of spasm was 10 to 40 seconds. Nitroglycerin was found to be most effective therapeutically and completely abolished both forms of spasms. Papaverine was also quite effective and lowered the resistance of the coronary vessels by an average of 9% during vasopressin spasm and 11% during nicotine spasm of the coronary vessels. It was evident that the therapeutic value of nitroglycerin against spasms of the coronary vessels was much greater than its prophylactic value, while papaverine showed similar prophylactic and therapeutic values.

It is, therefore, evident that some interaction between vasoconstrictor and vasodilator agent occur possibly on the coronaries themselves, since catecholamines (e.g. adrenaline) as well as nitrites are capable of antagonizing the vasoconstriction. It would also be logical to postulate that both catecholamines and the nitrites might have some similarity in their actions on the coronary vascular bed.

Associated with the vasoconstrictor actions of vasopressin is the unexplained phenomenon of tachyphylaxis which was first reported by Schaefer and Vincent (1899). The report of Gardier and Abreu (1958) that mid-cervical vagatomy, plus bilateral carotid sinus denervation would eliminate vasopressin tachyphylaxis, suggested a close connection between the action of the vasopressin and the autonomic nervous system. In dogs under pentobarbital anesthesia, denervating these buffer areas resulted in an increase in pressor response to the hormone which was reproducible within 10% on repeated

injections. This could also be maintained with constant infusions.

Since it thus appeared that sympathetic activity influenced the vasopressin responses, Gardier et al. (1962) using morphinized pentobarbitalized dogs, reported that non-reproducible pressor response of low magnitude were usually associated with high sympathetic tone. With low sympathetic tone, the responses were of appreciable magnitude and reproducible. Nasmyth and Bartelstone (1962) reported that in pithed rats and spinal cats, physiological amounts of vasopressin potentiated the pressor response to noradrenaline (no details available).

The ability of catecholamine depletion to modify the vasopressin response was reported by Nash (1962). In control dogs and dogs having sympathetic pathways modified by reserpine pre-treatment or by ganglionic blockade with chlorisondamine (3 mg/kg) coronary flow, blood pressure, peripheral resistance and contractile force were recorded. Following repeated injections of vasopressin, it was observed that whereas tachyphylaxis rapidly developed in the controls; in the reserpinized animals, there was failure to develop complete tachyphylaxis. Ganglionic blockade did not significantly alter the responses. However, the initial dose of vasopressin produced a greater increase in coronary resistance in the reserpine pre-treated than in the control animals, but similar reductions in contractile force. After four doses (at 30 minutes intervals) there was no myocardial effect and very little effect on coronary resistance whereas controls showed the same reduction in contractile force as with the

first dose and significant coronary effects in response to vasopressin. This greater attenuation of contractile force and coronary effects indicate that tachyphylaxis developed more rapidly in the presence of reserpine.

Since there was, in no instance, a depressor component in the vasopressin response after reserpine treatment as seen in control animals, if this is due to coronary constriction as shown by several workers, it is logical to assume that reserpine may have altered the effects of vasopressin on the coronary bed. This study also invites interpretation that vasopressin has an indirect effect through catecholamines and the data also allows speculation that vasopressin either causes the release of a resistant pool of catecholamine (noradrenaline) or that reserpine increases the permeability of the receptor site to the amines normally liberated from the nerve endings.

Nash (1963) reported an intimate relationship between catecholamines and vasopressin vascular effects. In dogs under pentobarbital anesthesia, it was shown that pyrogallol (1, 2, 3, trihydroxy benzene) a catechol-o-methyl transferase (COMT) enzyme inhibitor, can also prevent and interrupt vasopressin tachyphylaxis (no dosages given). It is widely known that one of the major routes of degradation of sympathomimetic amines is via o-methylation and the presence of pyrogallol will potentiate the duration of action of adrenaline and noradrenaline. Since prior to this, it had been the accepted concept that vasopressin is a direct acting vasoconstrictor with a mechanism unrelated to adrenergic receptors, it was of interest to find that a COMT inhibitor would modify this response.

The work of Gardier and Abreu (1958) and Gardier et al. (1962) would favour this concept of a catecholamine, and hence an adrenergic receptor relationship. However, the possibility of pyrogallol being able to alter in some way the receptors for vasopressin or to increase dissociation from the receptor site must not be ruled out, since other inhibitors of COMT do not have this effect (e.g. catechol), and it has previously been shown that pyrogallol possesses an antispasmotic action unrelated to COMT inhibition.

An opposing view is maintained by Gardier et al. (1965) who claimed that the most probable pharmacological action is a combination of direct and indirect actions, and from their study on the mechanism of action of antidiuretic hormone, they implied that a change in permeability was the indirect mechanism.

In a general summary of all the data presented, it is quite clear that nitroglycerin and the nitrites does increase coronary flow in isolated heart preparations. Evidence obtained in intact animals and human studies is quite confusing and so far have yielded no unified theory of the mechanism of action. It is the concensus of opinion (as has been repeatedly demonstrated) that nitroglycerin, and the nitrites, when administered intravenously, will antagonize coronary vasoconstriction induced by vasopressin. Recent evidences (Nash et al. 1962) have indicated that the mechanism of action of vasopressin, which was thought to be a direct musculotropic one, may be, in some way, related to a release of catecholamine (e.g. noradrenaline). In the light of these observations, it would be then logical to assume that presumably

the nitrites coronary vasodilation and vasopressin coronary constriction antagonism might be, in some way, related to adrenergic receptors in the coronary vascular bed.

III. METHODS AND MATERIALS

A. CORONARY FLOW AND HEART CONTRACTIONS MEASUREMENTS IN ISOLATED PERFUSED HEARTS OF MONKEYS

Warm (37 ± 1°C) McEwen's (1956) solution, and a modified Langendorff preparation were employed. McEwen's solution had the following composition per 1,000 ml: NaCl 7.6 gm, KCl 0.42 gm, $CaCl_2$ 0.24 gm, NaH_2PO_4 0.143 gm, $NaHCO_3$ 2.1 gm, dextrose 2.0 gm, sucrose 4.5 gm. The required quantity to make a volume of 20 litres was dissolved individually, and except for the $NaHCO_3$, was added to a vessel which contained about one-third of the required quantity of distilled water. $NaHCO_3$ was bubbled with CO_2 for 15 minutes before being added to the vessel. The total volume (20 litres) was then bubbled literally with a mixture of 95% O_2 and 5% CO_2 for 20 minutes. The pH of this solution, as determined routinely, was 7.3. Adequate oxygenation was maintained throughout all experiments. McEwen's solution definitely increased the useful life and stability of the preparation as compared to Locke or Tyrode solutions, which have been previously employed in this laboratory by other investigators.

The perfusing apparatus used in this investigation was similar to that described by Agrawal (1965), and a simplified diagram is shown in Figure 1. For measuring coronary inflow, a 100 ml/min maximum flow capacity rotameter[1] was used. Calibration was done by measuring the

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[1]The rotameter was obtained from Clifford Wilson Instruments, 325 West 42nd Street, Indianapolis, Indiana, U.S.A.

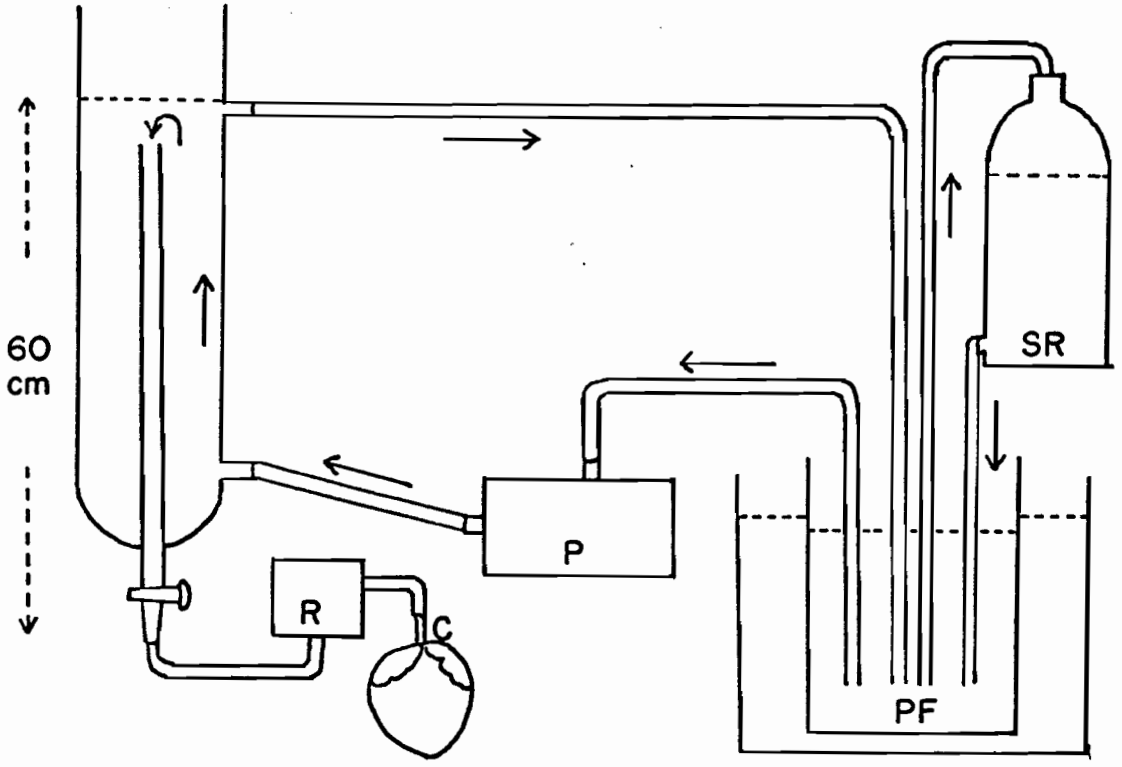
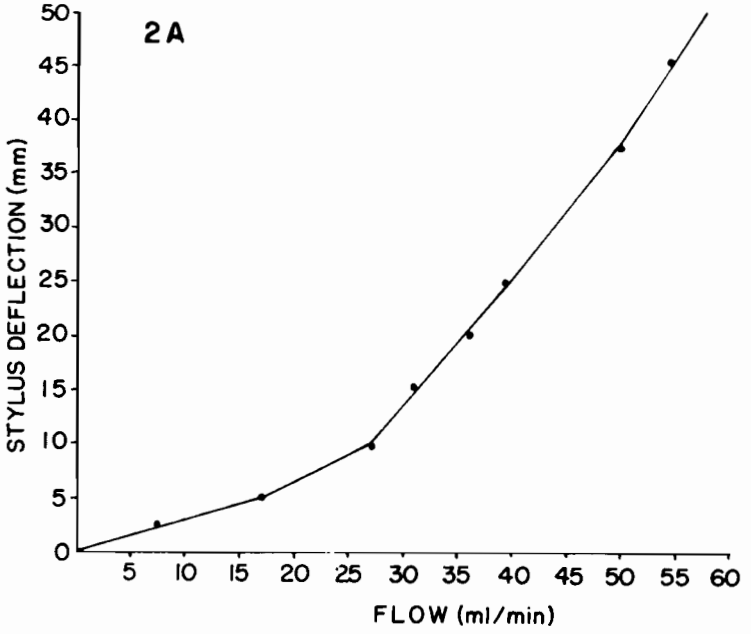


FIG.I DIAGRAM OF PERFUSION APPARATUS
C= AORTIC CANNULA WITH HEART P= PERISTALTIC PUMP
R= ROTAMETER SR= SUPPLEMENTARY RESERVOIR
PF= PERFUSING FLUID CONTAINER IN WATERBATH

volume of fluid collected in a graduate cylinder over a one-minute period, at various rates, and relating this to the recorder pen deflection. Several calibration points over the desired range were obtained and these were plotted to obtain a calibration curve as shown in Figure 2A. Zero flow was determined by stopping the flow completely. Mean coronary flow (ml/min) was determined by taking the average of maximum and minimum flow, as was determined by correlating the recorder pen deflection with the flow rates from the calibration curve.

For recording the amplitude of contractions, a Grass Model (F.T. 03) force displacement transducer was used. For calibration, gram weights were hung by a non-stretch fish line to the lug of the transducer. The recorder pen deflections, corresponding to increasing weights, were plotted to obtain a calibration curve for the transducer. Sensitivity of the recorder was adjusted so that a weight of 5 grams corresponded to a recorder pen deflection of 1 ml. Prior to the commencement of an experiment, diastolic tension of the isolated hearts was set between 3 and 5 grams and changes in isometric tension recorded. Contractile amplitude is expressed in 2 ways (a) as per cent change; an increase or decrease expressed as a percentage of pre-injection values; (b) as millimeters of recorder pen deflection. For comparative purposes, the changes expressed as percentages are more valid since there were variations in the weights of the isolated hearts used and it is possible that the larger hearts were capable of developing a greater force. Both coronary flow and amplitude of contraction were recorded concurrently on



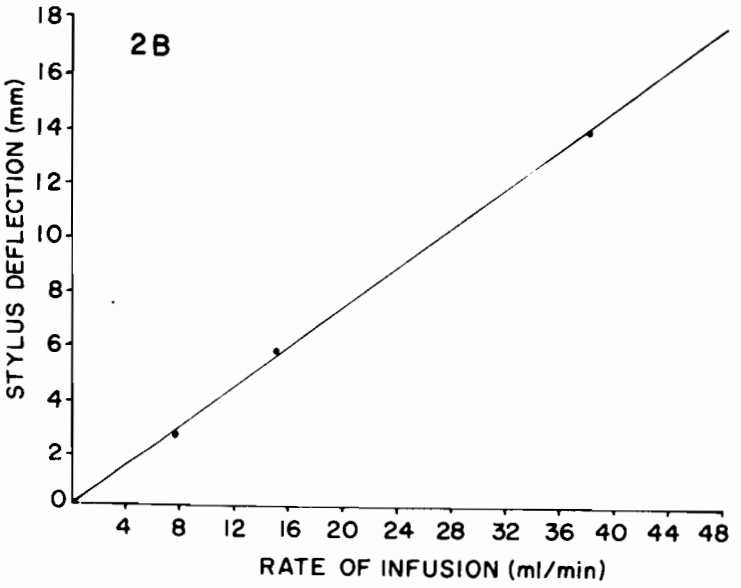


FIGURE 2A: Calibration curve of the rotameter used for reading out coronary flow in ml/min. Abscissa: flow of the perfusing fluid expressed in ml/min; Ordinate: the deflection of the writing pen from the zero base line.

FIGURE 2B: Calibration curve of the electromagnetic blood flowmeter used for reading out coronary flow in ml/min; Abscissa: rate of saline infusion expressed in ml/min; Ordinate: the deflection of the writing pen from the zero base line.

a Gilson Polygraph[1] at a speed of 10 mm/sec.

The heart rate was determined from the electrogram (EG). Lead II was recorded on a Sanborn Visocardiette. Two sterling silver wires (0.01 inch diameter) were connected, one to the aorta and the other to the apex of the ventricles. The aortic wire was then connected to the R.A. lead and the ventricular wire to the L.L. lead. The Sanborn recorder was calibrated for 1 mV output per 0.3 cm deflection.

Isolated hearts of 2 species of monkeys were used in this study. The original intent was to investigate the responses of the isolated perfused hearts of Macaca mulatta[2] (rhesus) but during the course of this investigation, 3 isolated hearts of Cercopithecus aethiops (vervet, African green) were available, hence they were also used. All hearts of vervet monkeys (3) and 1 heart of a rhesus monkey[3] were obtained from the Institut de Microbiologiè of the Université de Montréal, Laval des Rapides, Quebec. Two other hearts (rhesus) were obtained from monkeys purchased from the Institut de Microbiologiè through the McIntyre Animal Centre. These 6 hearts (3 rhesus, 3 vervets) were used to test the responses to catecholamines (see Results, Part One).

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[1]The Gilson Polygraph was obtained from Gilson Medical Electronics, Middleton, Wisconsin, U.S.A.

[2]Formerly Macacus rhesus.

[3]Courtesy of Dr. Gaston Boulay, Director, Institut de Microbiologiè d'Université de Montréal, Laval des Rapides, Quebec, Canada and Dr. Leslie Lord, Director, Animal Care, McGill University, Montreal, Canada.

The weights of vervet monkeys ranged from 2.5 to 3 kg (hearts 10-15 gm) and rhesus monkeys from 3 to 4 kg (hearts 12.5-15 gm). The animals were anesthetized with pentobarbital sodium (Nembutal); 60 mg/kg intraperitoneally. In all animals used in Part One (Results), the jugular veins were severed and the animals were completely exsanguinated. Following removal of the kidneys (applicable to hearts obtained from the Institut de Microbiologiè) the chest was opened by a median incision and the heart (with about 1 cm of the aorta) was immediately removed. This heart was then perfused with cold (20°C) McEwen's solution until free of blood (using a 20 ml syringe) and was then placed in a 2 litre capacity vacuum bottle, containing oxygenated cold (10°C) McEwen's solution.

After being cooled for 2 to 3 hours,[1] the hearts were suspended by the aorta to the perfusion cannula of the apparatus and perfusion was started using McEwen's solution at room temperature (23°C). The temperature of the solution was increased to 37 ± 1°C over a 30 minute period, and all hearts treated thus showed electrical activity within 10-20 minutes. Five out of 6 hearts treated thus showed ventricular fibrillation. In 2 hearts (1 rhesus, 1 vervet), oxytocin (Sandoz, synthetic, 10 I.U.) and in 3 hearts (1 rhesus, 2 vervets) procainamide hydrochloride (Pronestyl, 10 mg) were equally effective in restoring normal sinus rhythm. All 5 hearts showed normal sinus rhythm within

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[1] During this time, the hearts were transported from Laval des Rapides to Montreal. Hearts obtained through the McIntyre Animal Centre were also cooled for 2½ hours.

120 to 180 minutes. In one rhesus heart, normal sinus rhythm was restored without the aid of drugs within 60 minutes.

The isolated hearts of rhesus monkeys,[1] used in Part Two (Results section) were removed after perfusion of the lung *in situ* as indicated below. The animals were anesthetized with pentobarbital sodium (60 mg/kg) intraperitoreally and were then artificially respired with room air, using a Havard respiration pump. The chest of the animal was then opened by a median incision and the heart was exposed. A 16 gauge hypodermic needle (connected to a perfusion bottle filled with Tyrode's solution at room temperature) was then inserted into the apex of the left ventricle, through the interventricular septum and into the pulmonary vein. A small incision was then made in the right ventricle through which systemic blood escaped. The lungs were then perfused with Tyrode's solution until entirely free of blood (10-20 min).

On completion of the perfusion procedure, the heart and lungs were removed together. Heart and lungs were then separated, and the heart was placed in a 2 litre capacity vacuum flask filled with cold (10°C) oxygenated McEwen's solution. After attaching the heart by the aorta to the perfusion apparatus, perfusion, using McEwen's solution, was began at room temperature and the temperature of the solution was increased to 37 ± 1°C over a 30 minute period. Hearts treated thus,

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[1] Courtesy of Dr. B. A. Kovacs, Associate Professor, Department of Pharmacology, McGill University and Division of Allergy and Immunology, Royal Victoria Hospital, Montreal, Canada.

showed immediate electrical activity and normal sinus rhythm was restored within 30 minutes and without the aid of any chemical agents. None of these hearts showed ventricular fibrillation presumably due to the short (15-30 min) periods of cooling.

There was no failure to achieve a normal sinus rhythm in any of the hearts obtained in the course of this investigation. The preparation was considered satisfactory when three consecutive recordings, made at 5 minute intervals, gave a heart rate, amplitude of contraction, and coronary flow of equal value. All preparations were carefully examined for evidences of impaired aortic values (an excessive rate of inflow during the stabilization period or a sudden increase in coronary inflow) and, for any observable evidences of myocardial damages. Of a total of 11 hearts, 2 (rhesus) showed evidences of impairment and were discarded.

Injections were contained in 0.5 ml of McEwen's solution and were made obliquely in the rubber tubing with the tip of the needle (25 gauge) directed towards the aortic cannula, to prevent any changes in the perfusion pressure (60 cm H_2O). All administrations of catecholamines were made at 10 minute intervals and other agents at 5 minute intervals.

In order to achieve beta-adrenergic blockade (BAB), pronethalol was added to the reservoir of perfusion fluid to attain a final concentration of 0.12 ug/ml (Melville and Benfey 1965). The isolated hearts were then perfused with this solution for 30 minutes after which time, and while still continuing the perfusion, the various agents were tested. The complete experiment lasted for a period of 5 to 6 hours

including the period of stabilization, and at the end of this period, most of these hearts were quite viable. In fact, several of these were cooled and revived at varying periods, after which time normal responses could be induced in response to drugs. In one heart (rhesus) a normal sinus rhythm was present, on the third day (51 hours) after being alternately cooled and revived on successive days. This preparation was responsive to doses of adrenaline (10 ug), and noradrenaline (10 ug) although these responses were not comparable to those obtained on the first day.

B. CORONARY FLOW AND HEART CONTRACTIONS MEASUREMENTS IN OPEN-CHEST ANESTHETIZED DOGS WITH THE HEART BEATING IN SITU

An electromagnetic blood flowmeter was employed for measuring coronary flow. This type of flow measurement has several advantages over other methods (Wetterer 1963). It furnishes direct transformation of the mechanical magnitude into an electrical signal. Its interference with blood flow is very small and can be completely neglected. It delivers strictly linear calibration curves and equal sensitivities with opposite signal direction to forward and backward flow. Its calibration, in terms of average velocity of flow rate, is independent of the velocity profile and of the density, viscosity, and temperature of the fluid. The range of frequency to which it responds is theoretically unlimited and depends, in practice, on the electrical equipment used. Most important, this method of recording coronary flow is applicable to unopened blood vessels and, therefore, does not require damaging the blood vessels.

Male and female mongrel dogs (13-22 kg) were used in this study. Following anesthesia with pentobarbital sodium (30 mg/kg i.v.), the trachea was cannulated and the animal respired with room air, using a Havard pump (15-20 ml at a rate of 20 strokes/min). After cannulation of the femoral vein and artery, the chest was opened between the 4th and 5th ribs on the left side. The pericardium was then cut and the edges either sutured to the skin on either side of the opening of the chest, or otherwise suitably fastened to the operating table. This was done in such a way that the heart was brought into an accessible position

with a minimum of disturbance of its initial position. The left circumflex branch of the left coronary artery was dissected out, as near to its origin as possible. A loose ligature (size 25 thread) was passed under the exposed artery. By gently pulling on the ligature, the distal section of the exposed artery was emptied of blood and then slipped through a 1.5 mm probe which, in turn, was attached to the gated sine wave electromagnetic blood flowmeter.[1]

For the determination of zero flow, methacholine chloride (10-15 µg/kg) was injected intravenously. The flowmeter was calibrated at the end of each experiment by cannulating the same vessel upstream to the probe, and forcing saline at known rates through the vessel, using a Havard infusion pump. The flowmeter output deflection was then directly correlated to the flow in ml/min. It has been shown that the results were identical when blood or saline were used (Gillis 1965). A typical correlation curve, using saline, is shown in Figure 2B.

Systemic blood pressure (systolic and diastolic) was recorded by a Sanborn (model 267A) transducer, connected to the femoral artery by a polyethylene (PE 200) catheter. Heparin (2 mg/kg i.v.) was given to prevent clotting and the intra-arterial catheter was periodically flushed with heparinized saline to prevent clotting.

Electrocardiograms (Lead II) were taken during each experiment. This was used to (a) determine heart rate, (b) determine any disturbances

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[1]The electromagnetic blood flowmeter was obtained from Elliot Instrument Company, 2411 Eucalyptus Way, San Bruno, California, U.S.A.

of normal cardiac rhythm, (c) correlate the electrical changes of the cardiac cycle with the concomitant changes in pulsatile coronary flow. Since the lowest coronary flow recorded, occurred between the summit of the R wave and the end of the T wave (ventricular systole), the "mean coronary flow" was determined by adding one half of the pulsatile flow to the flow valve obtained during systole.

The force of left ventricular contraction was measured using the strain gauge arch technique (Boniface et al. 1953; Cotton and Bay, 1956). By this technique, the muscle segment was stretched to 30 to 50% of its normal length. This substantially reduced the relative importance of local bulging of the muscle segment, introduced by changes in the venous return and arterial pressure. Concurrently, the coronary flow, blood pressure, electrocardiograms and contractile force were recorded on a Sanborn 964 recorder. Unless otherwise stated, all drugs were administered intravenously and washed in with a volume of 5 ml of saline.

To achieve beta-adrenergic blockade, pronethalol (100 ug/kg/min) was infused into the femoral vein at a rate of 0.382 ml/min, using a 50 ml syringe and a Havard infusion pump.

C. ELECTROCARDIOGRAMS AND BLOOD PRESSURE RECORDINGS ON TRAINED UNANESTHETIZED DOGS

Male and female mongrel dogs (10-16 kg) were trained for a period of 6 weeks, at the end of which the animals were able to tolerate various operation procedures without visible signs of discomfort. The mouth was kept opened for periods of 5 minutes during which time swallowing was impaired. A canine mouth-speculum was used for this procedure. During the period of training, circular rings of polyethylene tubing (cut to approximately the size of a nitroglycerin hypodermic tablet) were placed at the base of the tongue for 5 minutes (to mimic the tablets used in the experiments). A catheter attached to a hypodermic needle (21G) was inserted into the brachial vein, for the intravenous administration of drugs. Under local anesthesia (Xylocaine HCl 2%), a Cournand needle (18G) was inserted into the femoral artery and connected to a Sanborn pressure transducer (267A) for recording arterial blood pressure. Heparin (2 mg/kg) was administered intravenously. Electrocardiograms (Lead II) were recorded using German silver plate electrodes covered liberally with electrode paste to ensure adequate contact. The blood pressure and electrocardiograms were recorded on a Sanborn 964 recorder.

All animals were fasted overnight before being used and no animals were used more than twice and never more than once in the same series of experiments. Before and after each experiment, recordings of all standard leads (I, II, III) were made to detect any irregularities in the electrocardiogram. All injections were made intravenously and were washed in with 2 ml of sterile saline.

D. DRUGS USED

The following drugs were used in the course of these studies:

(1) Glyceryltrinitrate (nitroglycerin) 9.36% in lactose, supplied by Charles E. Frosst and Company, Montreal, Canada.

(2) Nitroglycerin hypodermic tablets (1/100 grain), supplied by the Royal Victoria Hospital Pharmacy, Montreal, Canada.

(3) Trolnitrate phosphate (metamine) 5.6% in lactose, supplied by Thos. Leeming and Company, Parsippany, New Jersey.

(4) L-Anterenol Bitartrate (noradrenaline), supplied by Winthrop Laboratories, New York, U.S.A.

(5) L-Epinephrine Bitartrate (adrenaline), supplied by Winthrop Laboratories, New York, U.S.A.

(6) L-Isoprotererol d Bitartrate (isoprenaline), supplied by Winthrop Laboratories, New York, U.S.A.

(7) Pronethalol (1-2 naphthyl -2 -isopropylaminoethanol hydrochloride), supplied by Ayerst, McKenna and Harrison Limited, Montreal, Canada.

(8) Synthetic oxytocin, supplied by Sandoz Pharmaceuticals, Montreal, Canada.

(9) Synthetic vasopressin (Pitressin), supplied by Parke Davis and Company, Limited, Brockville, Ontario, Canada.

(10) Methacholine chloride (mecholyl), supplied by Merck and Company, Rahway, New Jersey, U.S.A.

Solutions of the drugs were always freshly prepared by dissolving these agents in 0.9% sodium chloride or McEwen's solution. All doses, used in these studies, have been expressed as their salts.

Statistical procedures employed are those of Steel and Torre (1960), and results were declared significant if they exceeded the 95% probability level.

IV RESULTS

PART I - COMPARATIVE EFFECTS OF NORADRENALINE, ADRENALINE AND ISOPRENALINE ON CORONARY FLOW AND HEART CONTRACTIONS AS RECORDED IN THE ISOLATED PERFUSED HEARTS OF MONKEYS

Comparative changes in coronary flow and heart contractions (rate and amplitude) were studied in isolated heart preparations of both Macaca mulatta (rhesus) and Cercopithecus aethiops (vervet) monkeys. Prior to the commencement of an experiment, an injection of 0.5 ml of McEwen's solution was given and recordings were made at 1 minute intervals, for 5 minutes. If there was any change in any parameters recorded, the preparation was considered not stabilized, and the experiment was started only when this procedure produced no change in any parameters.

Threshold doses of noradrenaline, adrenaline and isoprenaline were determined for the isolated hearts of both species of monkeys (2 experiments each) and appeared to be similar with respect to noradrenaline (0.01 µg) and adrenaline (0.01 µg). In the case of isoprenaline, the hearts of vervet monkeys appeared more sensitive since doses of 0.0004 µg, produced responses in these hearts comparable to the responses produced by doses of 0.004 µg in the hearts of rhesus monkeys.

A. Effects of Noradrenaline

The results obtained in experiments in which the effects of 1 µg of noradrenaline was tested in both species are summarized in Table I.

TABLE I

COMPARATIVE CHANGES IN MEAN CORONARY FLOW, HEART RATE AND CONTRACTILE AMPLITUDE IN HEARTS OF MACACA MULATTA (R) AND CERCOPITHECUS AETHIOPS (V) MONKEYS

Drug and Dose	Species	Procedure	Mean Coronary Flow ml/min		Heart Rate beats/min		Contractile Amplitude mm	
			Control	Max. Change	Control	Max. Change	Control	Max. Change
NAD	R	Control	26±1.7	20±1.2	103±8	134±17	9±3.1	24±2.7
		BAB	23±0.4	21±1.1	97±10	122±12	10±3.0	19±7.4
1.0 µg	V	Control	20±1.8	31±1.4	94±7	133±15	16±4.2	34±4.7
		BAB	16±2.4	20±2.3	80±16	90±16	9±1.0	14±2.0
ADR	R	Control	25±2.0	17±0.2	102±9	129±19	11±4.1	24±5.3
		BAB	24±0.7	21±1.0	98±9	119±19	9±2.9	18±5.3
1.0 µg	V	Control	20±3.0	32±4.0	82±5	143±30	11±3.4	31±9.0
		BAB	15±1.5	18±2.0	77±19	85±20	7±0.7	12±1.1

BAB = Values during Beta-Adrenergic Blockade with pronethalol.
All values are mean ± Standard Error of 3 experiments.

These are also shown graphically in Figure 3 and 4. Following injections of noradrenaline (1 µg) in rhesus monkeys (3 experiments), there was a prompt decrease in mean coronary flow from a control value of 26 ± 1.7 ml/min to a minimum of 20 ± 1.2 ml/min within 20 seconds. Concomitantly, there was marked myocardial stimulation with an increase to 24 ± 2.7 mm in contractile amplitude from a control value of 9 ± 3.1 mm, and this reached a maximum within 30 seconds. Heart rate was similarly increased from 103 ± 8 (Control), to a maximum of 134 ± 17 beats/min. It will be observed from Figure 3 (Control) that even after 5 minutes, mean coronary flow (% change) was still below control (preinjection) values although heart rate and amplitude had returned to control values. From an examination of this Figure, although the maximum decrease in coronary flow occurred before (10 secs) the maximum change in amplitude, one may conclude that the decrease in coronary flow may be due to the associated intense myocardial stimulation induced by this agent, since these changes appeared to occur simultaneously.

In contrast to the hearts of rhesus monkeys, following injections of noradrenaline (1 µg) in the heart preparations of vervet monkeys, there was a significant increase in mean coronary flow to a maximum of 31 ± 1.4 from a control value of 20 ± 1.2 ml/min in 50 seconds. Figure 4 shows that at no instant there was even a transient decrease in coronary flow as have often been reported to occur following catecholamine administrations in several species. The control flow was restored within 5 minutes. There was an associated increase in heart rate from 94 ± 7 to 133 ± 15 beats/min. The amplitude of contraction

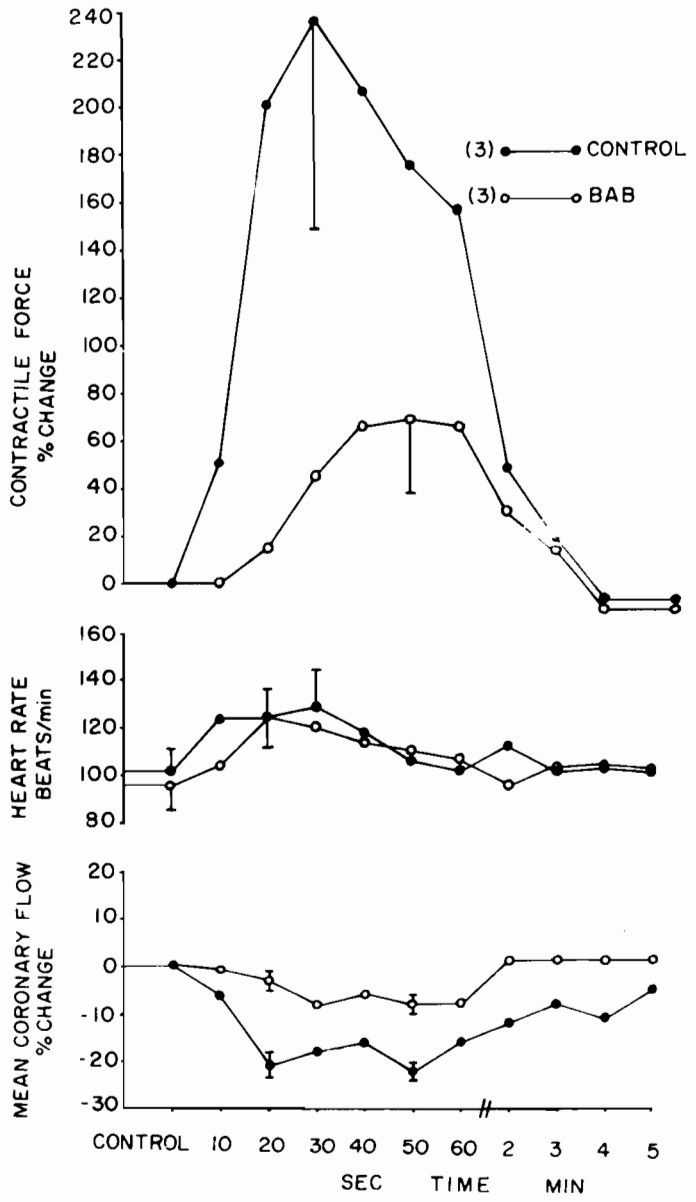

FIGURE 3: Isolated perfused heart of rhesus monkey. Effects of noradrenaline (1.0 µg) on contractile force, heart rate and coronary flow before (solid circles) and during (open circles) pronethalol perfusion(BAB). The abscissa denotes time (sec and min) after noradrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

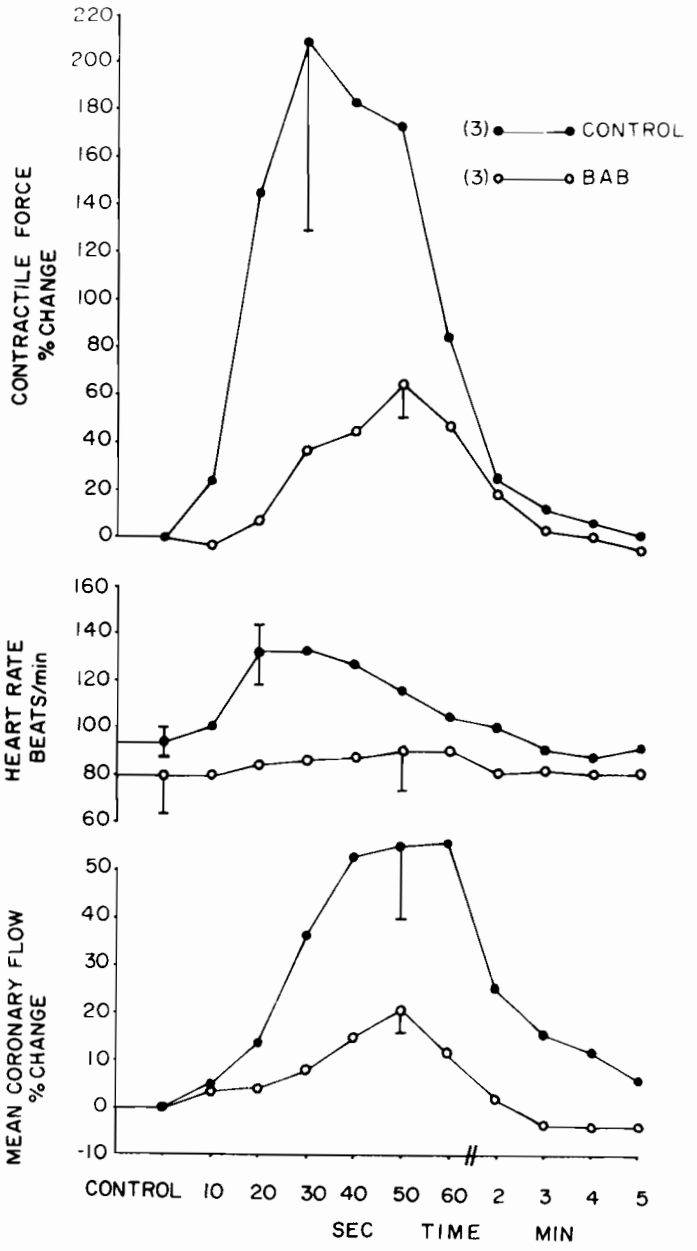

FIGURE 4: Isolated perfused heart of vervet monkey. Effects of noradrenaline (1.0 µg) on contractile force, heart rate and coronary flow before (solid circles) and during (open circles) pronethalol perfusion(BAB). The abscissa denotes time (sec and min) after noradrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

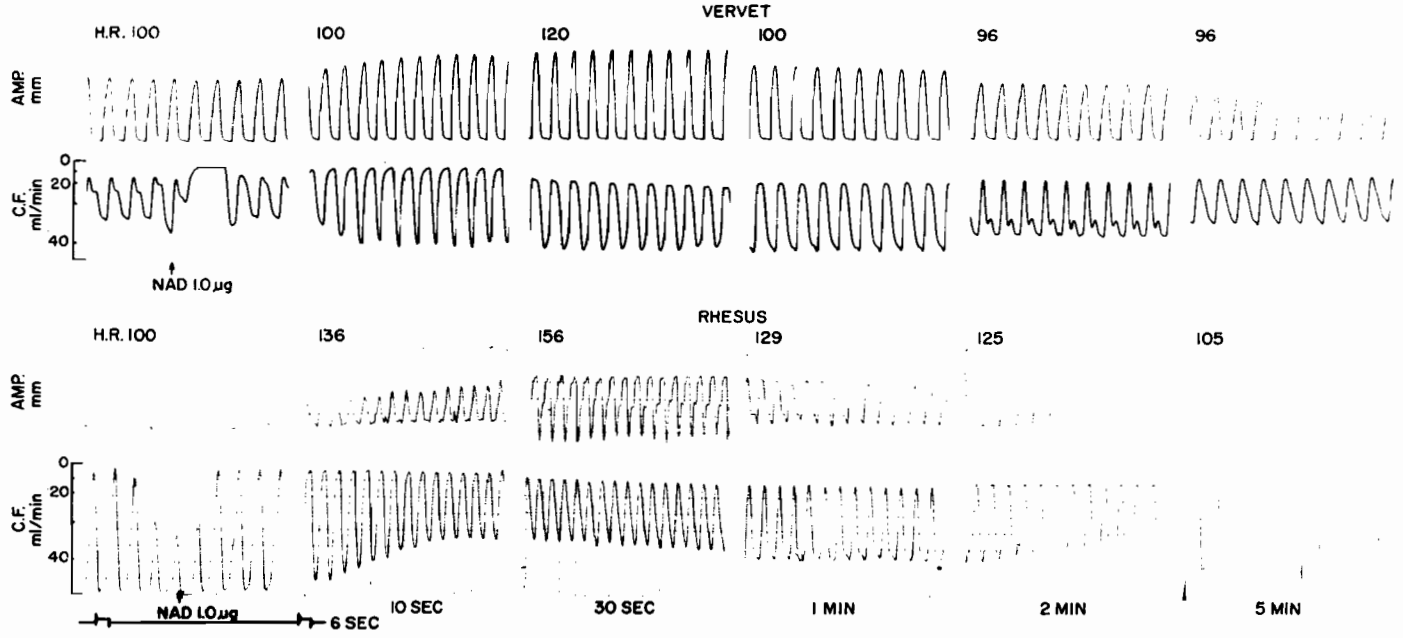

FIGURE 5: Isolated perfused hearts of monkeys. Experiments illustrating the comparative effects of noradrenaline (1.0 µg) on heart rate (HR), contractile amplitude (AMP) and coronary flow.

was similarly increased from 16 ± 4.2 (Control) to 34 ± 4.7 mm. Maximum contractile changes occurred within 30 seconds as was the case in the heart preparations of rhesus monkeys. Figure 4 (Control) shows that, in spite of the increases in both heart rate and contractile amplitude, the coronary flow was still markedly increased, hence it may be concluded that the increased contractions did not impede coronary flow. In neither species was there any secondary increase in coronary flow as are often seen in experiments using heart preparations of other animals. Comparative records of typical experiments are shown in Figure 5.

Following beta-adrenergic blockade (BAB) the responses to noradrenaline (1 µg) were also compared and the values obtained are summarized in Table I and shown graphically in Figures 3 (BAB) and 4 (BAB). In the hearts of rhesus monkeys, following administrations of noradrenaline, mean coronary flow still decreased for over 60 seconds although this was significantly less than the values obtained before blockade (see Figure 3 BAB). The changes in heart rate seen before blockade were not affected while changes in the amplitude of contraction were greatly reduced. The hearts of vervet monkeys showed an increase in coronary flow despite the blockade of beta receptors, although this was significantly less than the values obtained before blockade (Figure 4 - BAB). The changes in heart rate were completely abolished while the changes in amplitude of contraction were markedly reduced. Figure 6 shows comparative records of typical experiments.

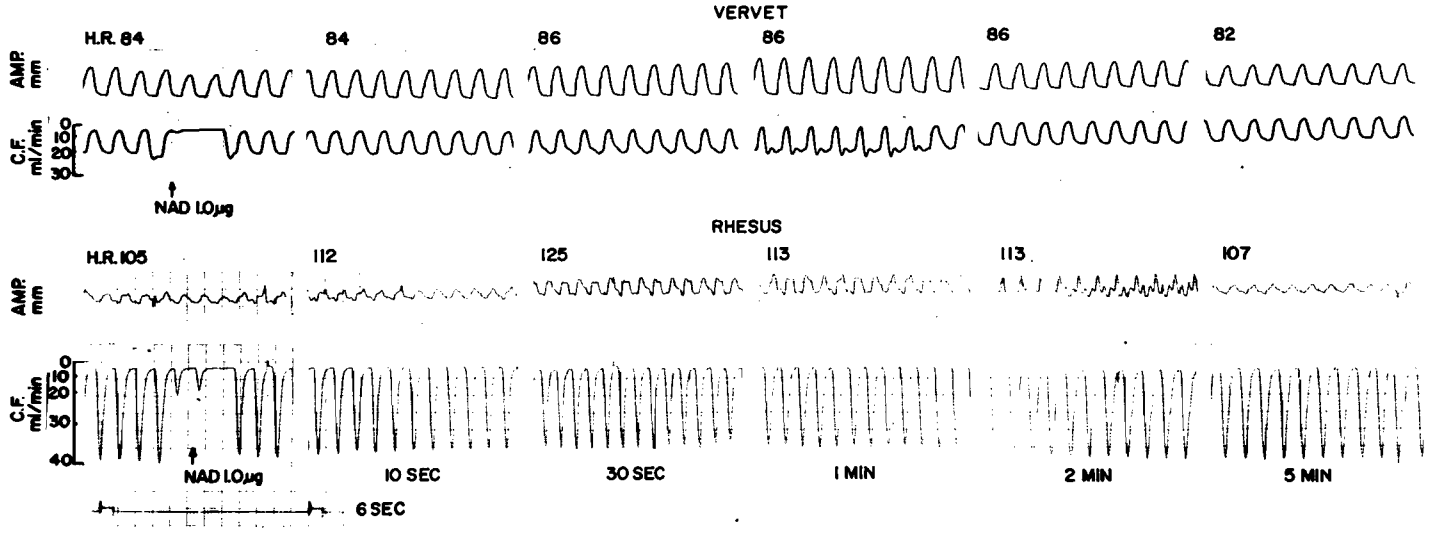

FIGURE 6: Isolated perfused hearts of monkeys. Experiments illustrating the comparative effects of noradrenaline (1.0 ug) on heart rate (HR), contractile amplitude (AMP) and coronary flow (CF) during pronethalol perfusion.

B. Effects of Adrenaline

Comparative changes in mean coronary flow and heart contractions in response to an injection of adrenaline (1 µg) were obtained and are summarized in Table I. Figures 7 and 8 show these changes graphically. In the isolated hearts of rhesus monkeys, following injections of adrenaline, there was a consistent decrease in mean coronary flow from a control value of 25 ± 2.0 to a value of 17 ± 0.2 ml/min within 20 seconds. Heart contractions were also affected. Heart rate increased from 102 ± 9 to 129 ± 19 beats/min and this coincided with the maximum decrease in mean coronary flow (20 secs) while maximum stimulation of contractile amplitude, from a control value of 11 ± 4.1 to 24 ± 5.3 mm occurred within 40 seconds. By comparison, there was less stimulation of heart contractions with adrenaline than with noradrenaline in this species while the mean coronary flow was more markedly decreased following adrenaline administration.

A similar injection of adrenaline (1 µg) produced a consistent increase in mean coronary flow in the hearts of vervet monkeys. (See Table I and Figure 8 - Control). This reached a maximum of 32 ± 4.0 from a control value of 20 ± 3.0 ml/min within 60 seconds, and returned to near control value within 5 minutes. The associated increase in heart rate was more pronounced, than was observed, following administrations of adrenaline. The change in amplitude was of the same magnitude as that induced by adrenaline, but when compared with heart preparation of rhesus monkeys, adrenaline induced a greater amplitude of contraction in the heart preparations of vervet monkeys. Figure 9 shows comparative records of typical experiments. It can, therefore, be inferred that noradrenaline

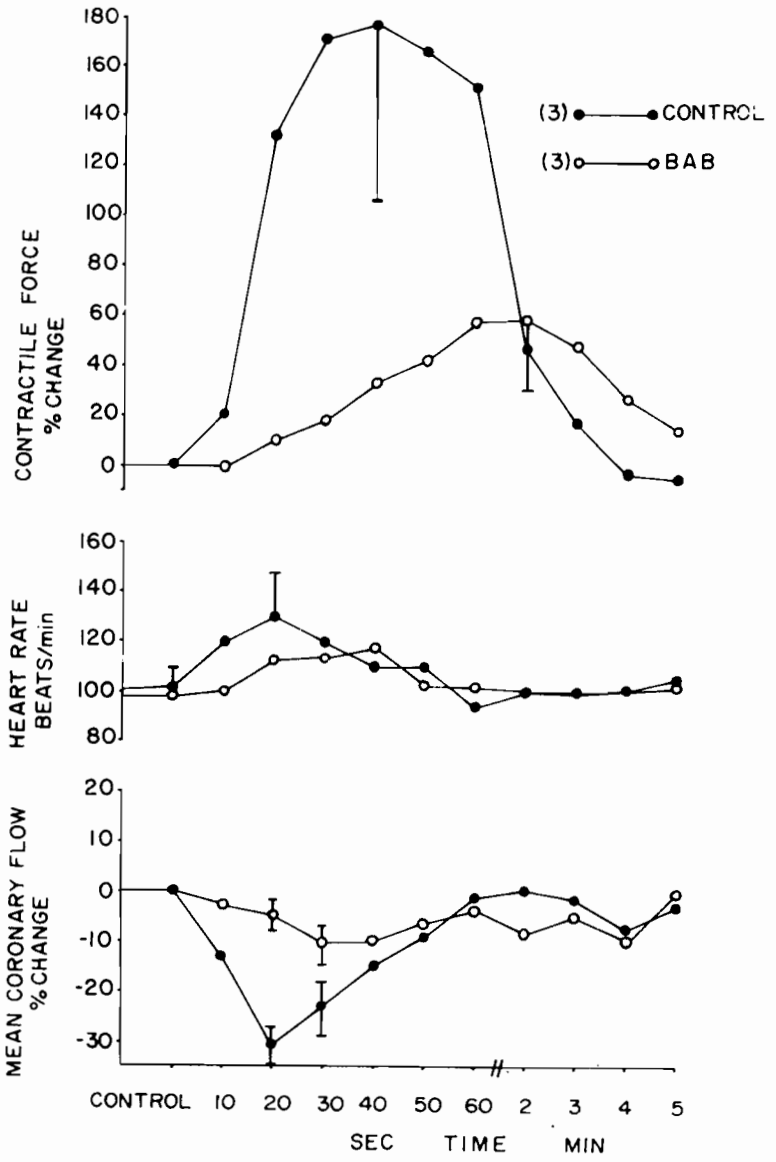

FIGURE 7: Isolated perfused heart of rhesus monkey. Effects of adrenaline (1.0 µg) on contractile force, heart rate and coronary flow before (solid circles) and during (open circles) pronethalol perfusion (BAB). The abscissa denotes time (sec and min) after adrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

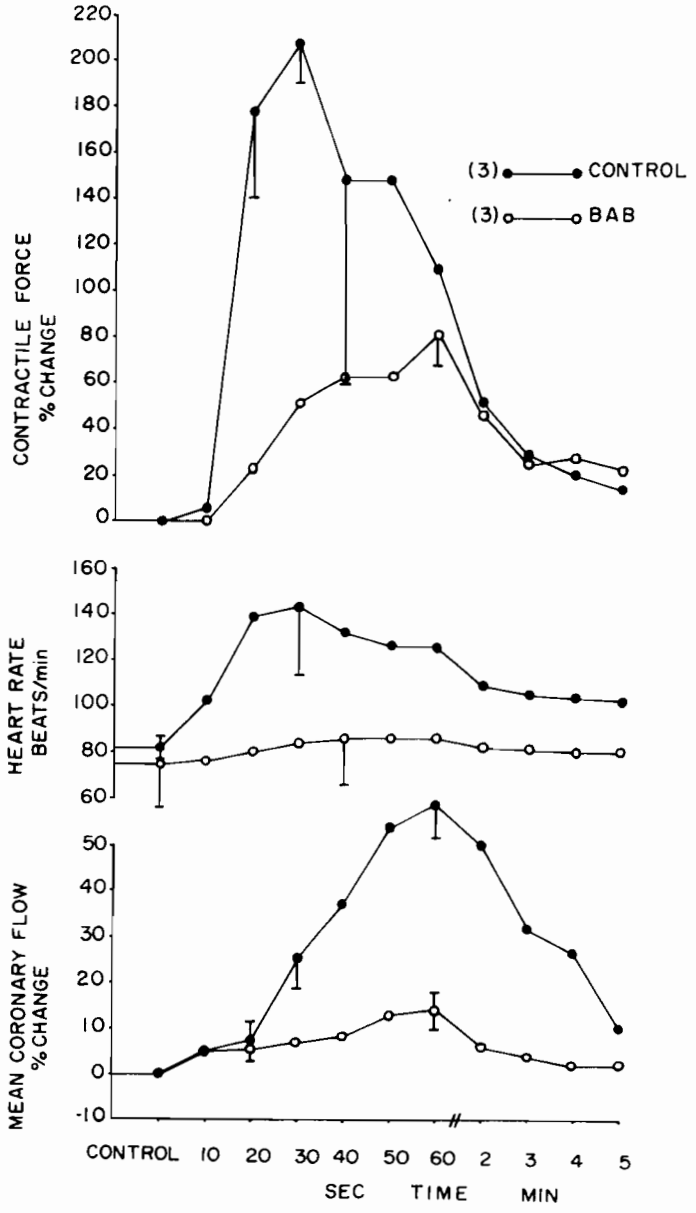

FIGURE 8: Isolated perfused heart of vervet monkey. Effects of noradrenaline (1.0 µg) on contractile force, heart rate and coronary flow before (solid circles) and during (open circles) pronethalol perfusion (BAB). The abscissa denotes time (sec and min) after noradrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

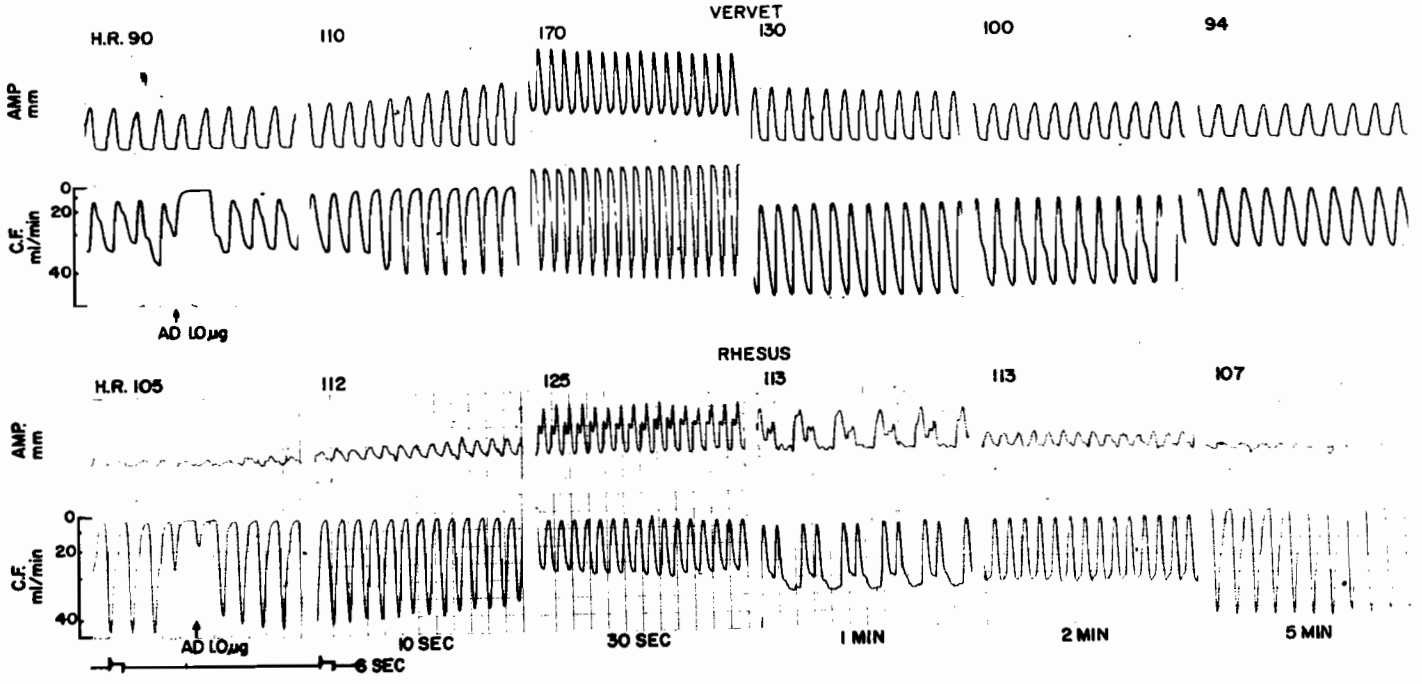

FIGURE 9: Isolated perfused heart of monkeys. Experiments illustrating the comparative effects of adrenaline (1.0 µg) on heart rate (HR), contractile amplitude (AMP) and coronary flow.

and adrenaline induced similar qualitative responses in heart contractions in both species. With respect to changes in mean coronary flow, whereas both agents caused increases in flow in the hearts of vervet monkeys, the responses shown by the hearts of rhesus monkeys was a consistent decrease.

A comparison of the responses to adrenaline during beta-adrenergic blockade is also summarized in Table I and shown graphically in Figures 7 (BAB) and 8 (BAB). The response of the coronary flow in the hearts of rhesus monkeys was still a consistent decrease following injections of adrenaline (1 µg). While heart rate changes were not significantly different from values observed before blockade, the amplitude of contraction was reduced with maximum changes occuring within 60 seconds after administrations of adrenaline (Figure 7 - BAB). In the hearts of vervet monkeys, the increase in coronary flow, observed before beta-adrenergic blockade, was greatly reduced. This would tend to indicate that most of the observed dilatation was due to the stimulation of beta-adrenergic mechanisms in this species. Changes in heart rate were completely abolished and the changes in amplitude of contraction were also significantly reduced (Figure 8 - BAB). Comparative records of typical experiments are presented in Figure 10.

C. Effects of Isoprenaline

A summary of the data obtained in response to injections of isoprenaline (0.04 µg) is presented in Table II, and the mean changes in coronary flow and heart contractions in both species are shown graphically in Figures 11 and 12. Isoprenaline induced a brief

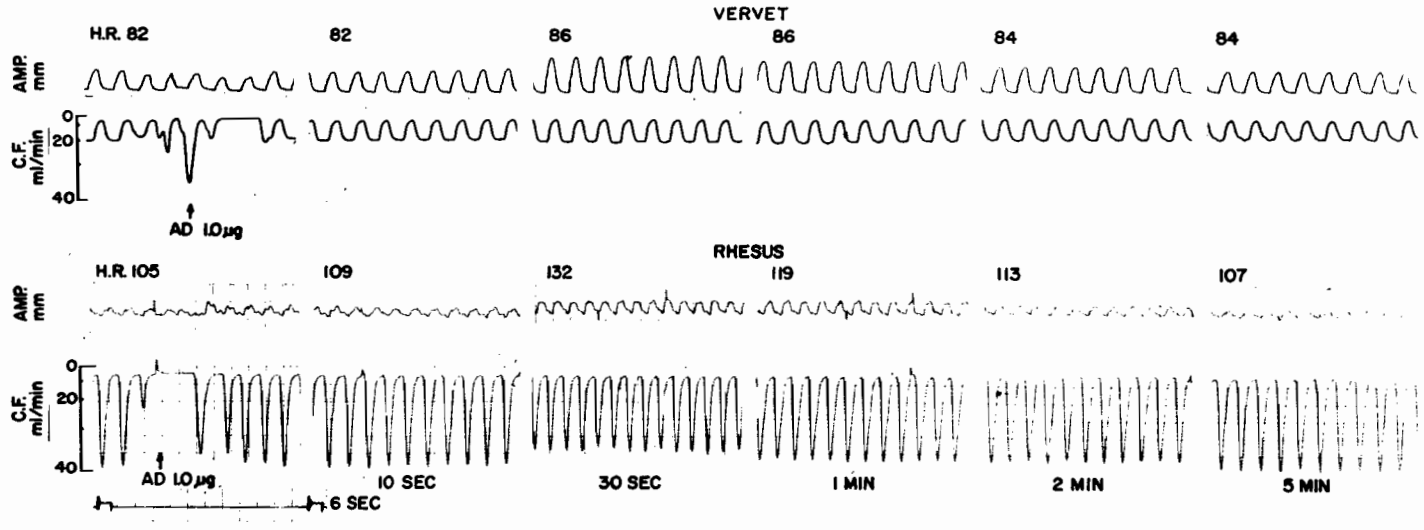

FIGURE 10: Isolated perfused hearts of monkeys. Experiments illustrating the comparative effects of adrenaline (1.0 µg) on heart rate (HR), contractile amplitude (AMP) and coronary flow (CF) during pronethalol perfusion.

TABLE II

COMPARATIVE CHANGES IN MEAN CORONARY FLOW, HEART RATE AND CONTRACTILE AMPLITUDE IN HEARTS OF MACACA MULATTA (R) AND CERCOPITHECUS AETHIOPS (V) MONKEYS

Drug and Dose	Species	Procedure	Mean Coronary Flow ml/min		Heart Rate beats/min		Contractile Amplitude mm	
			Control	Max. Change	Control	Max. Change	Control	Max. Change
ISO	R	Control	26±1.4	32±4.0	99±12	119±15	7±2.5	21±9.6
		BAB	24±0.7	24±0.7	94±7	94±7	8±2.8	8±2.8
0.04 ug	V	Control	21±4.3	31±2.8	90±13	131±26	8±2.0	22±9.9
		BAB	16±1.4	16±1.4	77±14	77±14	7±0.8	8±0.9
$CaCl_2$	R	Control	26±1.8	20±1.8	104±16	80±22	8±2.8	18±5.3
		BAB	24±1.0	19±1.0	94±7	72±15	7±2.5	12±4.0
5 mg	V	Control	23±4.1	15±3.4	96±17	116±37	8±2.3	19±1.5
		BAB	15±1.2	11±1.4	71±12	75±14	9±0.4	27±5.9

BAB = Values during Beta-Adrenergic Blockade with pronethalol.
All values are mean ± Standard Error of 3 experiments.

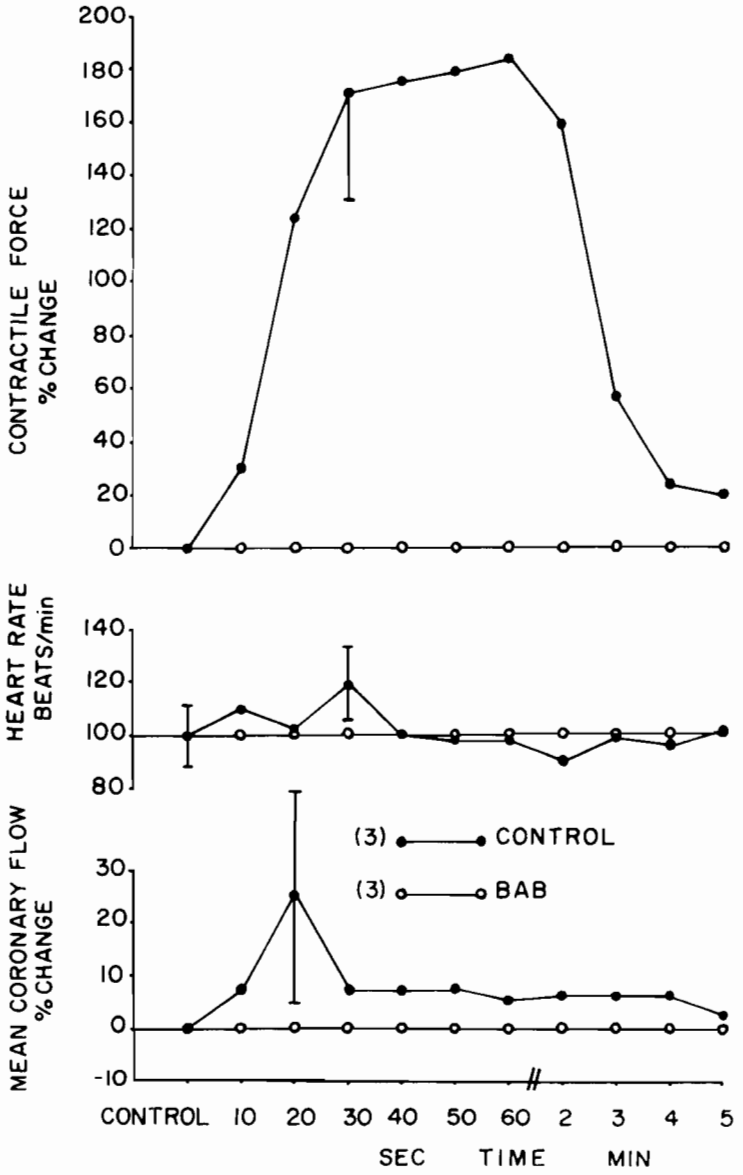

FIGURE 11: Isolated perfused heart of rhesus monkey. Effects of isoprenaline (0.04 µg) on contractile force, heart rate and coronary flow before (solid circles) and during (open circles) pronethalol perfusion (BAB). The abscissa denotes time (sec and min) after noradrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

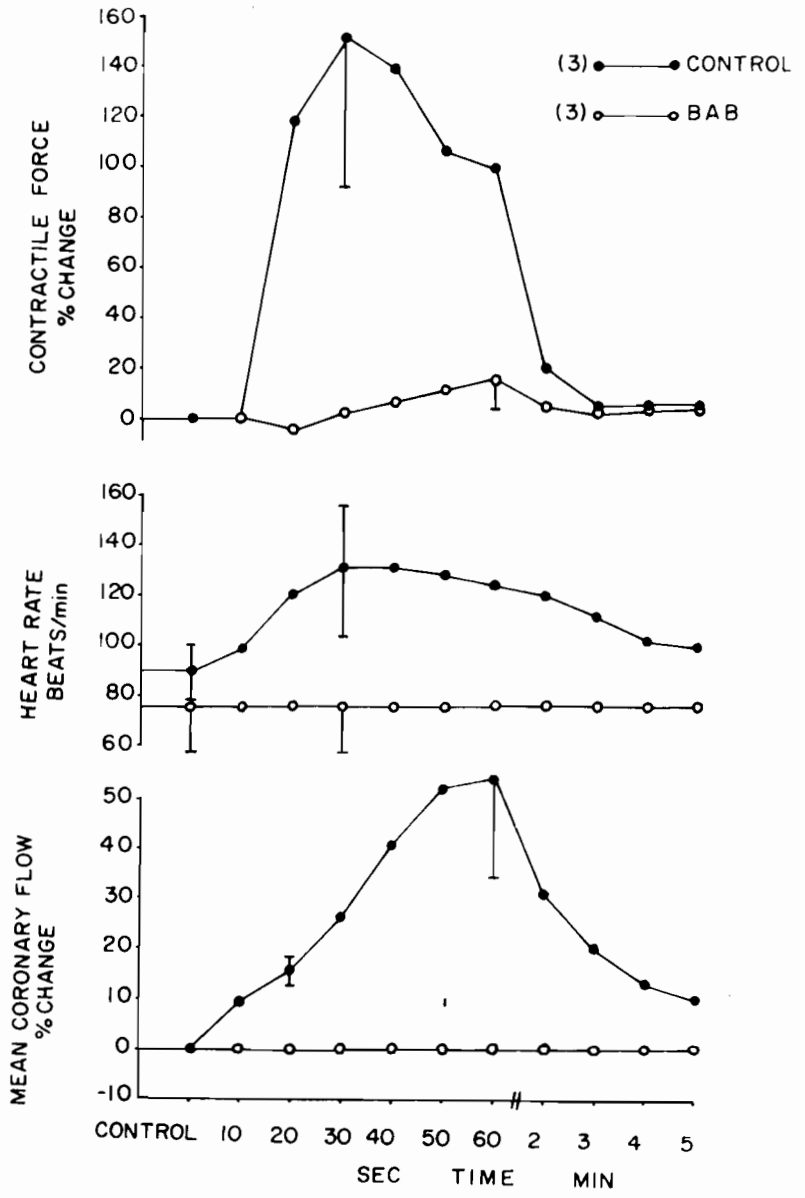

FIGURE 12: Isolated perfused heart of vervet monkey. Effects of isoprenaline (0.04 µg) on contractile force, heart rate and coronary flow before (solid circles) and during (open circles) pronethalol perfusion(BAB). The abscissa denotes time (sec and min) after noradrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

(statistically insignificant) increase in coronary flow in the hearts of rhesus monkeys. Heart rate was increased from a control value of 94 ± 12 to 119 ± 15 beats/min. The amplitude of contraction was markedly influenced and increased from a control value of 7 ± 2.5 to 21 ± 9.6 mm. The duration of this increase was for a much longer period than in responses to noradrenaline and adrenaline. Following isoprenaline, a pronounced increase in mean coronary flow was the constant response in the hearts of vervet monkeys. The maximum increase was 31 ± 2.8 from a control value of 21 ± 4.3 ml/min and this occurred within 60 seconds. Even after 5 minutes, the control flow was not restored. There was a gradual increase in heart rate to a maximum of 131 ± 26 from a control value of 90 ± 13 beats/min and even after 5 minutes the rate was still above the control value. Amplitude of contraction was less affected than in the hearts of rhesus monkeys and for a shorter time also. Figure 13 is a record of the comparative responses in both species.

Injections of isoprenaline (0.04 µg) during perfusion of the blocking agent (BAB) elicited no changes in either coronary flow or heart contractions in both species of monkeys (see Figures 11 - BAB, 12 - BAB, and 14).

In order to determine the degree of specificity of the inotropic blockade produced by pronethalol, the effects of the cardiac stimulant calcium chloride (5 mg) were compared before and during perfusion of the blocking agent in both species. The results obtained are tabulated in Table II. Figures 15 and 16 show graphically these changes. There was no evidence of blockade of the positive inotropic actions of calcium.

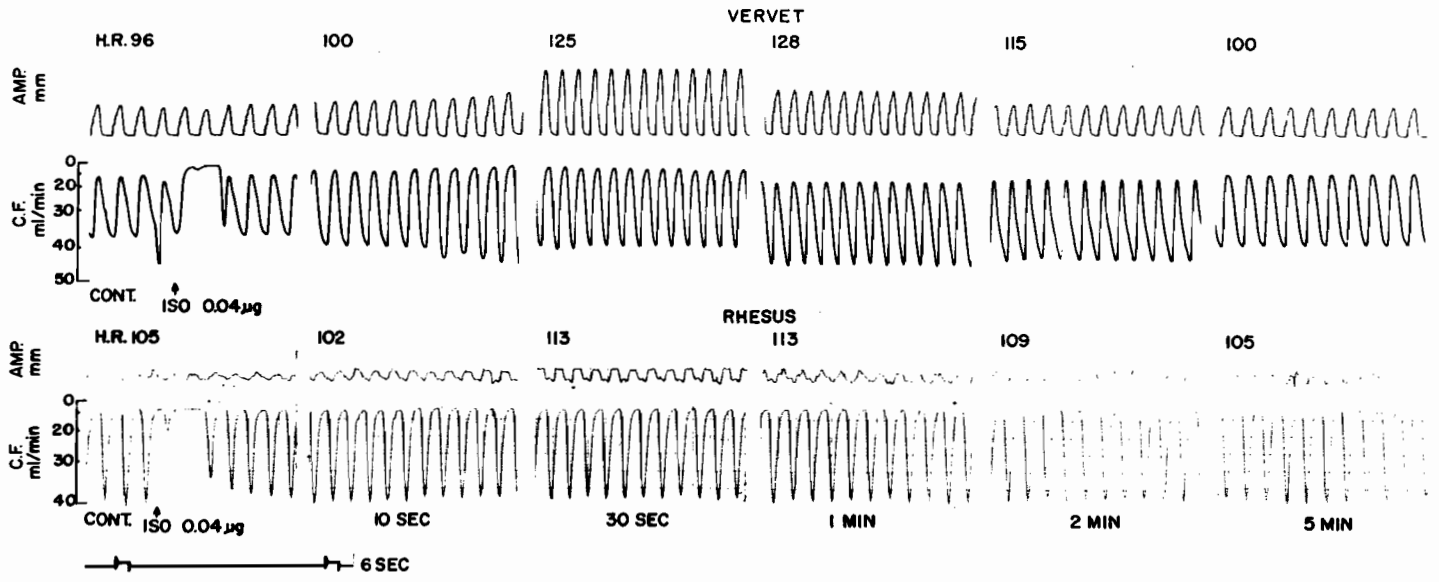

FIGURE 13: Isolated perfused hearts of monkeys. Experiments illustrating the comparative effects of isoproterenol (0.04 µg) on heart rate (HR), contractile amplitude (AMP) and coronary flow.

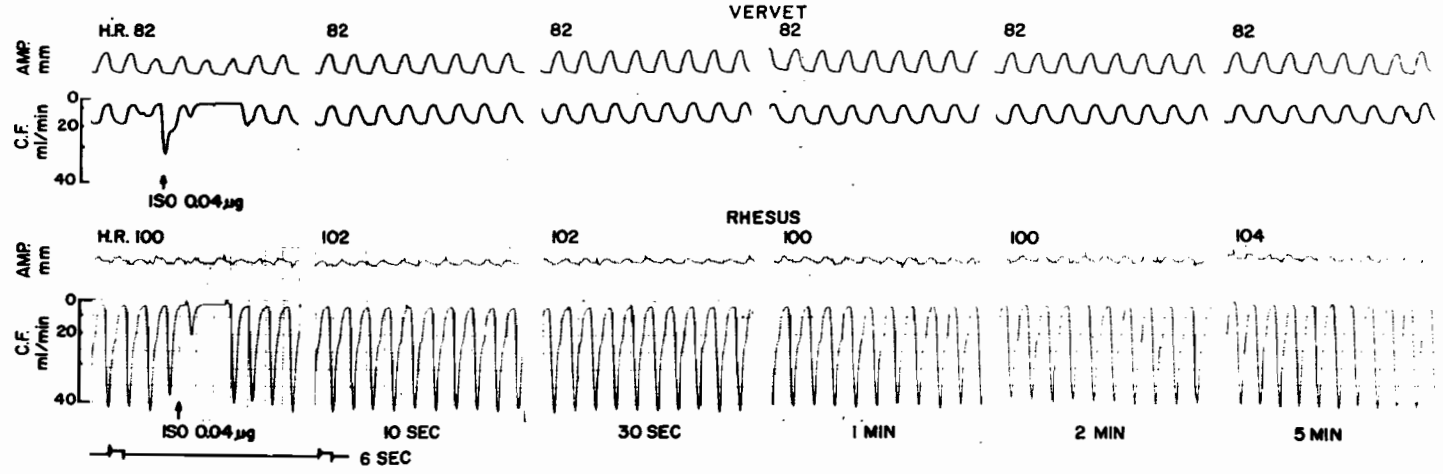

FIGURE 14: Isolated perfused hearts of monkeys. Experiments illustrating the comparative effects of isoprenaline (0.04 µg) on heart rate (HR), contractile amplitude (AMP) and coronary flow (CF) during pronethalol perfusion.

However, while the hearts of rhesus monkeys showed a decreased response after blockade, the hearts of vervet monkeys showed enhanced sensitivity as evidenced by the greater amplitude of contraction when compared to the control responses.

A comparison of the coronary flow values obtained before and during blockade shows that pronethalol (BAB) decreased mean coronary flow in the perfused hearts of both species of animals. A similar effect was reported by Gillis (1965) using the isolated perfused hearts of rabbits, when pronethalol (50 µg/min) was infused in the aortic cannula. In the present series of experiments, this decrease in coronary flow was more marked in the hearts of vervet monkeys than in the hearts of rhesus monkeys. Tables I and II also show that control of coronary flow was higher in the perfused hearts of rhesus than in similar heart preparations of vervet monkeys. This difference may be due to the fact that hearts isolated from rhesus monkeys were generally larger (12.5 to 16 gm) than hearts isolated from vervet monkeys (10 to 15 gm). However, this difference was not statistically significant.

Summarizing the data obtained from the hearts of both species of monkeys in responses to administrations of noradrenaline, adrenaline and isoprenaline, it has been observed that:

(1) Noradrenaline increased heart contractions similarly in both species of monkeys, but while augmentations of coronary flow occurred in the Cercopithecus aethiops, a decrease in coronary flow was the constant responses in the Macaca mulatta species.

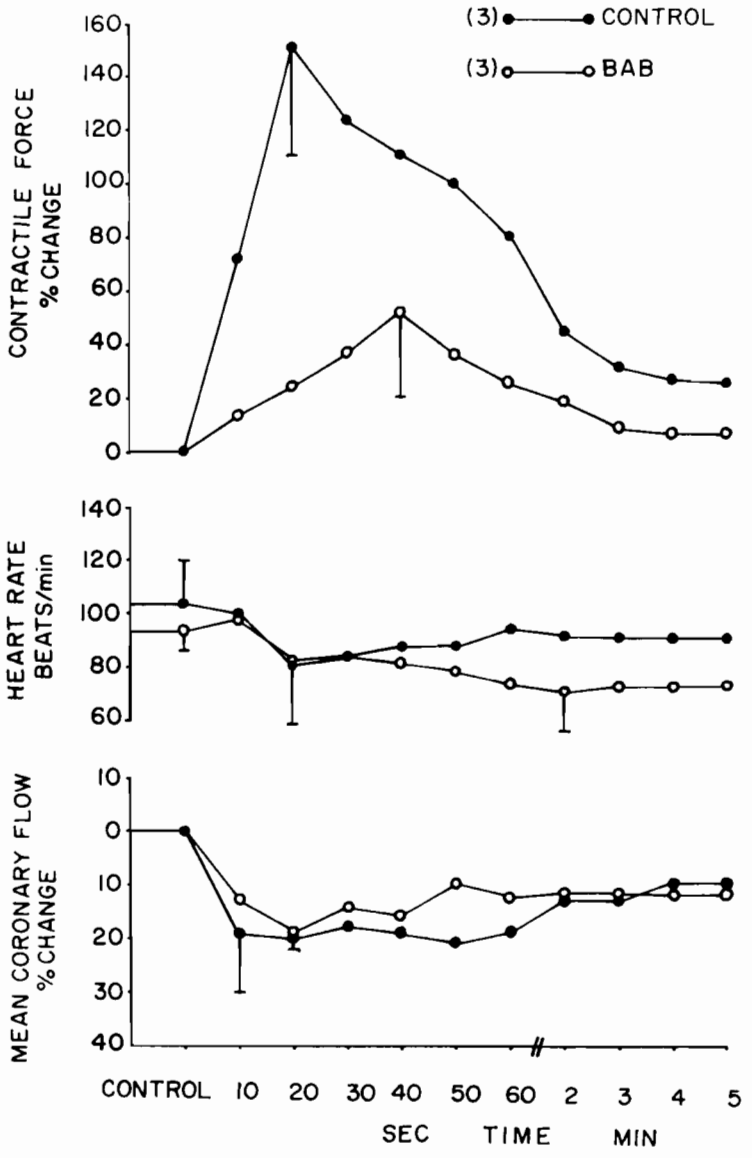

FIGURE 15: Isolated perfused heart of rhesus monkey. Effects of calcium chloride (5 mg) on heart rate, contractile force and coronary flow before (solid circles) and during (open circles) pronethalol perfusion (BAB). The abscissa denotes time (sec and min) after noradrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

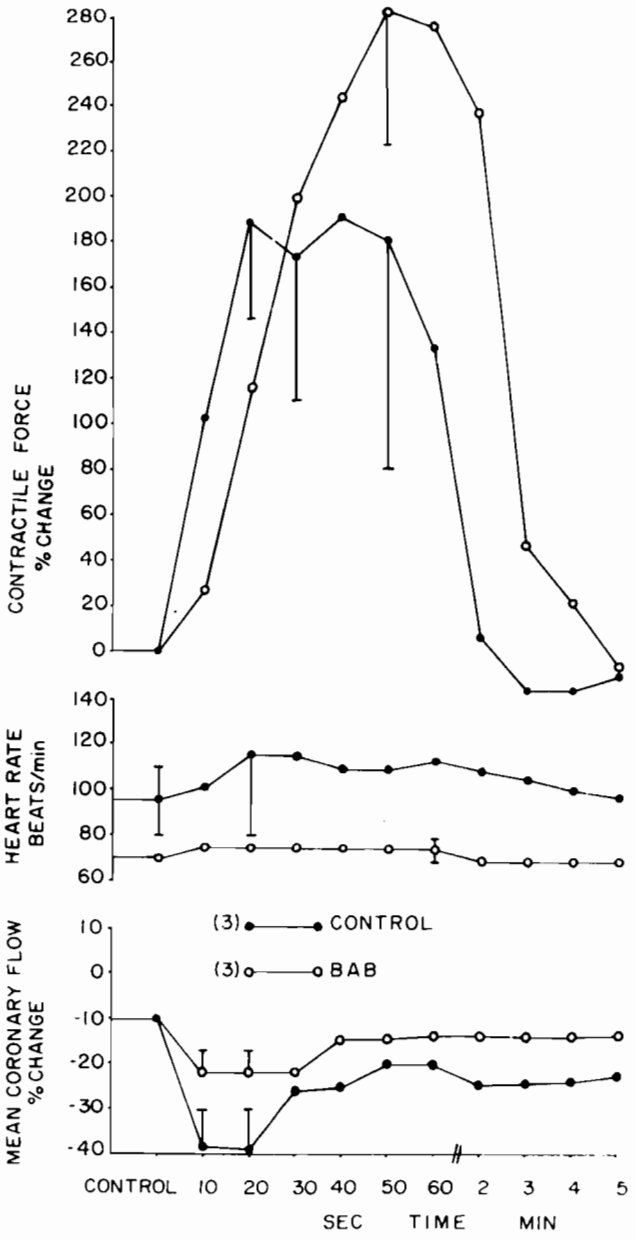

FIGURE 16: Isolated perfused heart of vervet monkey. Effects of calcium chloride (5 mg) on heart rate, contractile force and coronary flow before (solid circles) and during (open circles) pronethalol perfusion (BAB). The abscissa denotes time (sec and min) after noradrenaline. Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

(2) Adrenaline exerted qualitatively similar results to noradrenaline in both species.

(3) Qualitatively similar responses were induced by isoprenaline but vervet monkeys may be more sensitive.

(4) Adrenergic blockade (BAB) reduced the responses to noradrenaline and adrenaline, and completely abolished the responses to isoprenaline.

(5) Beta-adrenergic blockade reduced the mean coronary flow but did not affect the positive inotropic actions of calcium.

PART II - POSSIBLE ADRENERGIC INFLUENCES OF OTHER AGENTS ON CORONARY FLOW AND HEART CONTRACTION RESPONSES IN THE ISOLATED HEARTS OF MACACA MULATTA

The effects of these agents (nitroglycerin, trolnitrate and vasopressin) on the coronary circulation of various animals have been studied by several workers. I am aware of no reports of any investigations concerning the effects of these agents on the coronary circulation of rhesus monkeys. In the present study, the responses to nitroglycerin, trolnitrate and vasopressin were compared before (Control) and during beta-adrenergic blockade (BAB). The hearts of vervet monkeys were not used in this study.

A. Effects of Nitroglycerin

Table III summarizes the results obtained following injection of nitroglycerin (0.1, 1.0, 10 µg). In each experiment, the same sequence of doses was tested at 5 minute intervals. In the control responses, a dose of 0.1 ug increased mean coronary flow by 26%, while a dose of 1 µg induced an increase of 68%, and the maximum dose tested 10 µg, induced an increase of 90% in mean coronary flow. Heart contractions were not significantly affected by the range of doses administered.

Following perfusion with pronethalol (BAB), the responses were qualitatively similar but quantitatively markedly reduced. At the 0.1 µg and 1.0 µg dose levels, the reduction in the response was not statistically significant. However, at the 10 µg dose level, there was a difference of

TABLE III

CHANGES IN MEAN CORONARY FLOW AND HEART CONTRACTIONS FOLLOWING NITROGLYCERIN (NTG) AND TROLNITRATE (TROL) ADMINISTRATIONS

Drug and Dose	Procedure	Mean Coronary Flow ml/min		Heart Rate beats/min		Contractile Amplitude mm	
		Control	Max. Change	Control	Max. Change	Control	Max. Change
NTG 0.1 µg	Control	19±2.0	24±4.8 (26%)	77±8	77±8	15±0.3	15±0.3
	BAB	18±2.2	22±3.4 (22%)	97±12	97±12	9±1.0	9±1.0
NTG 1.0 µg	Control	19±2.2	32±5.8 (68%)	80±11	80±11	15±1.0	16±1.0
	BAB	22±3.7	30±5.6 (36%)	107±7	107±7	8±1.0	8±1.0
NTG 10.0 µg	Control	19±2.2	36±5.0 (90%)	83±14	83±14	15±0.3	15±0.3
	BAB	19±3.0	27±5.0 (42%)	97±7	97±7	8±1.0	8±1.0
TROL 1.0 µg	Control	21±3.0	22±2.6 (5%)	90±20	90±20	14±1.0	14±1.0
	BAB	20±1.0	21±1.0 (5%)	103±9	103±9	8±1.4	8±1.2
TROL 10.0 µg	Control	20±2.6	23±5.0 (15%)	90±20	90±20	14±1.0	14±1.0
	BAB	20±1.0	23±3.0 (15%)	100±20	100±20	8±1.0	8±1.0
TROL 100.0 µg	Control	20±2.6	33±5.1 (65%)	100±20	100±20	12±1.4	13±0.8
	BAB	20±0.9	25±3.8 (25%)	100±20	100±20	7±1.4	7±0.4

BAB = Values during Beta-Adrenergic Blockade with pronethalol.
All values are mean ± Standard Error of 3 experiments.

48% in the response when compared to the control value ($P < 0.01$). Heart contractions were not affected by nitroglycerin during beta-adrenergic blockade (BAB). Figure 17A shows a dose response curve for the doses of nitroglycerin tested (Control and BAB).

The threshold dose for nitroglycerin was found to be 0.1 µg since a dose of 0.06 µg was ineffective in producing a response. Figure 17A shows that the responses of the coronary flow to this agent were dose dependent (% changes). The results obtained agree well with similar data obtained by Lu and Melville (1950) and Gillis (1965). These investigators showed that in doses which increased the coronary flow quite strikingly, nitroglycerin exerted no significant inotropic or chronotropic effects on the isolated perfused hearts of rabbits. The present data, on the isolated hearts of rhesus monkeys, confirm these observations.

B. Effects of Trolnitrate

Based on the findings of Melville and Lu (1951) that trolnitrate exerted qualitatively similar actions to nitroglycerin on the isolated perfused hearts of rabbits, Gillis (1965) reinvestigated and confirmed these findings. Since it is not known if this finding is true for all species of animals, this agent was also tested on the isolated perfused hearts of rhesus monkeys. The procedure was similar to that adopted to test the effects of nitroglycerin. Doses of 1, 10 and 100 µg were compared before (Control), and during beta-adrenergic blockade (BAB).

Table III summarizes the results obtained. The observed effect was an increase in mean coronary flow with no significant effects on

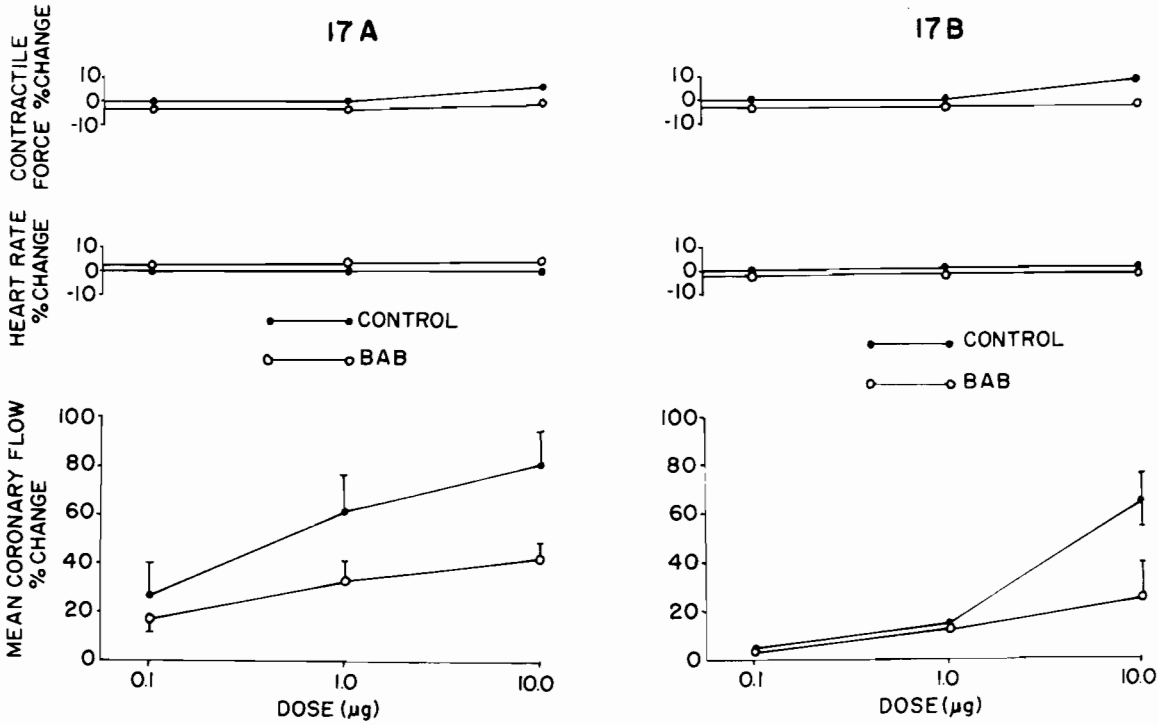

FIGURE 17A: Isolated perfused heart of rhesus monkey. Dose response curves for nitroglycerin (0.1, 1.0 and 10 µg) obtained before (solid circles) and during pronethalol (open circles) perfusion (BAB). Mean values of 3 experiments.

FIGURE 17B: Isolated perfused heart of rhesus monkey. Dose response curves for trolnitrate (1.0, 10 and 100 µg) obtained before (solid circles) and during pronethalol (open circles) perfusion (BAB). Mean values of 3 experiments.

heart contraction. Doses below 1 µg were ineffective in producing a response. At the 1 µg level, the increase in mean coronary flow was 5%, at the 10 µg level, 15% and at the 100 µg level, 65%.

Beta-adrenergic blockade (BAB) did not affect significantly the responses to 1 µg and 10 µg of trolnitrate, when compared to the responses observed during the control injections. However, at the 100 µg dose level, there was a difference of 40% in the observed responses when compared with values obtained before beta-adrenergic blockade ($P < 0.02$). Figure 17B shows the trolnitrate dose response curve. Unlike the responses to nitroglycerin, the increases in mean coronary flow when trolnitrate was administered were not dose dependent. Figures 18 and 19 show comparative records of typical experiments in which nitroglycerin and trolnitrate were administered, before (Control) and during beta-adrenergic blockade (BAB).

Comparing the responses to both agents, it can be observed that nitroglycerin appeared to be more potent. A dose of 1 µg of nitroglycerin induced 68% increase in mean coronary flow while an almost similar response (65%) was induced by a 100 µg dose of trolnitrate. It can, therefore, be inferred that, dose for dose, nitroglycerin appears to be 100 times more potent than trolnitrate in augmenting coronary flow in the isolated perfused hearts of rhesus monkeys. The observed responses after beta-adrenergic blockade are suggestive of some involvement of adrenergic mechanisms in the responses to both nitrites. It has been suggested that nitroglycerin exerts a direct action on the coronary vessels. The present investigation confirms this suggestion.

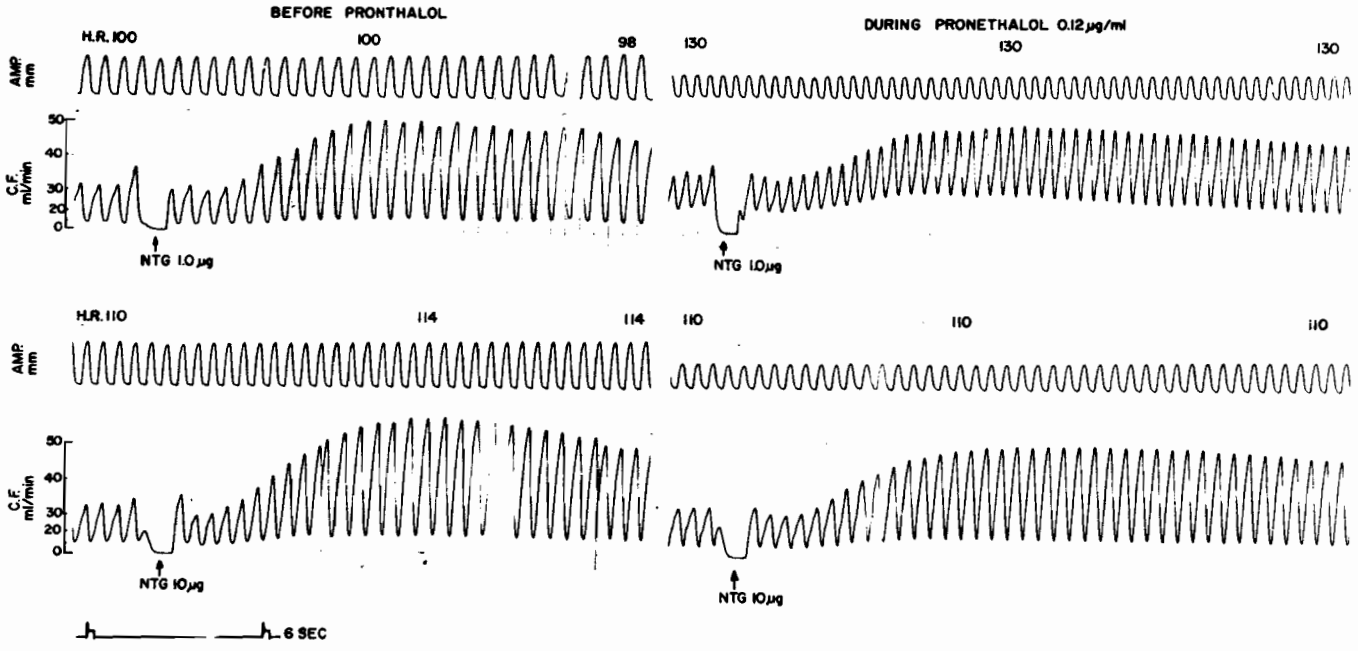

FIGURE 18: Isolated perfused heart of rhesus monkey. Comparative effects on heart rate (HR), contractile amplitude (AMP) and coronary flow (CF) before (right) and during (left) pronethalol perfusion (BAB) of nitroglycerin in doses of 1.0 µg (upper) 10 µg (lower).

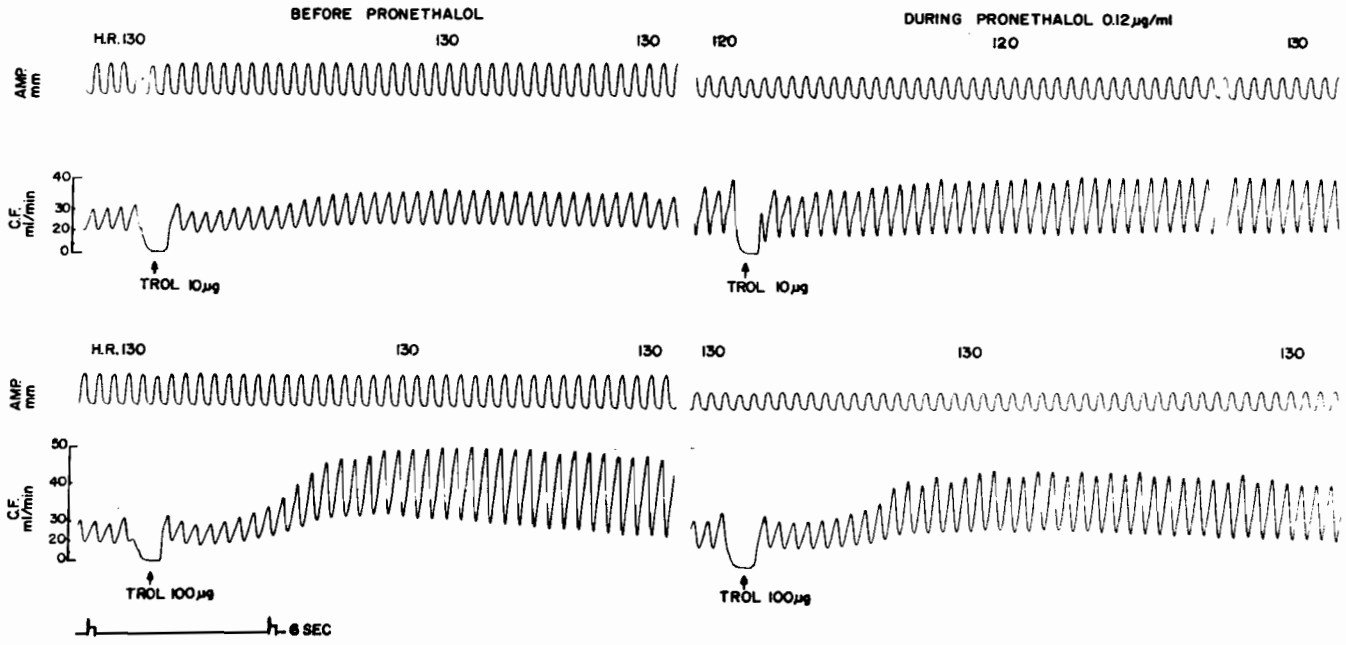

FIGURE 19: Isolated perfused heart of rhesus monkey. Comparative effects on heart rate (HR), contractile amplitude (AMP) and coronary flow (CF) before (right) and during (left) pronethalol perfusion (BAB) of trolnitrate in doses of 10 µg (upper) and 100 µg (lower).

In summary, it has been observed that:

(1) Nitroglycerin and trolnitrate increased coronary flow in the isolated hearts of rhesus monkeys, but did not exert any significant positive inotropic or chronotropic actions in the doses employed.

(2) A dose dependent increase was induced by nitroglycerin but not by trolnitrate.

(3) Nitroglycerin appeared to be, dose for dose, 100 times more potent.

(4) During beta-adrenergic blockade, augmentations of coronary flow induced by both agents were significantly reduced.

(5) The evidences obtained support the hypothesis that the actions of nitroglycerin and trolnitrate involve both adrenergic and non-adrenergic mechanisms.

C. Effects of Vasopressin

All experimental studies on the effects of this agent on the coronary circulation have demonstrated that vasopressin produces marked coronary constriction when injected into the isolated heart, heart-lung preparation, and also when injected into the intact animal. However, there have been no reports of the effect of this agent on the coronary circulation of the rhesus monkey and, in particular, the effects of beta-adrenergic blockade (BAB) on the observed coronary constriction, produced by vasopressin as observed in other species of animals. In this investigation, the response to a dose of 5 I.U. were

compared before (Control) and after beta-adrenergic blockade (Figure 20). It was observed that small doses (1 to 2 I.U.) produced only a transient decrease in coronary flow.

In 2 experiments, it was observed that, following injections of 5 I.U. of vasopressin, there was an immediate but transient (6 secs) increase in mean coronary flow. This was followed by a progressive decrease which persisted for over 5 minutes. Concomitant with the decrease in flow, there was an immediate depression of the contractile amplitude and this depression persisted for a longer duration than the decrease in mean coronary flow. Heart rate was reduced. Chloretone (chlobutanol 0.5%) was used as a preservative in the doses of vasopressin (Pitressin, Parke Davis and Company) administered. It has been previously shown by Melville (1933 b), that chloretone increased coronary flow in isolated rabbits hearts. In the present study, the transient increase in mean coronary flow was believed to be due to the dilatory property of this agent. Injections of 0.5 ml (0.5%) of chloretone produced a transient increase in mean coronary flow.

During beta-adrenergic blockade, the pronounced coronary constriction observed in the control responses was markedly reduced. The transient increase in mean coronary flow was also reduced and heart rate was unaffected. The degree of myocardial depression was not altered during beta-adrenergic blockade. The data presented are inadequate for quantitative conclusions to be made but tends to indicate that the observed responses of the coronary flow when vasopressin is administered may involve (in part) adrenergic mechanisms, while the observed myocardial depression is probably of non-adrenergic origin.

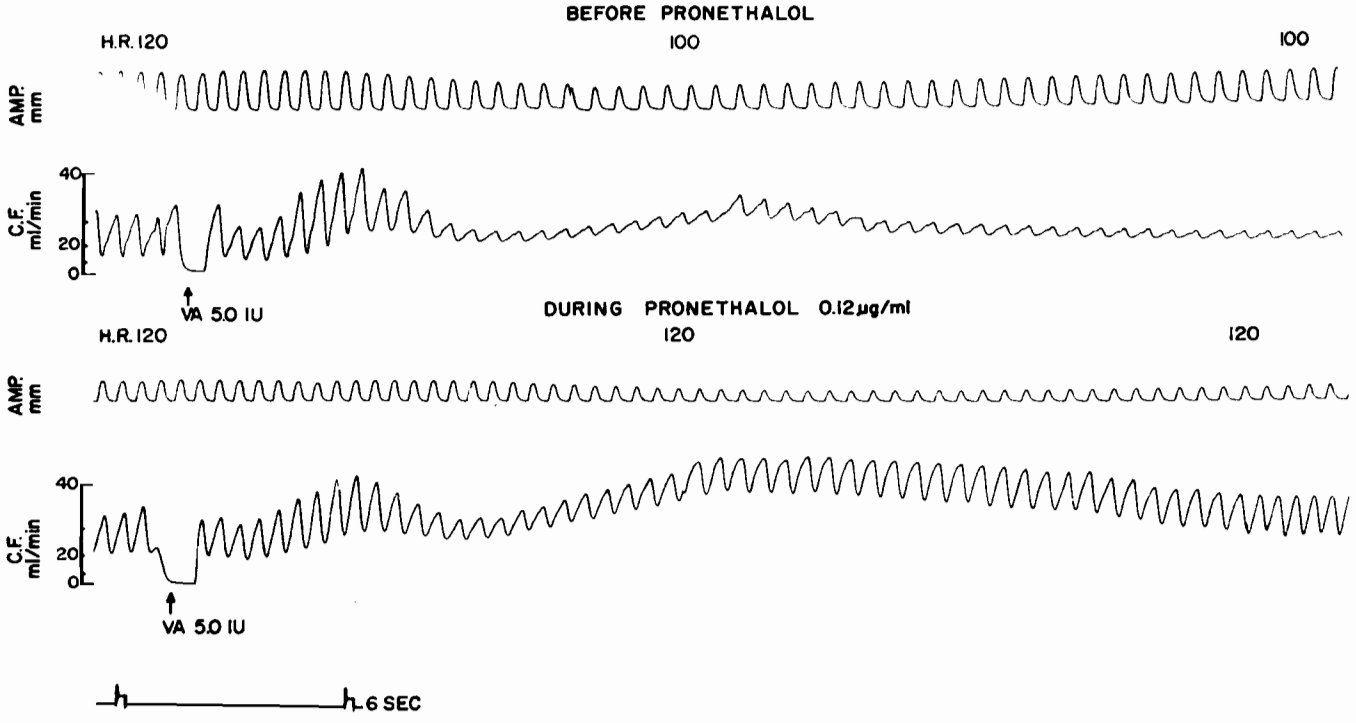

FIGURE 20: Isolated perfused heart of rhesus monkey. Experiment illustrating the effects of vasopressin (5 I.U.) on heart rate (HR), contractile amplitude (AMP) before (upper) and during (lower) pronethalol perfusion.

PART III - INFLUENCES OF NITROGLYCERIN AND TROLNITRATE ON CORONARY VASOCONSTRICTION INDUCED BY VASOPRESSIN

A. Influence of Intravenous Nitroglycerin on Responses to Vasopressin

One model of disturbance of the coronary circulation frequently used to study the effects of coronary dilator agents on the coronary circulation of the intact animal, is the intravenous injection of large doses of vasopressin. Angina pectoris is believed to be due to spasm of the coronary vessels and this use of experimental coronary spasm offers a reasonable approach to mimicking this condition in laboratory animal studies. It was, therefore, necessary to produce a model condition approximating coronary insufficiency in intact open-chest mongrel dogs, the experimental animals used in the investigation of this phase of the problem.

Figure 21 shows graphically the average coronary flow, (% change) blood pressure, heart rate and contractile force changes which were obtained in control dogs following a single injection of 1.0 I.U./kg of vasopressin, and Figure 22 is a record of an experiment. Following administration of vasopressin, there was an immediate reduction in coronary flow to a value of 53 $\pm$ 12% below control values within 60 seconds. This was followed by a sharp rise to a value above the control and then a progressive decline. Blood pressure changes indicated a diphasic response; a slight rise followed by a marked fall (presumed to be a direct resultant effect of the reduction in myocardial blood supply). When peripheral constriction became dominant, there was a sharp rise in blood pressure to overshoot the control values, which

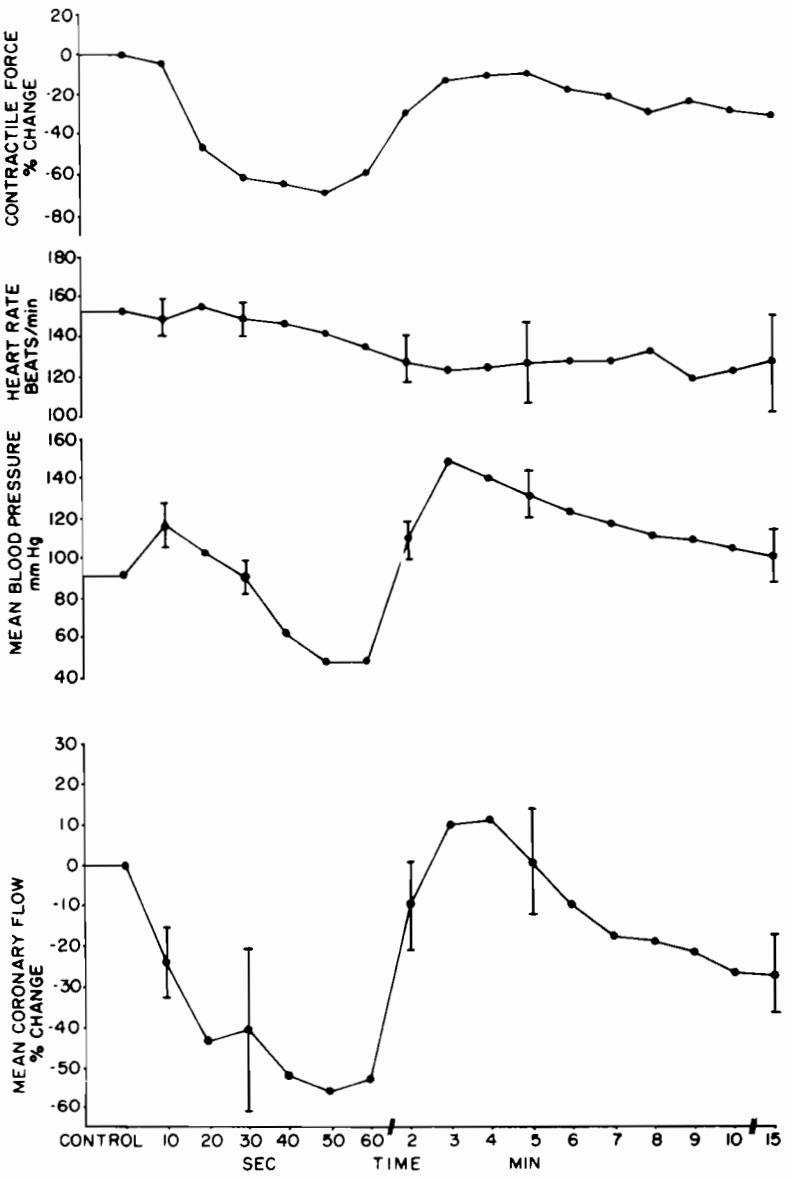

FIGURE 21: Anesthetized open-chest dog. Mean changes in contractile force, heart rate, blood pressure and coronary flow following an injection of vasopressin (1.0 I.U./kg). Vertical lines indicate the standard errors. Values are means of 3 experiments.

was followed by a progressive return to near control values over 15 minutes. Heart rate showed a progressive decrease to a value of about 30 beats/min below the control value. Depression of the myocardial contractile force was most striking and abrupt. Within 30 seconds after administration of vasopressin, there was a depression of 64% below control values, and not until the blood pressure and coronary flow increased was there a return to near control values.

The electrocardiographic changes following vasopressin administration are shown in Figure 22. The control pattern showed an upright T wave and following injection (30 secs) there was a diminution and an upright T wave which gradually increased in voltage. In some experiments, within 1 minute, there was marked bradycardia followed by ectopic ventricular beats. Gradual restoration of sinus rhythm always occurred and the control pattern was restored usually within 30 minutes. In some experiments, the ST segments formed the ascending line of a giant T wave. These electrocardiographic changes only occurred when coronary constriction was quite evident after vasopressin administration in these experiments. It is the concensus of opinion that the electrocardiographic changes, observed following vasopressin injection, provide a reliable indication of interference with myocardial blood supply, since these changes are a direct resultant of intense spasm of the coronary circulation leading to myocardial hypoxia. The changes observed in these experiments tend to confirm this. It is possible that the bradycardia observed may be partly due to reflex and partly due to a quinidine-like property this polypeptide is believed to possess (Brodeur and Beaulnes 1965).

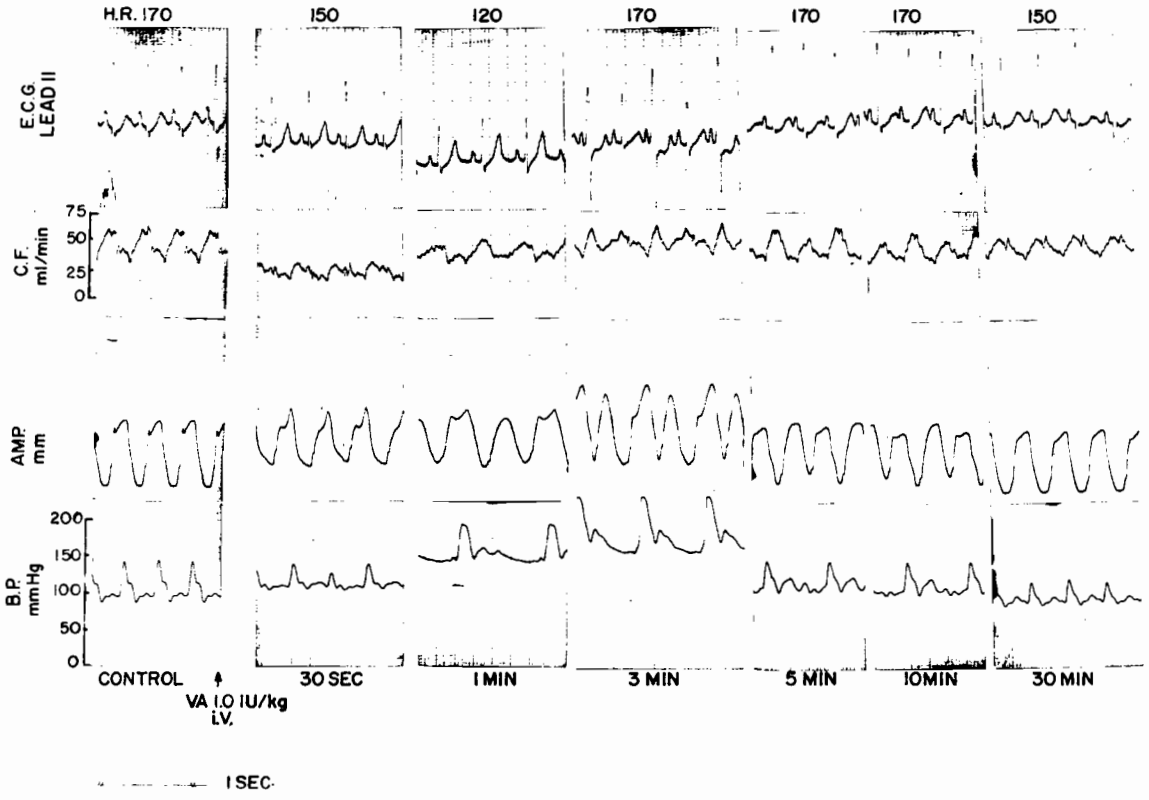

FIGURE 22: Anesthetized open-chest dog. Effects of vasopressin (1.0 I.U./kg) on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP).

Nitroglycerin is universally recognized as the yardstick against which all other coronary vasodilators are compared. Its mechanism of action is largely believed to be due to relaxation of the smooth muscles of the arterioles. A coronary dilator action has been demonstrated in the isolated heart. However, in the intact animal, only a transient increase in coronary flow is usually seen even when large doses are employed. Gillis (1965) used doses ranging from 0.1 µg to 100 µg intravenously in dogs. In all cases, only a brief increase, lasting from 10 to 20 seconds, could be obtained. In fact, following this brief increase, there was a marked decrease in flow which he assumed was probably due to the reduction in blood pressure.

In the present study, the response to 3 hypodermic tablets of nitroglycerin (1.95 mg) was determined when this was administered by rapid intravenous injection.[1] This dose was equivalent to approximately 108 µg/kg since the dogs averaged 18 kg in weight. The primary response to this dose was an immediate fall in blood pressure of 62 mm Hg below the control value, being maximal within 30 seconds, and with a progressive return to control values within 3 minutes. A transient increase in coronary flow was sometimes observed, but was always followed within 10 seconds by a marked decrease. This might be due to the fall in perfusion pressure which may have obscured the effect of a more pronounced dilatation. The decrease in mean coronary flow was maximal within 2 minutes (40%) and was followed by a progressive return to control

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[1] This procedure was employed as a basis of comparison in connection with similar experiments in which nitroglycerin was administered sublingually (see Part IV).

values within 7 minutes. That this decrease is secondary to the fall in blood pressure is seen from the fact that as the blood pressure recovered, the coronary flow also recovered. There was a reflex increase in heart rate which returned to the control pattern as the blood pressure recovered. There was no change in the contractile amplitude and the electrocardiogram showed no change from the control pattern.

The antagonism of chloroform - adrenaline fibrillation by nitroglycerin was shown by Melville (1948). He concluded that this was due to the coronary dilatation induced by this agent. The antagonism to vasopressin vasoconstriction has also been repeatedly demonstrated. Since nitroglycerin produces no significant dilatation in the present study, it was of interest to see if an antagonism to vasopressin vasoconstriction could be demonstrated under these experimental conditions. Figure 23 shows graphically the mean values of three experiments. In these experiments, nitroglycerin (3 tablets) was administered by rapid intravenous injection and was followed 30 seconds after, by an injection of vasopressin (1.0 I.U./kg). Examination of Figure 23 will show that in the first 30 seconds, the effects of nitroglycerin are manifested i.e. a decrease in coronary flow as a result of the changes in blood pressure. At 30 seconds, the coronary flow showed a progressive return to normal following the administration of vasopressin. There was complete antagonism of the coronary vasoconstriction and, in fact, there was even a slight increase in coronary flow as the peripheral vasoconstrictor action of vasopressin

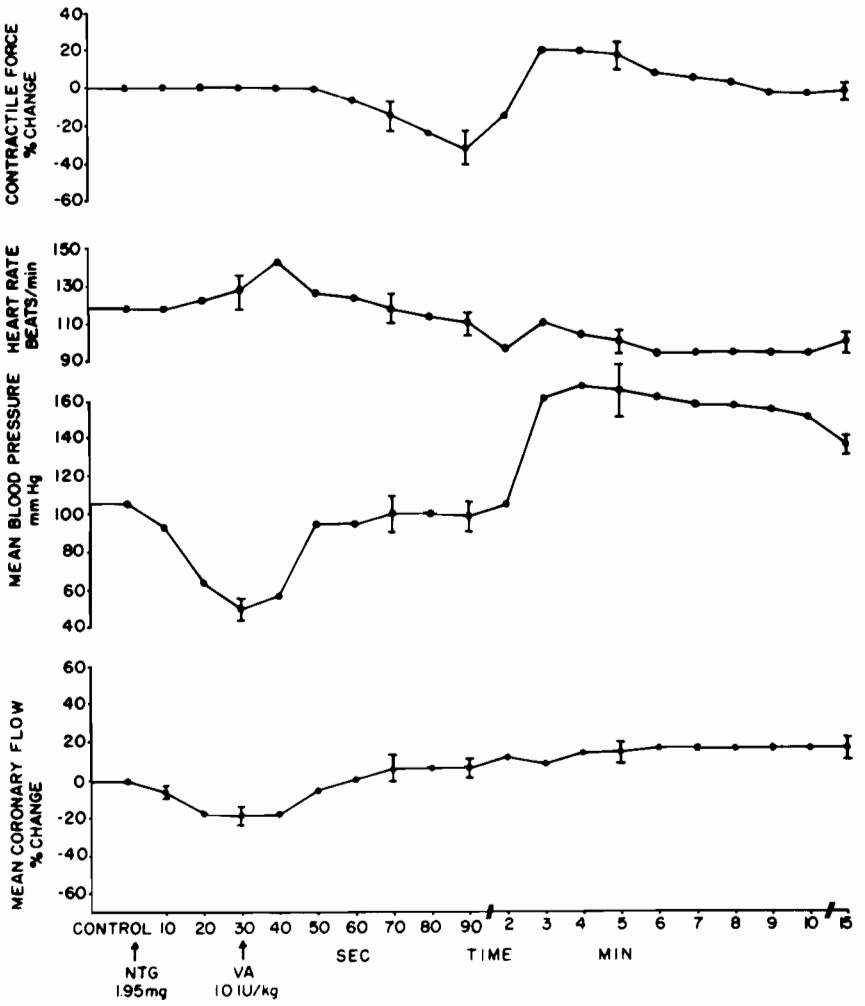

FIGURE 23: Anesthetized open-chest dog . Mean changes in contractile force, heart rate, blood pressure and coronary flow after nitroglycerin (1.95 mg i.v.) and vasopressin (1.0 I.U./kg i.v.). Values represent means and standard errors of 3 experiments.

became evident. The blood pressure showed the usual fall due to the peripheral dilatation caused by nitroglycerin. With the administration of vasopressin, it recovered to control values and after 1 minute increased, due to the peripheral vasoconstriction, which was not significantly antagonized. Heart rate changes indicated a brief increase, probably a reflex due to the blood pressure fall following nitroglycerin administration. This was followed by progressive reduction as the blood pressure increased, which may also be a reflex bradycardia due to the increased blood pressure.

While no changes in contractile force were observed, following nitroglycerin administration, intense myocardial depression was evident within 50 seconds following vasopressin administration. This cannot be attributed to any change in any of the other parameters as is evident from the Figure, and cannot be attributed to an impairment in myocardial nutrition as a result of coronary constriction. Examples of tracings taken during an experiment are shown in Figure 24. Although the coronary constriction was successfully antagonized in these experiments, marked ECG changes occurred. Within 20 seconds, an ischemic T wave was evident and was accompanied by progressive heightening of the ST segment to be almost as high as the QRS complex, within 4 minutes. The P wave was also slightly heightened and the PR interval showed some evidences of prolongation. Curiously, most of these changes were still pronounced after 30 minutes. This was significantly different from the responses to vasopressin alone, where all ECG changes were normalized within 15 to 30 minutes.

It is quite evident that the ECG changes and myocardial changes observed might not be due entirely to the vasoconstrictive actions of vasopressin on the coronary circulation.

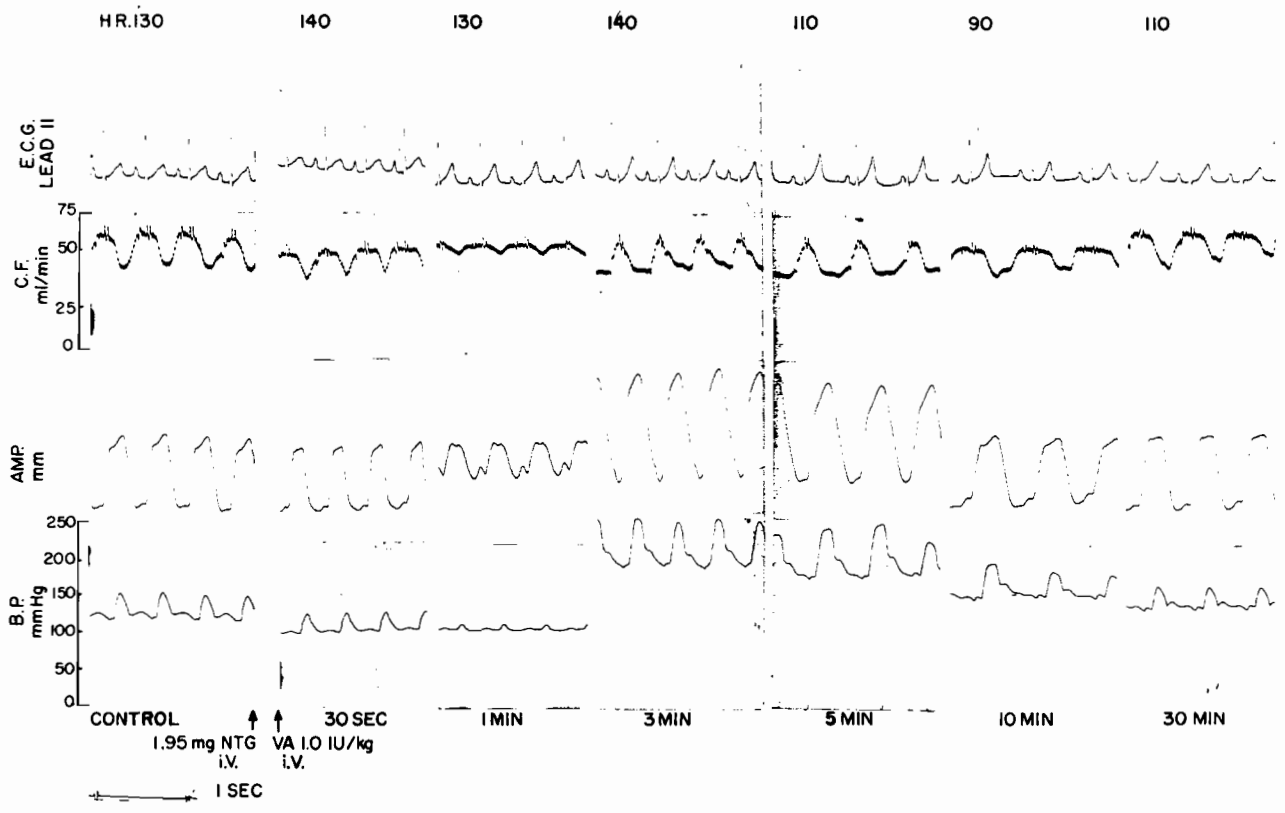

FIGURE 24: Anesthetized open-chest dog. Effects on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP) intravenous nitroglycerin (NTG) and vasopressin (VA). Vasopressin was administered 30 seconds after nitroglycerin.

B. Influence of Intravenous Trolnitrate on the Responses to Vasopressin

All experimental studies have indicated that trolnitrate exerts a qualitatively similar action to nitroglycerin. Nitroglycerin, however, dose for dose, is more potent. Since the vasopressin-induced coronary constriction was successfully antagonized by nitroglycerin, it was of interest to see if vasopressin vasoconstriction could also be successfully antagonized by trolnitrate. Control responses to a dose of 3.0 mg/kg were determined. A brief increase in coronary flow was observed but this was soon converted to a decrease, as a fall in blood pressure became evident. This was not pronounced as with nitroglycerin, but these changes were more prolonged. Changes in heart rate and contractile force were not observed to any marked degree.

In a separate series of experiments, trolnitrate (3.0 mg/kg) was administered intravenously and was followed 30 seconds after by vasopressin (1.0 I.U./kg). Figure 25 summarizes the results obtained graphically and an example of an experiment is represented in Figure 26. In Figure 25, the coronary flow showed no marked change although the blood pressure showed the usual decrease, due to peripheral relaxation of smooth muscles of the arterioles induced by trolnitrate. Heart rate was slightly increased and myocardial contractile force was slightly increased. Following administration of vasopressin, a brief increase followed by a 16% reduction in coronary flow occurred, which soon returned to control values. The blood pressure showed a sharp rise followed by a fall and a second rise subsequently, returning to control values within 8 minutes. The changes in coronary flow, followed the blood pressure changes quite closely and are either secondary to, or

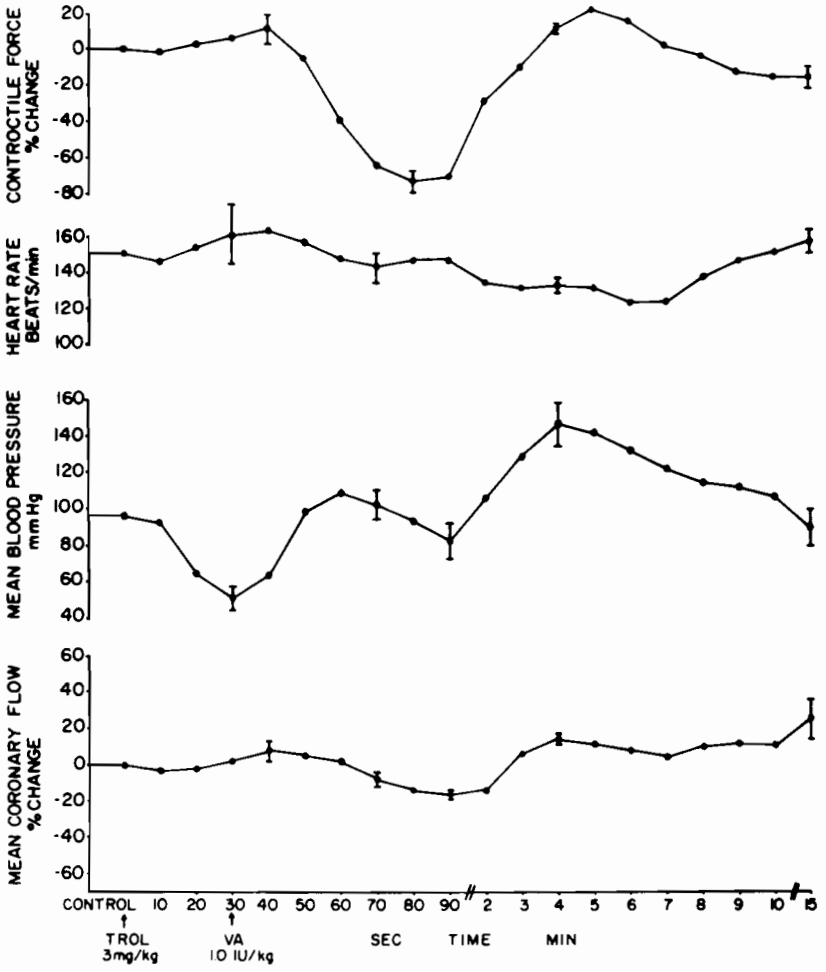

FIGURE 25: Anesthetized open-chest dog. Mean changes in contractile force, heart rate, blood pressure and mean coronary flow after trolnitrate (TROL) and vasopressin (VA). Values represent means and standard errors of 3 experiments.

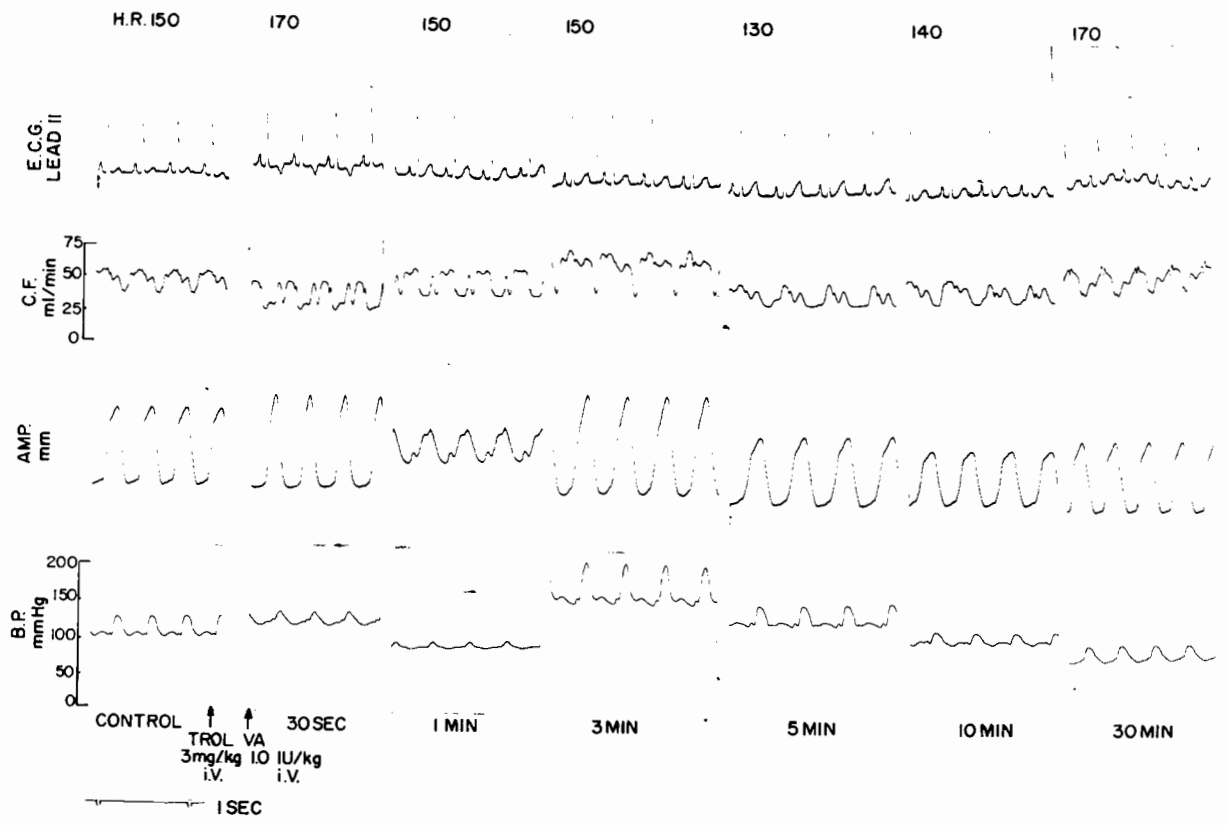

FIGURE 26: Anesthetized open-chest dog. Effects on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP) of intravenous trolnitrate (TROL) and vasopressin (VA). Vasopressin was administered 30 seconds after trolnitrate.

coincidental with these changes. While the heart rate was not markedly affected, the contractile force showed dramatic changes. This was evidenced by a rapid and extreme depression of a similar degree or in some cases to a greater extent than the depression induced by vasopressin alone. This was maximal within 80 seconds (66%) but returned to overshoot the control value within 5 minutes.

In Figure 26, it will be observed that as occurred in the response to vasopressin during nitroglycerin administration, significant ECG changes also occurred. Within 20 seconds following vasopressin, the control upright T wave was inverted followed by flattening and subsequent elevation within another 20 seconds. ST segment deviation persisted for over 10 minutes and within 15 minutes the control pattern was restored. No prolongation of the PR interval was observed.

Summarizing the data presented above, it was observed that:

(1) When administered intravenously, nitroglycerin and trolnitrate were capable of antagonizing vasopressin-induced coronary vasoconstriction in open-chest pentobarbitalized dogs.

(2) No significant antagonism of the peripheral vasoconstriction induced by vasopressin was observed.

(3) The observed decrease in heart rate was eliminated by both nitrites.

(4) The vasopressin-induced myocardial depression was not antagonized by either nitroglycerin or trolnitrate.

(5) Antagonism of the coronary vasoconstriction did not prevent the development of the typical ECG changes induced by vasopressin which were more pronounced with nitroglycerin pre-treatment than with trolnitrate.

These observations indicate that the ECG changes might be more directly related to the myocardial depression than to the changes in coronary flow induced by vasopressin.

C. Observations on the Role of Beta-Adrenergic Mechanisms on The Responses to Vasopressin

The influence of beta-adrenergic blockade on the response to nitroglycerin and trolnitrate was investigated by Gillis (1965). He demonstrated that adrenergic blockade reduced the initial increase in coronary flow, abolished the tachycardia, and the increased contractility, observed in control responses. No secondary decrease in coronary flow was reported to have occurred. In a second series of experiments, it was shown that nitroglycerin exerted significant beta-adrenergic blocking properties. During continuous infusions of nitroglycerin, a partial blockade of the inotropic responses to intravenous injections of adrenaline and noradrenaline were observed, but not to isoprenaline. Injections of $CaCl_2$ indicated no evidence of a blockade of the positive inotropic actions of this compound, suggesting a specific type of blockade by nitroglycerin.

The investigations of Nash (1964) indicated that the mechanism of action of vasopressin may involve directly or indirectly cardiac adrenergic mechanisms. On the basis of these observations, it was of interest to determine to what extent the effects of vasopressin could be modified by a blockade of beta receptors, and what was the effect of the blockade exerted by pronethalol on the antagonism of the vasopressin-induced coronary vasoconstriction previously observed.

To achieve a blockade of beta receptors, a continuous infusion of pronethalol (100 µg/min) was made for 30 minutes (see Methods), and while continuing the infusion, the various agents were tested. No striking effects on any of the parameters recorded were observed.

The coronary flow showed a slight decrease over a 30 minute period, blood pressure was unchanged and heart rate was slightly decreased (10 beats/min). Contractility changes were insignificant. To determine an effective blockade, an injection of isoprenaline (0.1 µg/kg) was made before commencement of pronethalol infusion and this was repeated after 30 minutes of infusion. The control injection produced a brief increase in coronary flow of about 20 seconds duration, which was followed by a return to the control value and a secondary rise. The blood pressure decreased by 20 mm Hg from the control value and remained at this value for the duration of the effect of this agent. There was a mild increase in heart rate (30 beats/min) which gradually returned to control values within 20 seconds. Contractility was enhanced, and within 10 seconds attained a value of over 70% above the control value. The control pattern was restored within 2 minutes, by which time all other parameters were back to their normal control values. After infusion of pronethalol for 30 minutes and while still continuing the infusion, a second injection of isoprenaline was made. There were no changes in any parameters. The electrocardiogram showed no distinct changes in response to either the control or the second injection of isoprenaline.

During beta-adrenergic blockade, injections of nitroglycerin (3 tablets IV) produced a mild decrease in coronary flow which returned to the control value within 1 minute. The blood pressure fall was not as pronounced as observed in the absence of blockade and the reflex tachycardia was abolished. Myocardial contractility was not affected.

After 30 minutes of blockade with pronethalol as above, and while continuing the infusion, an injection of vasopressin (1.0 I.U./kg) was

made. The responses to this injection are shown graphically in Figure 27, and Figure 28 shows a typical experiment. Figure 27 shows that while the reduction in coronary flow was not prevented by beta-adrenergic blockade, it was markedly reduced. Maximum effect was achieved within 90 seconds following the administration and this was of a magnitude of 36 $\pm$ 12%. This mild vasoconstriction was, however, short lived, the control pattern being restored within 5 minutes. No secondary rise due to peripheral vasoconstriction was observed, as in previous experiments without blockade. The blood pressure showed the usual diphasic response, though again this was less marked than during vasopressin administration in the absence of blockade. Maximum decrease occurred at the same time the coronary flow was at its lowest value. This was succeeded by a secondary rise (54 mm Hg), and a quick return to near control values. Heart rate values showed a progressive decrease but this was not pronounced, i.e. not exceeding 30 beats/min. The contractile force curve showed the greatest changes. This was reduced by 66 $\pm$ 14% within 60 seconds after vasopressin injection. Comparing this with values obtained in the absence of blockade, a greater depression of contractility occurred in response to vasopressin during beta-adrenergic blockade. It should be also noted that the control pattern was not restored for the duration of the experiment, still being reduced by over 20% at 15 minutes.

In Figure 28 the ECG showed no marked changes. The control pattern shows an inverted T wave. Twenty seconds after vasopressin administration, there was a further deepening of the ST segment which persisted for over 3 minutes. The control pattern was restored soon after.

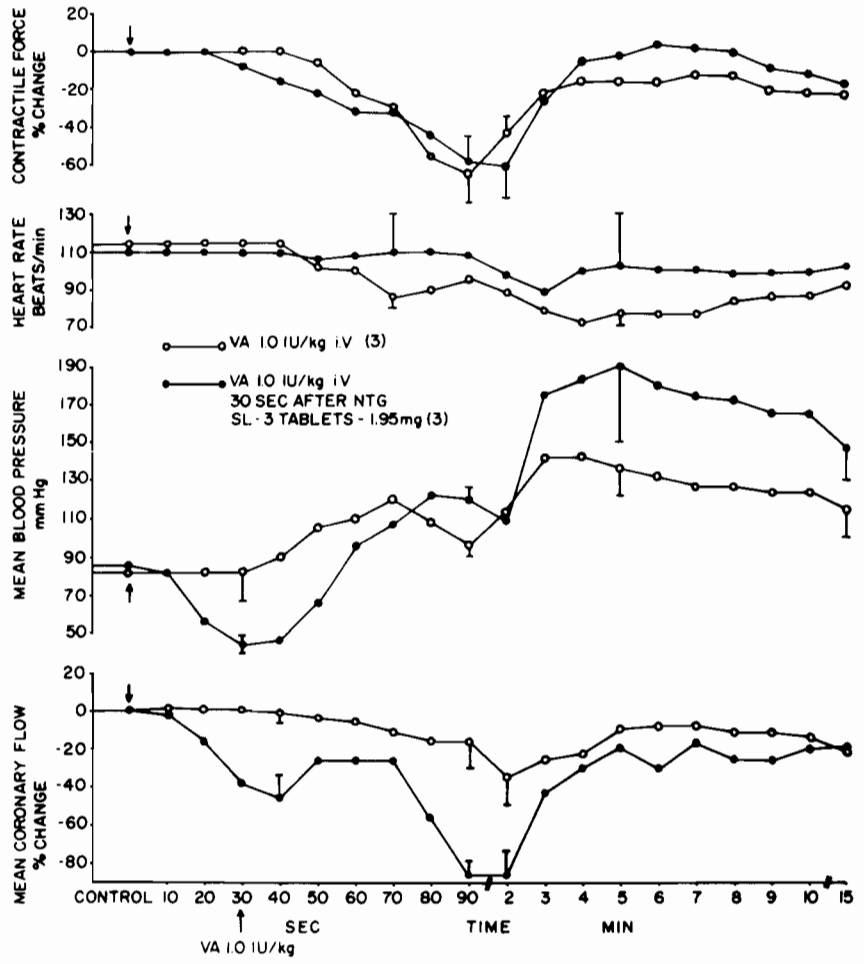

FIGURE 27: Anesthetized open-chest dog during beta-adrenergic blockade with pronethalol. Mean changes in contractile force, heart rate, blood pressure and mean coronary flow when vasopressin (VA) is injected alone (open circles) or 30 seconds after an injection of nitroglycerin (NTG) (closed circles). Arrows indicate the point at which nitroglycerin was administered. Number of experiments in parenthesis.

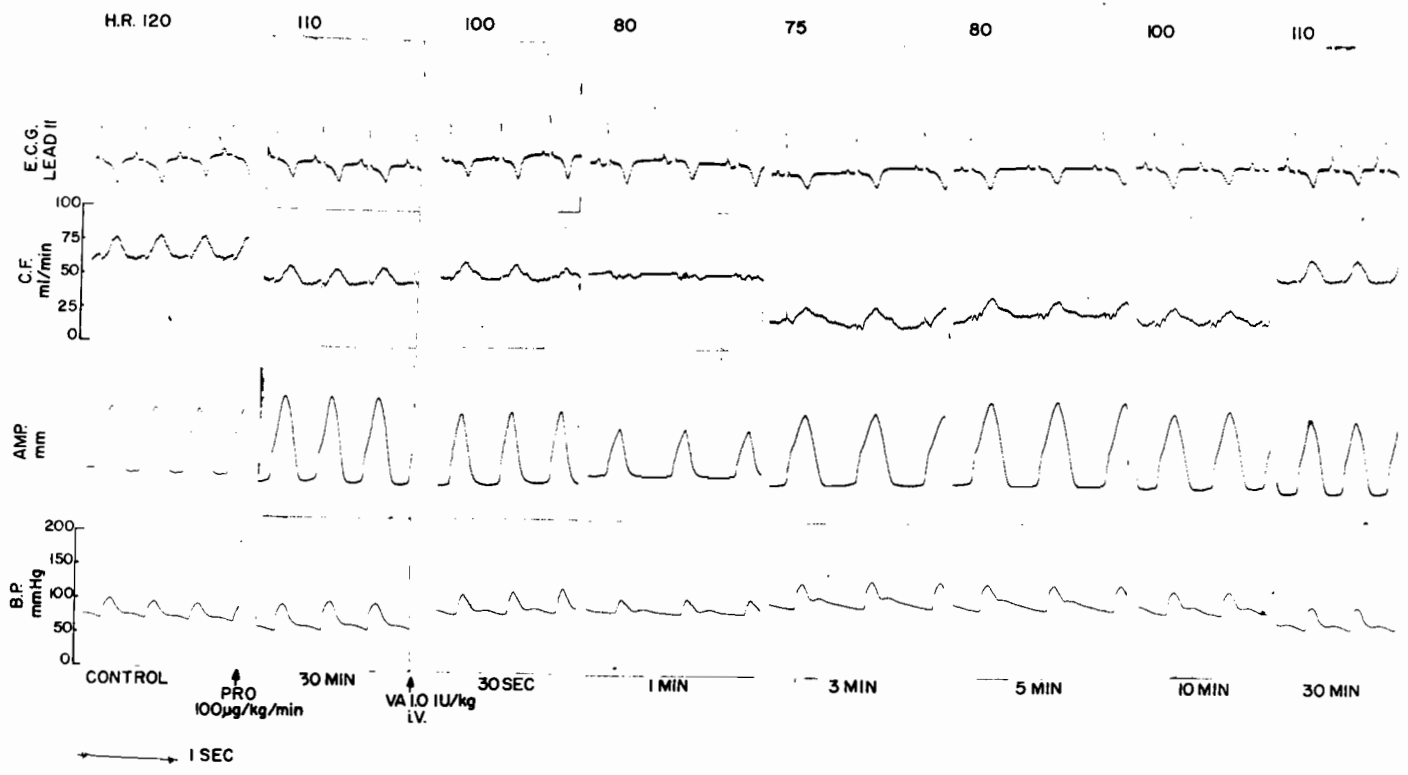

FIGURE 28: Anesthetized open-chest dog during beta-adrenergic blockade with pronethalol (PRO). Experiments illustrating the effects on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP) of vasopressin (1.0 I.U./kg).

That this might not be due to the reduced coronary constriction will be seen in Part IV, where a 28% reduction in coronary flow induced by injection of 0.5 I.U./kg of vasopressin in the absence of blockade, resulted in marked ECG changes. Pronethalol is believed to be capable of blocking the ECG changes occurring in patients with myocardial ischemic diseases and also possesses anti-arrhythmic properties. The present observations may be due to either of these actions.

Since there was a reduction in the vasoconstriction induced by vasopressin during adrenergic blockade, it was of interest to determine to what extent the antagonism previously observed could be influenced by beta-adrenergic blockade with pronethalol. Consequently, in this series of experiments, after 30 minutes of blockade and while still continuing the infusion, an injection of nitroglycerin (3 tablets) was made intravenously, fcllowed within 30 seconds by vasopressin (1.0 I.U./kg). These results are summarized graphically in Figure 27. Following the administration of nitroglycerin, mean coronary flow decreased to a value of 38% below the control value. With the vasopressin injection there was a further decrease to 46% of the control value followed by a slight increase and then a second decrease more dramatic than the first to attain a value of 86 $\pm$ 8% within 60 seconds of administration of vasopressin. The control flow was not restored for the duration of the remainder of the experiment. The blood pressure showed an initial fall after the nitroglycerin administration (38 mm Hg). With the administration of vasopressin, there was a sharp rise from 44 mm to 122 mm Hg within 60 seconds of administration (90 secs from control).

This was succeeded by a mild fall and a second and much more marked rise to a value of 190 mm Hg. For the duration of the experiment, the blood pressure remained elevated but at a lower value than the maximum. The heart rate was not markedly affected but the contractile force showed identical changes to those which occurred when vasopressin alone was administered in the absence of blockade. A maximum decrease of 60% below control value within 2 minutes occurred, but in this case there was a progressive recovery back to the control.

In the record of an actual experiment shown in Figure 29, the electrocardiographic response was most striking. Since the ECG observed during the vasopressin response (during blockade) showed no marked changes, it was assumed that the ECG changes in this series of experiments would not be pronounced. However, the figure presented indicates the contrary. Typical T wave changes were observed soon after the administration of vasopressin, which were succeeded by progressive heightening of the ST segment. The PR interval was prolonged and within 4 minutes after control, and this was followed by several ectopic ventricular beats. Unlike the response to vasopressin seen in Figure 22, even after 30 minutes, marked ST segment elevation was still quite evident.

The results of this series of experiments indicate a potentiation of the vasopressin response by nitroglycerin in the presence of pronethalol. Since progressive worsening of the ECG occurred after the coronary flow was restored to near control values, one would be tempted to doubt that these ECG changes are the resultant effects of

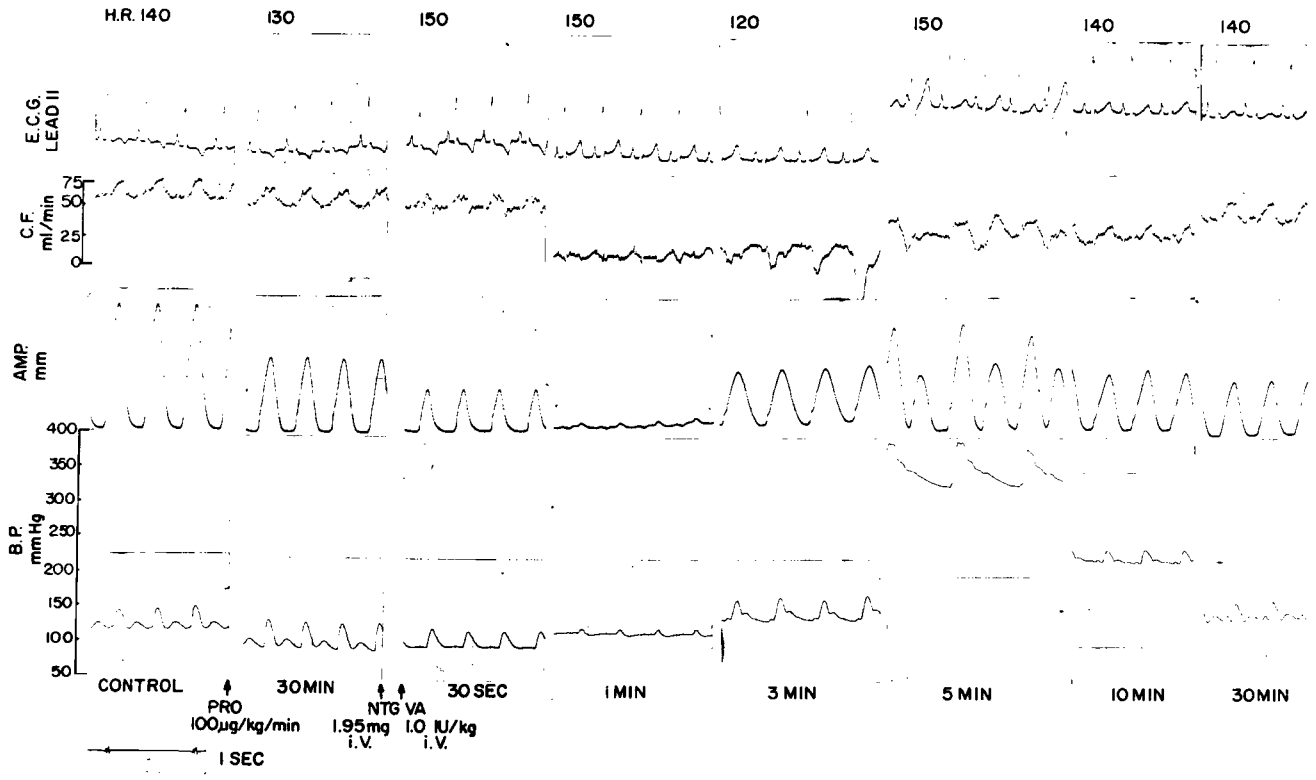

FIGURE 29: Anesthetized open-chest dog during beta-adrenergic blockade with pronethalol (PRO). Experiment illustrating the effects on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP) of vasopressin (VA 1.0 I.U./kg i.v.) when administered 30 seconds after nitroglycerin (NTG 1.95 mg i.v.).

vasopressin-induced coronary constriction and particularly in the fact that these changes persisted for such a long period. Identical results were obtained when the procedure was repeated using trolnitrate (3 mg/kg i.v.).

In summary, it was observed that during beta-adrenergic blockade with pronethalol, in open-chest pentobarbitalized dogs:

(1) coronary flow, blood pressure, heart rate and myocardial contractility were not significantly affected by the blockade.

(2) the responses to isoprenaline (0.1 µg/kg) were abolished.

(3) the decreases in coronary flow in response to nitroglycerin and trolnitrate were significantly reduced.

(4) cardiovascular changes induced by vasopressin were reduced and the ECG changes abolished.

(5) administrations of vasopressin after nitroglycerin and trolnitrate administrations resulted in pronounced coronary and peripheral vasoconstriction, and marked myocardial depression.

(6) typical vasopressin arrhythmia resulted after nitrites vasopressin administrations.

It is concluded that beta-adrenergic receptors may be of great importance in the antagonism to vasopressin-induced coronary vasoconstriction.

PART IV - EFFECTS OF SUBLINGUAL ADMINISTRATION OF NITROGLYCERIN

Nitroglycerin is used clinically by sublingual administration. In an attempt to confirm its coronary dilatation by sublingual administration, Gillis (1965), using anesthetized open-chest dogs failed to obtain evidence that coronary dilatation occurs when nitroglycerin is administered sublingually. He suggested that to establish the fact that sublingual nitroglycerin does relieve vasospasm, it would be necessary to administer nitroglycerin sublingually during experimentally-induced coronary vasoconstriction with an agent such as vasopressin. This aspect of the study is concerned with a further investigation of this problem.

A large dose of nitroglycerin, 3 hypodermic tablets (1.95 mg), was used in this study and both anesthetized open-chest and trained unanesthetized dogs were used.

A. Effects of Sublingual Nitroglycerin in Anesthetized Open-Chest Dogs

After operation procedures were completed (see Methods), the mouth of the animal was cleansed with about 5 ml of saline to remove any food particles and excess saliva. The tablets were then placed at the base of the tongue and the mouth closed to prevent displacement of these tablets. Control recordings were made continuously for 2 minutes and thereafter intermittently for 15 minutes. The results indicated a very slight coronary dilator response (3 experiments). This was maximal at 80 seconds (5% above control value), and continued slightly above control value for over 15 minutes. Blood pressure decreased by 22 mm Hg,

this being maximal within 2 minutes of administration, and returning gradually to control values. Heart rate and contractile force were not affected.

Since clinical relief of pain is usually achieved from 1 to 3 minutes after nitroglycerin administration, and the experimental data indicate maximum effect (5%) within 2 minutes, it was assumed that if sublingual nitroglycerin is capable of antagonizing the vasospasm of angina, then its antagonistic action would be manifested, if an attempt was made to constrict the coronary vessels with vasopressin. Hence, 1 minute after sublingual nitroglycerin, 1.0 I.U./kg of vasopressin was administered by rapid intravenous injection. Figures 30 and 31 show these results. (The curve representing the response to 1.0 unit of vasopressin is included for comparison).

It can be seen that there was no antagonism of the coronary vasoconstriction. The changes in coronary flow showed no significant difference from those obtained with vasopressin alone, being decreased by over 50%. Blood pressure was similarly affected, being decreased for even a longer time and to a slightly greater degree than when vasopressin alone was injected. Heart rate was progressively decreased and contractility was markedly depressed (86% as compared with 70% with vasopressin). In Figure 31, a typical experiment is depicted. The blood pressure tracing shows a maximum reduction at 2 minutes followed by progressive recovery to control values. Contractile amplitude also showed maximal depression within 2 minutes. In most experiments, the ECG showed prompt changes tc an elevated T wave within 30 seconds and

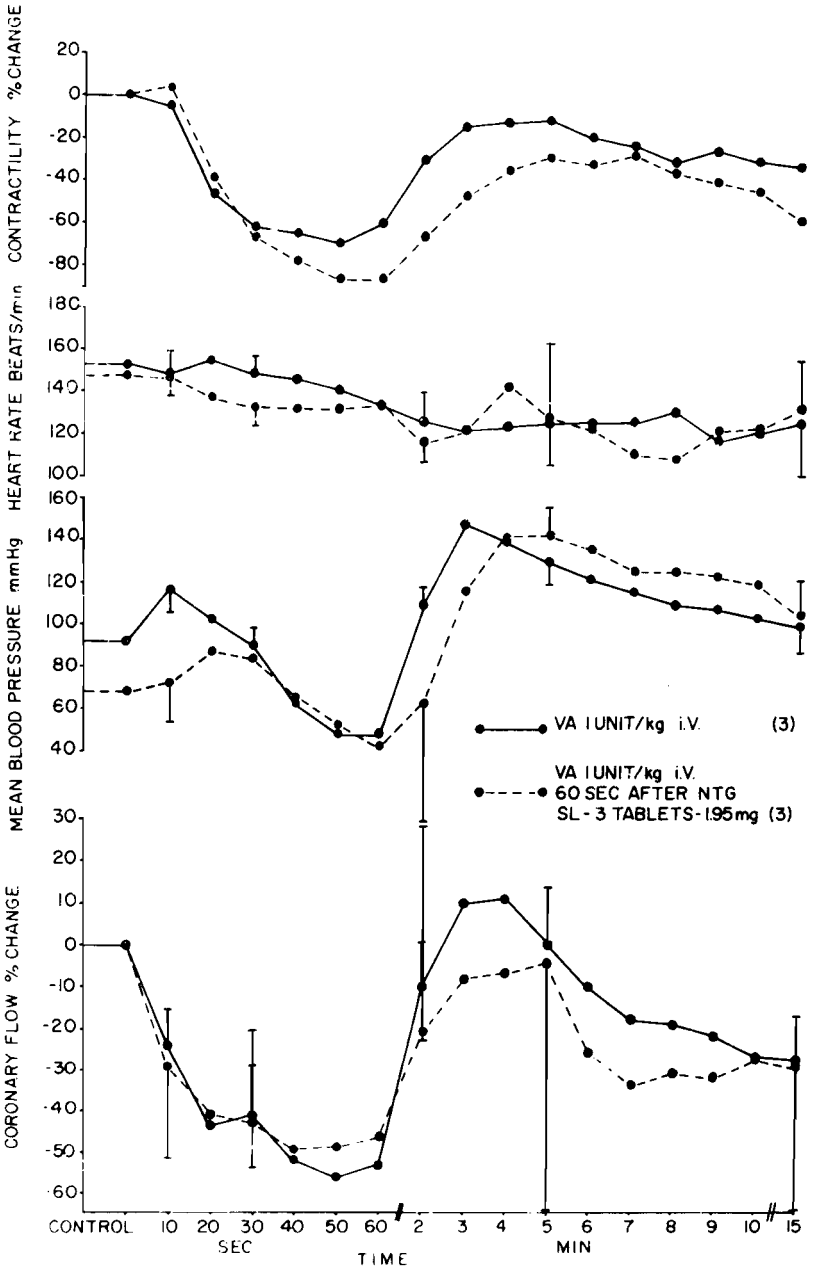

FIGURE 30: Anesthetized open-chest dog. Mean changes in contractile force, heart rate, blood pressure and mean coronary flow following vasopressin alone (solid curve) and vasopressin administered 60 seconds after sublingual nitroglycerin (broken curve). Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

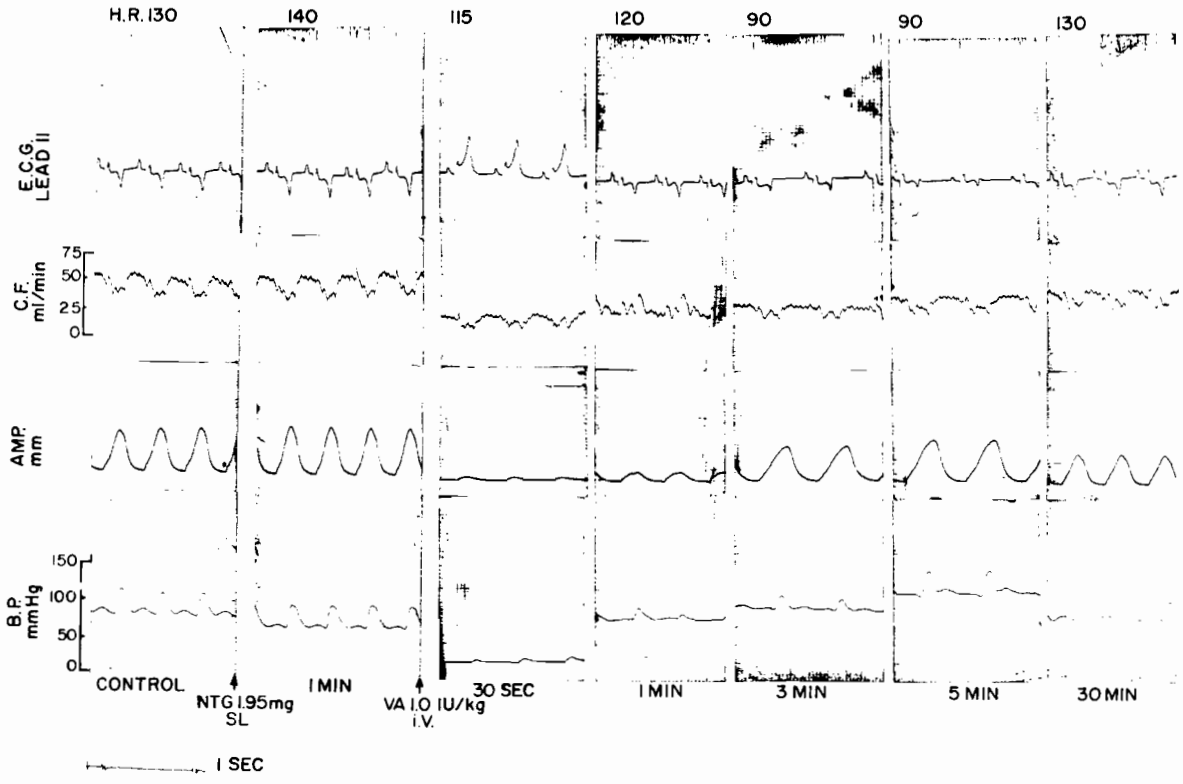

FIGURE 31: Anesthetized open-chest dog. Experiment illustrating the effects on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP) when vasopressin (VA 1.0 I.U./kg) is administered 60 seconds after sublingual nitroglycerin (NTG 1.95 mg).

within 1 minute a slightly elevated ST segment and upright T wave were evident. This usually continued for over 10 minutes, but returned to control within 30 minutes.

Since these results were largely negative, it was felt that if the vasoconstriction was reduced, then the antagonism would be more marked, hence 0.5 I.U./kg of vasopressin was next investigated. It can be seen from Figure 32 that injection of 0.5 I.U./kg of vasopressin resulted in only a 30% decrease in coronary flow which was maximal within 2 minutes and returning to control levels within 4 minutes. The effect on the blood pressure showed the characteristic diphasic response being maximally depressed within 60 seconds (16 mm Hg), and rising to overshoot the control value within 3 minutes and remained elevated for over 15 minutes. The heart rate was progressively decreased and the degree of myocardial depression (70%) was identical to that induced by 1.0 I.U./kg of vasopressin. In Figure 33, portions of the records obtained during a typical experiment are shown. Marked lowering of the blood pressure and myocardial depression were seen after 1 minute and maximum blood pressure elevation within 5 minutes. The ECG record shows that within 30 seconds, there was a diminution of the P wave with an upright T which gradually increased in voltage. This continued for over 10 minutes with a return to the normal pattern within 30 minutes.

Using the same dose of nitroglycerin (3 tablets), the identical procedure, as outlined for 1.0 I.U./kg of vasopressin during sublingual nitroglycerin, was repeated, using 0.5 I.U./kg of vasopressin. Figure 32 also represents graphically the mean values of 3 such experiments, and Figure 34 shows sections of the tracings obtained.

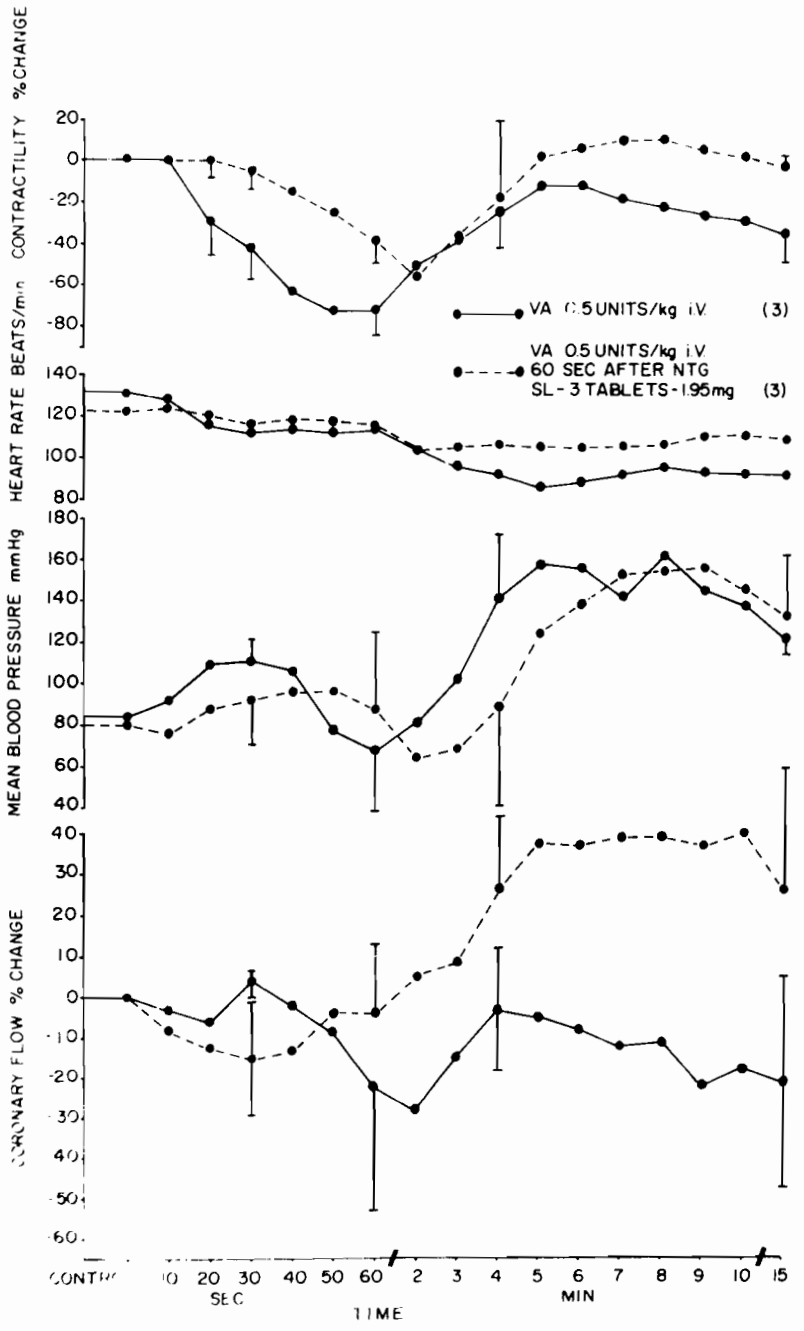

FIGURE 32: Anesthetized open-chest dog. Mean changes in contractile amplitude, heart rate, blood pressure and mean coronary flow following vasopressin alone (solid curve) and when vasopressin is administered 60 seconds after sublingual nitroglycerin (broken curve). Vertical lines indicate standard errors of the means. Number of experiments in parenthesis.

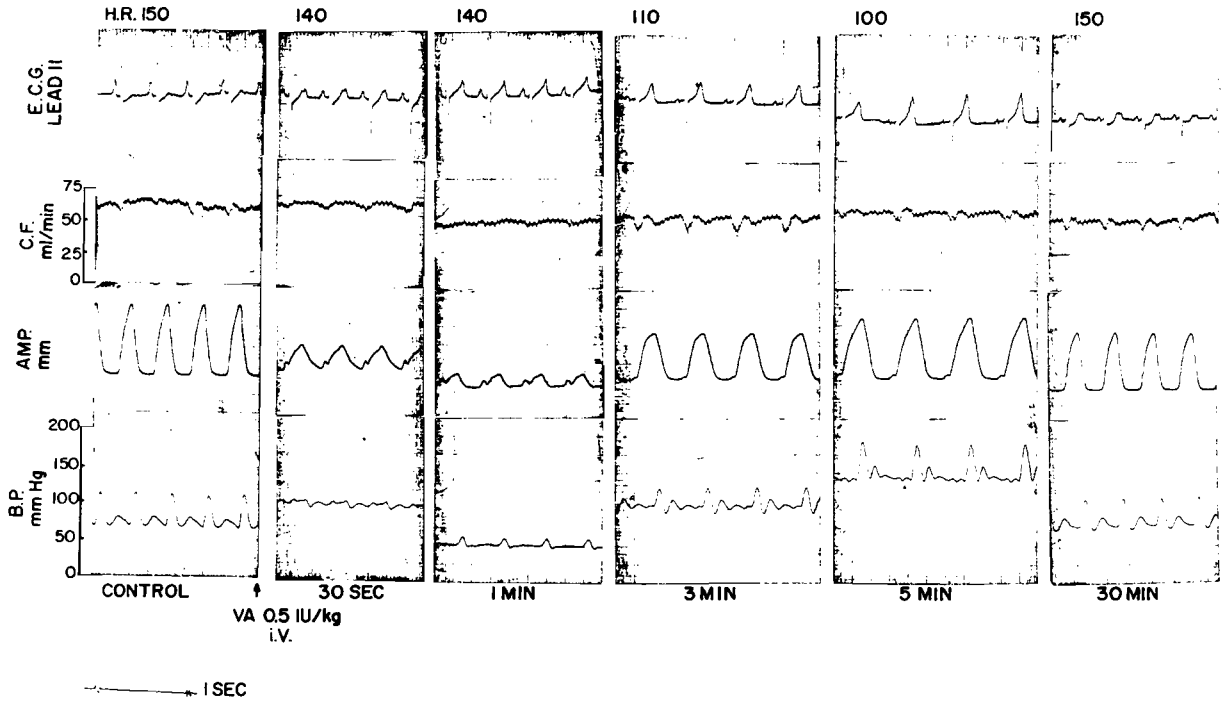

FIGURE 33: Anesthetized open-chest dog. Experiment illustrating the effects on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP) of vasopressin (0.5 I.U./kg).

It will be observed that there was a 15% decrease in mean coronary flow during the first minute after vasopressin administration followed by a 35% increase above control values and even after 15 minutes, coronary flow was still elevated. The blood pressure curve was not significantly different from that occurring during vasopressin administration. Maximum depression (22 mm Hg) occurred within 2 minutes, at which time the coronary flow was slightly above control values. The rise in pressure after 3 minutes may be responsible for the dramatic increase in coronary flow due to hyper-irrigation of the coronary vascular bed. The heart rate values were not significantly different from those observed in the responses to vasopressin alone, and the myocardial depression was delayed, being maximal within 2 minutes and was also reduced (but not significantly). Figure 34 shows that there were no changes in the general pattern of the ECG. In all (3) experiments, any ECG changes which occurred were only transient changes. The figure depicts a transient reversal of the T wave occurring within 3 minutes followed by elevation and occasional extra systole probably due to the elevated blood pressure. These changes were followed by a progressive return to the normal pattern within 10 minutes in all instances.

It can, therefore, be concluded that some antagonism does occur to vasopressin-induced vasoconstriction when nitroglycerin is administered sublingually, but this antagonism is only demonstrable when the vasoconstriction is less intense. However, there was the possibility that the anesthetic may have influenced the responses observed and that only the resultant effects of all the factors involved were being observed.

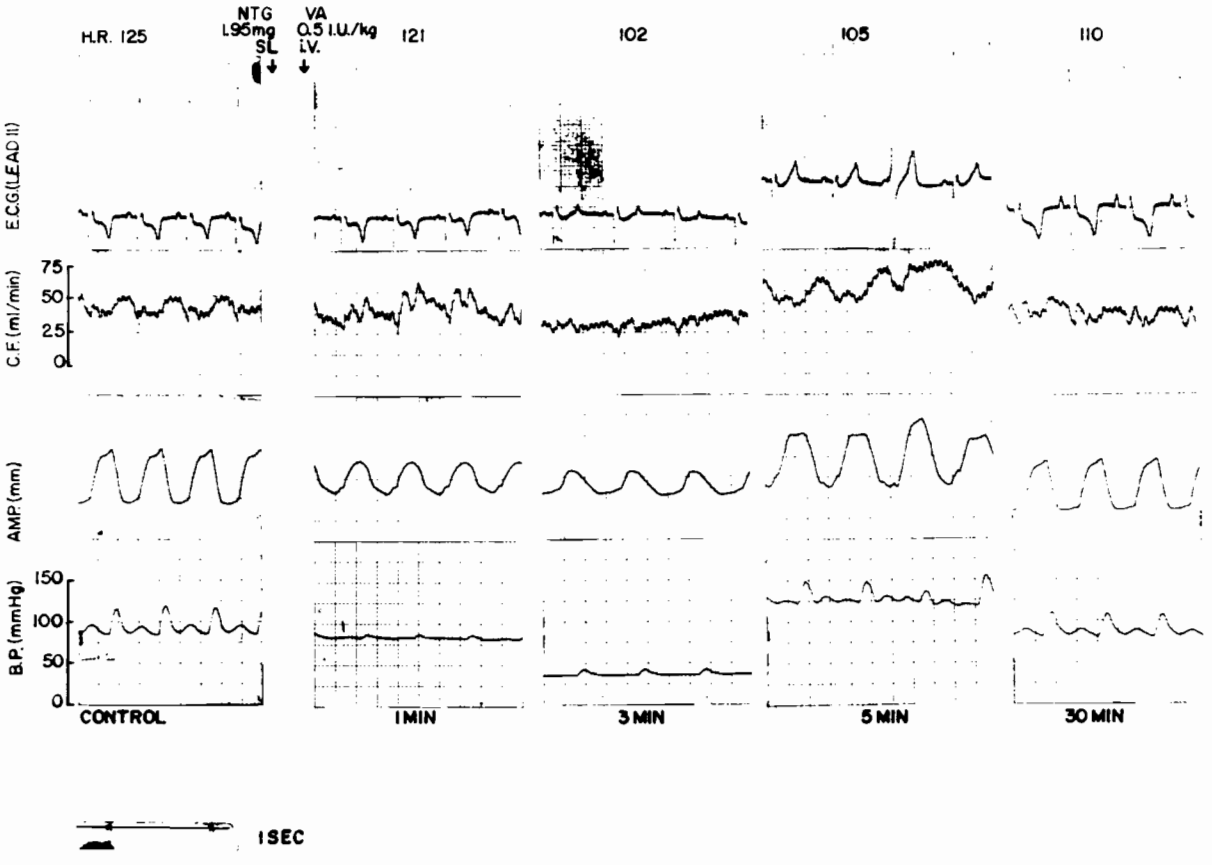

FIGURE 34: Anesthetized open-chest dog. Experiment illustrating the effects on heart rate (HR), electrocardiograms (ECG), coronary flow (CF), contractile amplitude (AMP) and blood pressure (BP) when vasopressin (VA 0.5 I.U./kg) is administered 60 seconds after sublingual nitroglycerin (NTG 1.95 mg).

To eliminate such a possibility, and since nitroglycerin is not administered under anesthesia, it was assumed that if the anesthesia was eliminated, a more pronounced antagonism could be demonstrated. Also, since during anesthesia metabolism is depressed, the use of unanesthetized animals might ensure more adequate absorption.

B. Effects of Sublingual Nitroglycerin in Normal Unanesthetized Dogs

In this series of experiments, dogs underwent a period of training (see Methods). Following initial preparations, the electrodes were put in place, the catheter for intravenous administration of drugs, and the Cournand (18G) needle for recording femoral arterial pressure inserted. Control readings (Leads I, II and III) were taken intermittently during a period of 5 minutes.

Sublingual administrations of nitroglycerin (3 tablets) produced no marked changes in the ECG, heart rate or blood pressure as were recorded. However, when a dose of 0.5 I.U./kg of vasopressin was administered (without prior administration of nitroglycerin), marked changes occurred. These are shown graphically in Figure 35. Within 30 seconds after vasopressin administration, marked T wave elevation occurred, and this increased to a maximum of 14 $\pm$ 1.3 mm (5 min) from the control value of 3 mm (average deviation, without regard to direction from the isoelectric line). In some experiments, slight widening of the QRS complexes occurred. The heart rate was depressed from a control value of 102 $\pm$ 12 to 20 $\pm$ 6 beats/min, within 40 seconds. Within 5 minutes, there was almost a complete recovery. Immediately after vasopressin, there was an immediate and progressive elevation of the blood pressure from a control value of 76 $\pm$ 10 mm Hg to a maximum of 150 $\pm$ 8 mm Hg within 4 minutes. Figure 36 is a record of an experiment.

Direct observations of the animal showed extreme paling of the skin, apnea, and a complete cessation of salivation. After 1 minute, a semi-comatose state ensued. This persisted for over 8 minutes but within 15 minutes animals showed complete recovery.

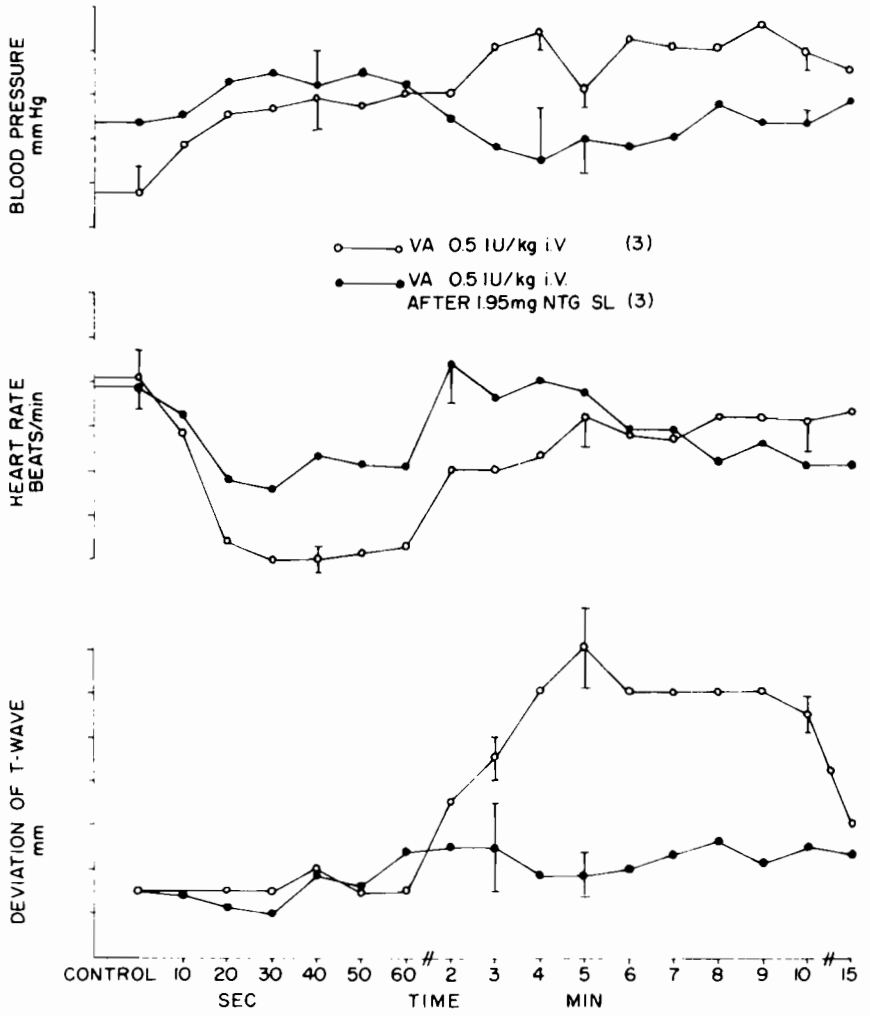

FIGURE 35: Normal unanesthetized dog. Mean changes in blood pressure, heart rate and deviation of T wave (from the isoelectric line) following injections of vasopressin (open circles) alone and when administered 60 seconds after sublingual nitroglycerin (closed circles). Vertical lines indicate standard errors of the means. Number of experiments in parenthesis. T wave was bi-directional.

The effects of vasopressin when administered after sublingual nitroglycerin was next investigated. Approximately 1 minute after placing 3 hypodermic tablets of nitroglycerin at the base of the animals tongue, an injection of vasopressin (0.5 I.U./kg) was made intravenously. The responses observed are also summarized graphically in Figure 35, and in Figure 36, a record of an experiment is shown. The ECG changes observed were not as pronounced as those observed following the injection of vasopressin alone. Maximum T wave changes occurred within 60 seconds after administration of vasopressin. The marked elevation observed in the control response was significantly reduced. In one experiment, there was widening of both the QRS complex and of the ST segments for 30 seconds. However, within 5 minutes, the control ECG pattern was largely restored except for a slight elevation of the T wave which persisted for over 15 minutes.

The bradycardia, although not completely eliminated, was significantly reduced, but no significant antagonism of the peripheral vasoconstriction induced by vasopressin was observed. Direct observations of the animal showed similar paling of the skin and apnea as were observed during the control injections. There was also a cessation of salivation but these changes were of a shorter duration (5 min) than those which were observed during the control injection of vasopressin. A semi-comatose state did not occur.

The data presented may be summarized thus:

(1) When administered sublingually, nitroglycerin produced a slight (5%) but prolonged coronary vasodilator response (15 min).

(2) This vasodilatation failed to antagonize the coronary vasoconstriction and other cardiovascular changes induced by vasopressin (1.0 I.U./kg i.v.).

(3) When the degree of coronary constriction was reduced (0.5 I.U./kg vasopressin), some antagonism of the coronary vasoconstriction was observed.

(4) When administered sublingually to normal unanesthetized dogs, nitroglycerin did not significantly affect the heart rate, blood pressure, or ECG.

(5) Significant antagonism of the ECG changes and bradycardia induced by vasopressin (0.5 I.U./kg) were observed when the normal anesthetized animals were pre-treated with nitroglycerin, but the peripheral vasoconstriction induced by vasopressin was not affected.

It is concluded that sublingual nitroglycerin will antagonize vasopressin coronary constriction, but this is not demonstrable in anesthetized animals. It is also doubtful if the antagonism is due to a coronary vasodilatation induced by sublingual nitroglycerin.

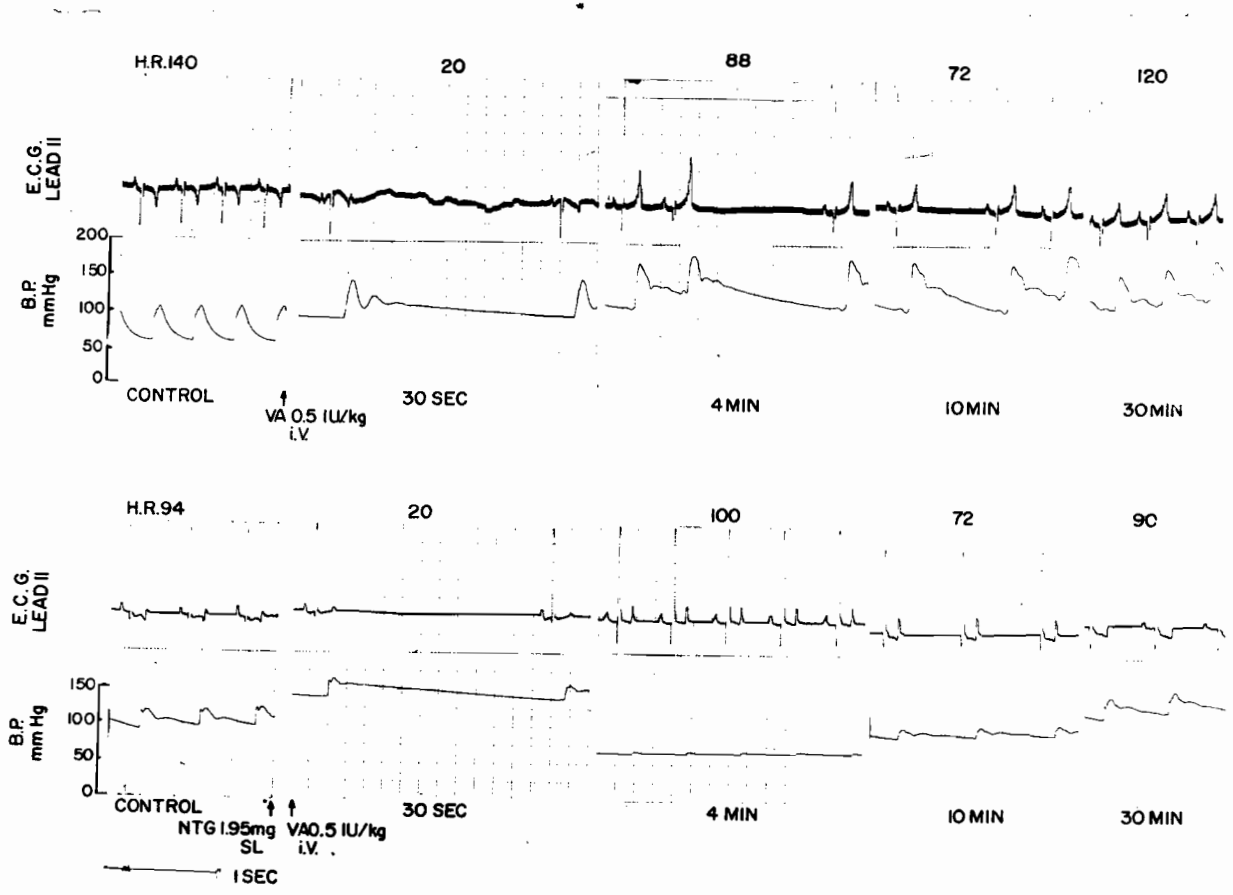

FIGURE 36: Normal unanesthetized dog. Experiments illustrating the effects on heart rate (HR), electrocardiograms (ECG) and blood pressure (BP) when vasopressin (VA 0.5 I.U./kg) is administered before (upper) and 60 seconds after sublingual nitroglycerin (NTG 1.95 mg sl).

V. GENERAL DISCUSSIONS

A. Cardiac Adrenergic Mechanisms in Species Responses to Catecholamines

Multiple factors are known to influence the flow of blood in the coronary vascular bed, and these complicate the study of the effects of drugs on the coronary circulation. The studies of Gillis (1965) indicate that the rate of coronary flow can be profoundly influenced by adrenergic mechanisms. The principal problem in evaluating the role of these adrenergic mechanisms in the control of the coronary flow is one of distinguishing direct effects on the coronary vessels i.e. effects mediated by specific adrenergic mechanisms in the coronary vascular bed and the secondary influences of the catecholamines on the heart such as those due to mechanical alterations in extravascular coronary compression, as well as modifications in coronary flow due to concomitant changes in heart rate, blood pressure and myocardial metabolism.

Two common methods employed in the investigation of the role of adrenergic mechanisms in the control of the coronary circulation include the injection of catecholamines and pharmacological blockade of receptors. There is general agreement that administrations of adrenaline and noradrenaline induce coronary vasodilatation, and any diminution of the coronary flow is due to mechanical factors. Thus Hammouda and Kinosita (1926), using arecoline - arrested hearts of rabbits, concluded that adrenaline had a pure dilator effect on the heart which was not affected by changes in heart rate but was

affected by changes in amplitude of contraction. The maximum change in amplitude was observed to coincide with the period of minimum coronary flow hence they suggested that the decrease in coronary flow was not due to direct vasoconstriction but compression of the coronary vessels.

In the present study, it is quite clear that a difference in the responses to noradrenaline and adrenaline occur in the isolated heart of rhesus and vervet monkeys. In the hearts of vervet monkeys, noradrenaline produced an immediate and marked increase in coronary flow which was accompanied by increases in heart rate and very striking increases in contractile amplitude. There was no transitory decrease in coronary flow as had been reported to occur in the isolated hearts of other species. The heart rate was maximally increased before changes in contractile amplitude and coronary flow were at their maximum. These latter occurred simultaneously. When compared to the isolated rabbit heart, the observed increase in heart rate of vervet hearts in response to a similar dose of noradrenaline (1 µg) was very mild. These observations establish a pure dilator effect of noradrenaline in the isolated hearts of vervet monkeys.

Observations made on the isolated hearts of rhesus monkeys demonstrated similar increases in contractile amplitude and heart rate as were observed in the hearts of vervet monkeys. However, with respect to coronary flow, a decrease was the constant response and this decrease was at its lowest value before maximum increases in heart rate or contractile amplitude had occurred. No secondary increase in coronary

flow was observed; in fact, the control flow was not restored even after heart rate and amplitude were restored to their control values. Lu and Melville (1951) and Gillis (1965), using isolated rabbit hearts, noted a primary decrease which was followed by a secondary increase. This secondary increase was postulated to be due to a metabolic effect resulting from the intense cardiac stimulation induced by noradrenaline. The primary vasoconstriction these workers observed was attributed to myocardial compression as was suggested by Hammouda and Kinosita (1926). In the present observations, it is quite evident that the primary event was an induced vasoconstriction and not a secondary reduction in coronary flow resulting from either the intense myocardial stimulation or compression. From the present study, it appears then, that noradrenaline which is more well known as an alpha-adrenergic receptor stimulant dilates the coronary vessels of one species but constricts the coronary vessels of the other species of monkeys.

When adrenaline (1 µg) was administered, the observations were quite similar to those made when noradrenaline was administered. The same inter-relationships of coronary flow, heart rate and contractile amplitude, as was observed following noradrenaline, occurred in both species. Adrenaline induced a greater increase in mean coronary flow in the hearts of vervet monkeys and a greater decrease in the hearts of rhesus monkeys when compared to noradrenaline. In the latter species, the decrease in coronary flow was the primary event. As noted, when noradrenaline was administered, no secondary increase in coronary flow occurred.

During beta-adrenergic blockade, the responses to both noradrenaline and adrenaline were significantly reduced in the hearts of vervet monkeys but were not completely abolished. Maximum increases in coronary flow and heart contractions occurred simultaneously in responses to both amines. When considered in terms of coronary flow per beat change, it was observed that the per cent change in mean coronary flow per beat was increased during beta-adrenergic blockade. This indicates that the induced increase in heart rate had a retarding effect on the coronary flow. Hammouda and Kinosita (1926) suggested that the increase in coronary flow, observed in isolated rabbit hearts in response to adrenaline, was not affected by changes in heart rate. In the hearts of rhesus monkeys, while amplitude of contraction and mean coronary flow were significantly reduced during beta-adrenergic blockade, the increase in heart rate induced by both noradrenaline and adrenaline was not affected.

It would appear then, that in the isolated hearts of vervet monkeys, the activation of beta receptors i.e. receptors responsible for mediating positive inotropic and chronotropic responses were partly responsible for the observed increases in coronary flow and heart contractions. However, during beta-adrenergic blockade, there were significant increases in both mean coronary flow and the amplitude of contraction although these were less than values obtained before blockade. Assuming complete blockade, it must be assumed that these latter responses were independent of beta-adrenergic mechanisms. In

the hearts of rhesus monkeys, since a significant reduction in the degree of the coronary flow response was observed during blockade, it also appears that beta-adrenergic mechanisms were partly responsible for this decrease. The increase in heart rate was not affected and there was still an increase in contractile amplitude during blockade although the latter was significantly ($P < 0.01$) less than values observed before blockade. Assuming complete blockade, it must be concluded that these increases in rate and amplitude and the decrease in mean coronary flow which were not abolished by blockade are independent of beta receptor stimulation. The increase in heart rate might, in some way or other, be responsible for the decrease in coronary flow which was not abolished during beta-adrenergic blockade in these experiments.

With regard to the effect of isoprenaline on coronary flow, beta-adrenergic mechanisms appeared to have been the only important mechanism of action since all responses observed before blockade were completely abolished in both species. In the control responses, sustained and significant increases in mean coronary flow were observed in the hearts of vervet monkeys. On the other hand, isoprenaline produced no significant increases in either mean coronary flow on heart rate in rhesus monkeys, although a sustained increase in the amplitude of contraction was observed.

From the above observations, it is concluded that (1) catecholamine-induced stimulation of beta-adrenergic mechanisms are important in mediating the increases in coronary flow observed in the hearts of vervet monkeys. (2) coronary constriction observed in the hearts

of rhesus monkeys is not wholly mediated by beta-adrenergic mechanisms. (c) increases in heart rate produced by noradrenaline, adrenaline, but not by isoprenaline in the hearts of rhesus monkeys are independent of the stimulation of beta-adrenergic mechanisms. (d) catecholamine-induced increases in cardiac contractile force are largely due to the stimulation of beta-adrenergic mechanisms. These findings might offer a possible basis for the marked variability i.e. increases and decreases in coronary flow in responses to catecholamine as previously reported in the literature. The implication of these findings also is that the catecholamine may decrease rather than increase coronary flow in man.

B. Possible Adrenergic Mechanisms in Coronary Drug Responses

All experiments, using isolated hearts of various species of animals, demonstrate that the "nitrites" increase coronary flow. Gillis (1965), using isolated rabbit hearts, reported a dose-dependent increase in mean coronary flow in response to nitroglycerin (0.1 to 100 µg). The threshold dose for this increase was 0.1 µg and doses above 100 µg did not produce a further increase in coronary flow. He also showed that nitroglycerin, in doses which strikingly increase coronary flow, exerted no inotropic or chronotropic effect on the isolated rabbit hearts. Prior to this, Melville and Lu (1950) had suggested that the coronary vasodilator action of nitroglycerin and trolnitrate was probably due to a direct action of these nitrites on the coronary vessels. In the present study, using isolated hearts of rhesus monkeys, dose-response curves were obtained for both nitroglycerin (0.1, 1.0, 10 µg) and trolnitrate (1.0, 10, 100 µg) before, and during beta-adrenergic blockade. The threshold doses and dose-response relationships observed for nitroglycerin and trolnitrate agreed quite well with the findings of Gillis.

During beta-adrenergic blockade, the vasodilator responses to the nitrites were significantly reduced. In the isolated hearts of rabbits, Gillis concluded that beta-adrenergic blockade did not significantly modify the coronary vasodilatation produced by these agents. Since the differences observed in these responses before and during beta-adrenergic blockade were quite significant (nitroglycerin $P < 0.01$, trolnitrate $P < 0.03$), it must be concluded that beta-adrenergic mechanisms are involved in these responses observed in the hearts of rhesus monkeys.

Data obtained from intact dogs (discussed later) would also confirm this conclusion in this species.

Earlier studies conducted on both isolated perfused hearts of rabbits and dogs and intact animals indicate that vasopressin leads to powerful coronary vasoconstriction by a direct musculotropic mechanism, (Rossler 1930; Goldenberg and Rothberger 1932; Deitrich 1933). More recent studies, however, suggest that the mechanism of action of vasopressin might be in some way or other be related to catecholamines, that is to say, might involve adrenergic mechanisms in the coronary vascular bed (Gardier and Abreu 1958; Nash 1962, 1963). The present study on isolated hearts of rhesus monkeys and in intact open-chest dogs, confirm a relationship between the vascular effects of vasopressin and adrenergic receptors. In the isolated rhesus monkey hearts, it was observed that during beta-adrenergic blockade with pronethalol, the coronary vasoconstriction and bradycardia, induced by vasopressin, was markedly reduced or abolished. The associated myocardial depression was unaffected.

It is concluded that the blockade of beta receptors are of some importance in reducing the coronary vasoconstriction induced by vasopressin. Gillis (1965), using isolated rabbit hearts, concluded that beta-adrenergic receptors were largely responsible for the decrease in coronary flow in response to noradrenaline since this decrease was abolished by beta-adrenergic blockade. In the present study, since beta-adrenergic blockade reduced vasopressin-induced coronary vasoconstriction, and in conjunction with the previous observations (Section A), it is concluded that

beta-adrenergic receptors are involved in mediating both increases and decreases in coronary flow. Gillis (1965), using isolated rabbits hearts, also suggested that pronethalol exerted significant myocardial depressant actions in single injections. This depressant action was not pronounced when continuous infusions were made. It is, therefore, not surprising that the myocardial depressant action of vasopressin was not affected during the beta-adrenergic blockade with pronethalol in the hearts of rhesus monkeys.

From the above studies, it is concluded that (1) the coronary vasodilatation induced by both nitroglycerin and trolnitrate in the isolated hearts of rhesus monkeys involves beta-adrenergic mechanisms. (2) beta-adrenergic mechanisms are important in the coronary vasoconstriction induced by vasopressin. (3) beta-adrenergic mechanisms are involved in mediating both increases and decreases in coronary flow.

C. Adrenergic Mechanisms in the Antagonism Between the Nitrites and Vasopressin on the Coronary Circulation

It has been the accepted concept that previous dilatation of the coronary vessels in the intact animal can antagonize coronary vasoconstriction induced by vasopressin. In this study, experiment with nitroglycerin and trolnitrate were performed on pentobarbitalized, open-chest dogs in which coronary flow was measured. Antagonism of the vasopressin-induced coronary vasoconstriction was demonstrable. However, since nitroglycerin and trolnitrate induced only transient coronary vasodilatation in this preparation, these findings would not suggest that the antagonizing effect of the vasopressin-induced coronary constriction is due to coronary vasodilatation, produced by the nitrites, as suggested by Melville (1933 a) and Kareva (1963). In fact, even adrenaline has been classified as a coronary vasodilator because of the fact that it will also antagonize effects of vasopressin vasoconstriction (Charlier 1961).

In the present study, during vasopressin-induced vasoconstriction, coronary blood flow was reduced (over 70%) and this was associated with bradycardia, various cardiac arrhythmias, peripheral vasoconstriction, as well as intense myocardial depression. On the other hand, when administered intravenously, nitroglycerin and trolnitrate induced only transient (10 - 20 secs) coronary dilatation and with large doses, this was succeeded by coronary vasoconstriction or a reduction in the coronary blood flow presumably as a result of the intense associated peripheral vasodilatation. A transient vasodilatation has been demonstrated in dogs

by the recent studies of Bergamaschi and Glasser (1963) and by Gillis (1965). In the former study, it was shown that increasing the dosage of nitroglycerin resulted in a reduction in the degree of induced coronary vasodilatation. In Gillis' investigations, transient nitroglycerin-induced dilatation was followed by a marked reduction in coronary flow. No consistent coronary vasodilatation was observed in the present study but a reduction in coronary flow was quite evident. The observed results conform quite well to similar observations made by Marchetti et al. (1964). They concluded that an observed coronary vasodilatation is less easily detected when the hypotensive effects of nitroglycerin on the general circulation are allowed to occur since the hypotension opposes the increase in coronary flow.

Nitroglycerin and trolnitrate were equally effective (the latter at a higher dose level) in antagonizing the coronary vasoconstriction induced by vasopressin. Since it is impossible to conceive that such a transient inconsistent vasodilator action could be responsible for the complete antagonism of the intense coronary vasoconstriction observed in these experiments, it must be concluded that this antagonism involves some mechanism not wholly dependent on coronary vasodilatation. Marchetti et al. (1964) concluded that the mechanism of action of nitroglycerin on the coronary circulation involved lowering of the arterial blood pressure and reducing the oxygen consumption of the myocardium, the latter as a consequence of the reduced cardiac work rather than by a direct action. It was suggested that concurrent coronary vasodilatation occurred with the lowering of the arterial pressure.

It was observed in the present study that the peripheral vasoconstriction induced by vasopressin was not antagonized by the nitrites but the primary fall in arterial blood pressure, which is believed to be the direct resultant of the intense cardial action, was antagonized. This observation contradicts the hypothesis of Scriabine and McShane (1965) that nitroglycerin stops an anginal attack by a peripheral vasodilator action resulting in a reduction of cardiac work and myocardial metabolic demands. However, it seems quite possible that nitroglycerin acts to maintain rather than to increase coronary blood flow. Eckenhoff and Hafkenschield (1947) suggested that in the absence of observed coronary vasodilatation, nitroglycerin and amyl nitrite were capable of improving the nutrition of the cardiac muscle cell. In the present study, an adequate coronary blood supply was maintained by both nitroglycerin and trolnitrate when these were administered before injections of vasopressin.

An interesting observation was the fact that although both nitroglycerin and trolnitrate successfully antagonized vasopressin-induced vasoconstriction, typical ECG changes still occurred. These changes have been postulated to be a direct resultant of interference with myocardial blood supply hence nutrition, but the present observations indicate that the ECG changes appear to be more closely related to the myocardial depressant action of vasopressin which, it was shown, was not antagonized by nitroglycerin or trolnitrate.

The effect of beta-adrenergic blockade on the responses to the nitrites was investigated by Gillis (1965). He observed that during

blockade, the transient vasodilator response was somewhat reduced, and while the hypotensive action was unaffected, both the positive inotropic and chronotropic responses were abolished. He concluded that in view of the fact that the hypotensive effects were unaffected that the secondary decrease in coronary flow which appeared to be directly related to the fall in blood pressure in control injections of nitroglycerin must have been mediated in some way or other by beta-adrenergic receptors. In the present studies, observations on the isolated rhesus monkey hearts support the findings that beta-adrenergic blockade significantly reduces the coronary vasodilator response to the nitrites. In view of the observations that beta-adrenergic mechanisms may be important in the action of the nitrites, it was believed that by preventing the stimulation of beta receptors, the ability of the nitrites to antagonize vasopressin-induced vasoconstriction might be reduced or abolished. Control injection of vasopressin during beta-adrenergic blockade indicated a reduction in the degree of coronary vasoconstriction and an increase in the pressor response to this agent. The mild control ECG changes observed may have been due to a combination of two factors (a) mild reduction in coronary flow and (b) the anti-arrhythmic actions of the blocking agent pronethalol. Murray et al. (1963) suggested that the anti-arrhythmic actions of pronethalol involved beta-adrenergic mechanisms. Brodeur and Beaulnes (1964) suggested anti-arrhythmic properties for vasopressin. They suggested that since vasopressin antagonized chloroform-adrenaline fibrillation in dogs, its anti-arrhythmic action was probably due to both a quinidine-like property and the induced coronary constrictor

action resulting in myocardial anoxia which depressed the metabolic processes of the myocardium.

In the presence of both of the nitrites, during beta-adrenergic blockade, vasopressin-induced coronary and peripheral vasoconstriction were most pronounced and were identical to the effects observed when vasopressin was administered in the absence of pronethalol infusion in these experiments. These observations suggest that whatever antagonistic actions the "nitrites" exerted against vasopressin-induced coronary vasoconstriction, these were abolished during the blockade of beta-adrenergic receptors.

Finally, the potentiation of the myocardial depressant action of vasopressin observed in these experiments could be a resultant of the myocardial depressant actions of both pronethalol and vasopressin. In view of the fact that intense cardial arrhythmias occurred when vasopressin was administered after nitroglycerin and trolnitrate in these experiments, it would appear that a blockade of beta-adrenergic receptors might not be the mechanism through which pronethalol exerts its anti-arrhythmic actions on vasopressin-induced cardial arrhythmias. In fact, this was recently demonstrated by Lucchesi (1965) who evaluated the relative potencies of the dextro and laevo isomers of pronethalol in producing beta-adrenergic blockade and in preventing experimentally induced cardial arrhythmias. It was demonstrated that the dextro isomer, a compound 40 times less active in producing beta-adrenergic blockade was more effective in preventing several types of experimental arrhythmias. The observations made on the isolated hearts of rhesus monkeys (see Part A) showed that the catecholamine-induced tachycardia was not affected

by beta-adrenergic blockade with pronethalol. This, in association with the failure of pronethalol to antagonize vasopressin arrhythmia in the present study, confirms the suggestion of Lucchesi (1965) that beta-adrenergic inhibition is not the mechanism through which pronethalol produces its anti-arrhythmic action.

In conclusion (1) nitroglycerin and trolnitrate induce no pronounced coronary vasodilatation in the intact open-chest dog. (2) nitrites will antagonize vasopressin-induced coronary vasoconstriction and this antagonism is dependent in some way or other on beta-adrenergic mechanisms. (3) the anti-arrhythmic actions of pronethalol on vasopressin-induced cardial arrhythmias might not involve beta-adrenergic mechanisms.

D. Sublingual Nitroglycerin and Vasopressin Antagonism

Anginal pain, believed to be due to coronary arteriolar vasospasm and nitroglycerin when administered sublingually, is reported to be most effective in relieving this vasospasm on the assumption that it produces a coronary dilator effect. However, most experimental evidence has failed to confirm a coronary vasodilator action of sublingual nitroglycerin. Eckenhoff and Hafkenschield (1947) observed no consistent coronary vasodilator response to nitroglycerin when administered sublingually, subcutaneously and intramuscularly.

The present study also failed to confirm any significant coronary vasodilator action when nitroglycerin was administered sublingually in anesthetized dogs. While it is conceivable that a transient increase in coronary flow might improve the functions of the heart during anginal attacks, it seems unlikely that these insignificant changes in coronary flow can explain the therapeutic value of nitroglycerin (Gillis et al. 1965). Gillis (1965) also suggested that to establish the fact that sublingual nitroglycerin does relieve the vasospasm of angina, it would be worthwhile to administer nitroglycerin sublingually during experimentally induced coronary vasospasm.

In anesthetized open-chest dogs, no significant antagonism of vasopressin-induced coronary vasoconstriction was observed following sublingual nitroglycerin in the present study. This observation was indicative of little or no prophylactic useful effect of sublingual nitroglycerin. However, since clinically nitroglycerin is not administered under anesthesia, one might argue that adequate absorption

did not occur in the anesthetized animals, since during anesthesia absorption from the mucosa of the gastrointestinal tract is depressed. This might also be due to some depression of metabolism during anesthesia. In experiments in which nitroglycerin was administered sublingually to trained unanesthetized dogs, significant antagonism of the vasopressin-induced ECG changes was observed. If the ECG changes can be interpreted as indicating disturbances in the myocardial blood supply, then sublingual nitroglycerin was effective in maintaining an almost adequate blood supply to the cardiac muscles. It is impossible to conclude that the degree of antagonism shown was the resultant of a coronary vasodilator-coronary constrictor antagonism. Changes in coronary flow were not determined in these animals. The observations of Essex et al. favour a concept of an antagonism by coronary dilatation. They demonstrated increases of up to 100% in coronary flow for over 3 minutes in trained unanesthetized dogs in response to sublingual or intravenous administrations of nitroglycerin.

It is concluded, therefore, that when administered sublingually in unanesthetized dogs, nitroglycerin is capable of exerting significant antagonism against coronary constriction induced by vasopressin although the mechanism of this antagonism is not demonstrated.

VI SUMMARY AND CONCLUSIONS

Experiments were performed to determine whether a species variation exist in the responses of the coronary circulation to catecholamines; and the possible role of cardiac adrenergic mechanisms in the mediation of these responses. Whether the coronary flow responses to the nitrites are dependent on cardiac adrenergic mechanisms, the role of beta-adrenergic mechanisms in the antagonisms shown by the nitrites to coronary vasoconstriction induced by vasopressin; and whether sublingual nitroglycerin is effective in relieving vasopressin-induced spasm of the coronary vessels.

The results can be summarized as follows:

(1) Using oxygenated McEwen's solution, preparations of isolated hearts of monkeys were kept viable and responsive to drugs for long periods (up to 36 hours) with no signs of deterioration.

(2) Noradrenaline increased heart contractions (both rate and amplitude) in isolated perfused hearts of Macaca mulatta and Cercopithecus aethiops, but while augmentation of coronary flow occurred in the latter, a decrease in coronary flow was a constant response in the former.

(3) Adrenaline exerted qualitatively similar actions to noradrenaline in both species of monkeys.

(4) In the isolated perfused hearts of monkeys, isoprenaline produced cardioacceleration and an increase in coronary flow which was more pronounced and prolonged in the isolated heart of vervet monkeys.

(5) Beta-adrenergic blockade with pronethalol reduced coronary flow and heart rate, but did not affect the amplitude of contraction in the isolated perfused heart of monkeys.

(6) Beta-adrenergic blockade with pronethalol reduced the changes in amplitude of contraction and mean coronary flow induced by noradrenaline and adrenaline in the isolated hearts of both species of monkeys but, while the heart rate changes were abolished in the isolated hearts of vervet monkeys, they were unaffected in the hearts of rhesus monkeys.

(7) All responses to isoprenaline were abolished in both species after beta-adrenergic blockade.

(8) Beta-adrenergic blockade did not affect the positive inotropic actions of calcium on the isolated hearts of monkeys.

(9) Glyceryltrinitrate (nitroglycerin) and trolnitrate increased coronary flow in the isolated hearts of rhesus monkeys but did not exert any significant positive inotropic or chronotropic actions.

(10) A dose-dependent increase in mean coronary flow was induced by nitroglycerin but not by trolnitrate in the hearts of rhesus monkeys.

(11) Nitroglycerin was observed to be,dose for dose, 100 times as potent as trolnitrate on the isolated hearts of rhesus monkeys.

(12) During beta-adrenergic blockade, augmentations of mean coronary flow induced by nitroglycerin and trolnitrate, on the isolated hearts of rhesus monkeys were significantly reduced.

(13) The coronary vasoconstriction induced by vasopressin on isolated hearts of rhesus monkeys was reduced during beta-adrenergic blockade, while the induced myocardial depression was unaffected.

(14) When administered intravenously, nitroglycerin and trolnitrate were capable of antagonizing vasopressin-induced coronary vasoconstriction in open-chest artificially respired pentobarbitalized dogs.

(15) In anesthetized dogs, antagonism of the coronary vasoconstriction by nitroglycerin and trolnitrate did not prevent the development of typical ECG changes which were more pronounced and persistent with nitroglycerin as the antagonist than with trolnitrate.

(16) The "nitrites" abolished vasopressin-induced bradycardia but not the peripheral vasoconstriction nor the associated myocardial depression in anesthetized dogs.

(17) During beta-adrenergic blockade in anesthetized dogs, coronary flow, heart rate, blood pressure and myocardial contractility were not significantly affected by the blockade.

(18) The secondary decrease in flow induced by nitroglycerin was significantly reduced during beta-adrenergic blockade in anesthetized dogs.

(19) In anesthetized dogs, coronary vasoconstriction induced by vasopressin was reduced and the ECG changes observed were either only transient ischemic-like ones or completely abolished.

(20) During beta-adrenergic blockade in anesthetized dogs, administration of vasopressin after intravenous nitroglycerin resulted in pronounced coronary and peripheral vasoconstriction with marked myocardial depression.

(21) Vasopressin-induced ECG changes were intensified when administered after nitroglycerin during beta-adrenergic blockade in anesthetized dogs.

(22) When administered sublingually in anesthetized dogs, nitroglycerin produced no significant increase in coronary flow but produced a mild decrease in blood pressure and a slight increase in contractile amplitude but no change in heart rate.

(23) Sublingual nitroglycerin did not significantly antagonize vasopressin-induced coronary vasoconstriction or ECG changes in anesthetized dogs.

(24) Sublingual nitroglycerin produced no significant changes in blood pressure, heart rate or ECG pattern in normal unanesthetized dogs.

(25) In normal unanesthetized dogs, significant antagonism of the ECG changes and bradycardia induced by vasopressin were observed when nitroglycerin was administered sublingually but the associated peripheral vasoconstriction was unaffected.

From the foregoing observations, the following conclusions are drawn:

(1) Catecholamine-induced stimulation of beta-adrenergic mechanisms are important in mediating increases in coronary flow as observed in the hearts of vervet monkeys.

(2) Coronary constriction observed in the hearts of rhesus monkeys is not mediated by beta-adrenergic mechanisms.

(3) Increases in heart rate produced by noradrenaline, adrenaline, but not by isoprenaline in the hearts of rhesus monkeys are independent of the stimulation of beta-adrenergic mechanisms.

(4) Catecholamine-induced increases in cardiac contractile force are largely due to the stimulation of beta-adrenergic mechanisms.

(5) Catecholamines may decrease rather than increase coronary flow in man.

(6) Coronary vasodilatation induced by both nitroglycerin and trolnitrate in the isolated hearts of rhesus monkeys involve beta-adrenergic mechanisms.

(7) Beta-adrenergic mechanisms are involved in the coronary vasoconstriction induced by vasopressin.

(8) Beta-adrenergic mechanisms are involved in some way or other in mediating both increases and decreases in coronary flow.

(9) Nitroglycerin and trolnitrate will antagonize vasopressin-induced coronary vasoconstriction and this antagonism is dependent in some way or other on beta-adrenergic mechanisms.

(10) Sublingual nitroglycerin is capable of antagonizing vasopressin-induced coronary vasoconstriction.

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