AN ABSTRACT OF THE THESIS OF

CLAUDE LANE GRIFFIN for the Doctor of Philosophy in Pharmacology
(Name) (Degree) (Major)

Date thesis is presented July 6, 1965

Title ADRENERGIC RECEPTORS FOR GLYCOGENOLYSIS AND CARDIAC CONTRACTILE FORCE

Abstract approved [redacted]
(Major professor)

It is common to characterize adrenergic receptors by placing them in one of two categories, alpha or beta. The receptors responsible for adrenergically induced increase in cardiac contractile force and glycogenolysis are usually classified as beta receptors, but such a classification is not definitely established. Therefore, an attempt was made in the present study to characterize the nature of the receptors responsible for increases in cardiac contractile force and glycogenolysis induced by various adrenergic amines.

The effects of rapid injections of phenylephrine and norepinephrine (amines which excite only alpha receptors) and epinephrine (amine which excites alpha and beta receptors) and isoproterenol (amine which excites beta receptors) were measured in dogs, on blood pressure and cardiac contractile force before and after phenoxybenzamine or Hydergine (alpha receptor blocking agents) or dichloroisoproterenol (beta receptor blocking agent) or

d-tubocurarine (neuromuscular blocking agent). Cardiac contractile force was measured by a strain gauge sutured on the ventricule wall and average blood pressure was measured from the carotid artery. Phenoxybenzamine did not alter the cardiac contractile effects of any of the amines and d-tubocurarine did not alter the cardiovascular responses of any of the amines. Hydergine inhibited the cardiac contractile force response of phenylephrine and norepinephrine, and dichloroisoproterenol inhibited this response of all four of the amines. Phenoxybenzamine and Hydergine blocked the blood pressure responses of all of the amines except isoproterenol. Dichloroisoproterenol blocked the blood pressure response of only isoproterenol.

These results show that both alpha or beta adrenergic amines can increase cardiac contractile force and that dichloroisoproterenol can inhibit this response of all four of the amines, while Hydergine can produce a selective blockade with two of the amines. This led to the conclusion that the cardiac receptor responsible for adrenergically induced increases in cardiac contractile force can be excited by either alpha or beta adrenergic amines and it is therefore suggested that this receptor be reclassified as an undifferentiated receptor.

The glycogenolytic effects of adrenergic amines, in dogs, were determined by measuring the elevation of serum levels of

of glucose and lactic acid produced by slow infusions of the four adrenergic amines before and after blocking agents. Serum levels of glucose and lactic acid were significantly elevated by epinephrine and isoproterenol, but not by phenylephrine or norepinephrine. Dichloroisoproterenol or Hydergine significantly reduced the hyperglycemic and hyperlactic acidemic responses of epinephrine, but d-tubocurarine or phenoxybenzamine did not. The hyperglycemic and hyperlactic acidemic responses of isoproterenol were inhibited by d-tubocurarine or dichloroisoproterenol, but not by Hydergine.

These results show that only adrenergic amines which could excite beta receptors (epinephrine and isoproterenol) elevated serum levels of glucose and lactic acid, and that Hydergine, an alpha blocking agent, inhibited the effects of epinephrine, but dichloroisoproterenol inhibited the effects of both epinephrine and isoproterenol. These results suggest that adrenergically induced glycogenolysis, in the dog, occurs by excitation of a receptor that is closely related to the beta receptor, but cannot be categorically identified as a beta receptor.

Isoproterenol stimulated glycogenolysis, as determined by glucose uptake and lactic acid production, in isolated liver and skeletal muscle tissues, from dog. Dichloroisoproterenol inhibited these effects of isoproterenol in only skeletal muscle. These results indicate that isoproterenol is an effective glycogenolytic

agent in both types of tissues and that d-tubocurarine can separate isoproterenol induced glycogenolysis in liver and skeletal muscle by selectively inhibiting the amine's effect in skeletal muscle.

Phenoxybenzamine, by itself, significantly elevated serum glucose levels in intact dogs. Since this effect was not observed in adrenalectomized animals, it was concluded that the hyperglycemia induced by phenoxybenzamine occurred from a release of epinephrine from the adrenal glands.

The present study has been concerned with the proposal that all adrenergically induced responses occur due to the excitation of either of two receptors. It seems more likely that each particular physiological or biochemical response induced by adrenergic amines has its own particular receptor and that categorically identifying adrenergic receptors as either alpha or beta is an over-simplification of what is an extremely complex system.

ADRENERGIC RECEPTORS FOR GLYCOGENOLYSIS AND CARDIAC CONTRACTILE FORCE

by

CLAUDE LANE GRIFFIN

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1966

APPROVED:

[redacted]

Professor of Pharmacology

In Charge of Major

[redacted]

Head of Department of Pharmacology

[redacted]

Dean of Graduate School

Date thesis is presented July 6, 1965

Typed by Opal Grossnicklaus

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INTRODUCTION

Historical Development of Adrenergic Concepts

It was Oliver and coworkers, in 1898, who first showed that an extract of the adrenal glands given intravenously, could evoke a marked pressor response (82). Lewandowsky (57) four years later first noted a specific correlation between the actions of sympathetic nerve-stimulation, and the resultant effect of application of supra-renal extract. Lewandowsky's observations were confirmed and expanded by Langley (52) who also surmised that electrical stimulation of sympathetic nerves corresponds identically with the effects induced by an extract of the supra-renals. He also pointed out that since the effect seen with an adrenal extract continued even after denervation, the active constituent of the extract must be attacking the effector organ directly, not the effector nerve.

It was through the work of J. J. Abel that the active constituent of the crude extract, used by Langley, was purified. Abel named it epinephrine (1). Working with the purified substance, Elliot (35, 36) not only confirmed and extended Langley's work, but he also suggested that evidence was available to encourage accepting the hypothesis that epinephrine was liberated at the ends of peripheral

nerve fibers when impulses traversing down the fiber reached the periphery. Elliot failed to pursue this line of thought mainly because of discouragement or more correctly, lack of encouragement, from Langley, his professor.

Twenty years passed before Elliot's hypothesis was recognized as being correct. The now classical experiment of Otto Loewi (58), which presented positive evidence that chemical transmission occurred, was responsible for bringing the first real semblance of order into the field. The endeavors of Dale, which preceded that of Loewi, must also be regarded as classical. Dale, working with the physiological effects of ergot alkaloids on the effects of epinephrine and sympathetic nerve stimulation (31), described the nature of the adrenergic receptor. The work of Dale's was the first real attempt at characterizing the receptive substance which epinephrine united with to induce it responses. Dale even explained the dichotomous nature of epinephrine (its ability to produce both excitation and inhibition) which he observed with ergot alkaloids. This concept is still accepted today.

No other important work was done regarding the nature of adrenergic receptors on the mechanism of action until 1933. Cannon and Rosenblueth (19), in an attempt to re-evaluate Langley's (52) previous work, obtained data which suggested that an excitatory substance called sympathin, which was released during sympathetic

nerve stimulation, was not only different from adrenalin, but also consisted of two types. Their work led them to believe that an exogenous substance, A (adrenalin) or an endogenous substance, M (from local nerve stimulation) united in the cell with a substance which they designated as H. They also advanced the idea that H was either inhibitory (I) or excitatory (E). Thus the combination of the nerve stimulation would result in ME or MI. Since, in their terminology, sympathin was a chemical mediator released from sympathetic nerve endings, it followed that it became either sympathin E or sympathin I when it combined with the H, the receptive substance in the cell. Thus, their substance H appeared to be what Langley called the receptive substance. Their sympathin E and I appeared to be what Langley called the "Chief" substance (19). Cannon's and Rosenblueth's hypothesis remained more or less unchallenged until 1948. In 1948, Ahlquist (4) proposed a theory that was diametrically opposed to the sympathin E and I theory of Cannon and Rosenblueth. In his work, Ahlquist, demonstrated that although there were two types of receptors, they could not be classified simply as excitatory or inhibitory because these receptors produced either action depending upon where they were found. After experimenting with six different amines on different organs, Ahlquist concluded that there are two distinct types of adrenergic receptors, which are determined by their relative responsiveness to the tested amines. He named

those receptors concerned mainly with the excitatory responses alpha receptors (4). The excitatory responses referred to as alpha responses were vasoconstriction, stimulation of the uterus, and stimulation of the nictitating membrane. (One alpha inhibitory response which was included was intestinal relaxation). Those responses which were mainly responsible for the inhibitory responses were designated as beta. The beta responses included vasodilation uterine relaxation, bronchial relaxation and one excitatory response (myocardial stimulation) (4). Ahlquist and Levy have made some slight alterations to this nomenclature pertaining to the intestinal tract (5).

Subsequently, there have been several attempts to make major alterations to Ahlquist's dualistic receptor concept. Lands (51) was concerned with the previously suggested concept that stimulation of either receptor could produce excitation or inhibition, and that this response was determined by the particular organ after the union of the agonist with the receptor. He proposed (51) the following classification: Ac receptors for excitatory responses; Ar receptors for inhibitory; and Acr receptors for undifferentiated receptors. He further concluded that the receptor in the heart was an Acr type with affinity for agonists that activated either Ar or Ac receptors.

In 1959, while summarizing previous work on the adrenergic receptor, Furchgott (42) suggested a fairly strong modification of

Ahlquist's classification. He proposed that alpha receptors are responsible for contraction of smooth muscles, beta receptors are responsible for the relaxation of smooth muscle other than that of intestine and beta receptors also increase cardiac rate and strength of cardiac contraction, gamma receptors for glycogenolysis, and delta receptors for inhibition of intestinal smooth muscle. It should be noted, that shortly after this classification was advanced, Ahlquist and Levy (5) suggested that both alpha and beta receptors are present in the ileum and that both subserve relaxation and inhibition. Furchgott (42) fully realized that his classification which put glycogenolysis in a separate class of receptors (gamma), implied that the stimulation of glycogenolysis is not associated with alpha or beta receptors.

More recently, Belleau (11) suggested two possible mechanisms of action for catecholamines on the receptor. He suggested that the pharmacological response to the amines occurred only after a chemical modification had occurred at the receptor site. He proposed an alternative that suggested that the response occurred only from an electrostatic interaction between the amine and the receptor site.

From the foregoing discussion, it may be seen that there is considerable disagreement concerning the nature of the receptors first considered by Dale. There is general agreement about which adrenergic receptors are excited to produce ectopic beats in the

heart, vasoconstriction, and vasodilation. However, there is considerable disagreement about the adrenergic receptors which are excited to produce increases in cardiac contractile force and glycogenolysis.

It is common to use blood glucose level as an index of glycogenolysis and to aid in characterizing the nature of the glycogenolytic receptor. Glucose is the chief, and for practical purposes the only, transport form of carbohydrate. Carbohydrates enter the blood from the gastrointestinal tract largely as glucose. In the post-absorptive state, glucose is the carbohydrate which the liver supplies to all the other tissues of the body. For these reasons the level of glucose in the blood is normally higher than in any other tissue or fluid of the body.

The average normal level of glucose in the blood does not vary appreciably with the species of animal. In most mammals it is very similar, ranging from 75-95 milligrams per 100 cc of whole blood. It is customary to express these amounts as milligrams percent. Strictly speaking this is incorrect because whole blood is not homogeneous, nor is it of the same specific gravity as water.

Because the peripheral tissues are constantly removing sugar from the blood, samples of arterial or capillary blood will show a level of sugar a few milligrams percent higher than that of simultaneously drawn samples of venous blood (40).

Normal urine contains a small amount of glucose. An average adult human excretes from 500 to 750 milligrams of glucose in the 1500 milliliters of urine excreted in 24 hours (44, 83). In clinical medicine such urine is termed "sugar-free" because the routine methods for the qualitative detection of sugar are not sufficiently sensitive to indicate its presence in this concentration. That the concentration of glucose in normal urine is far below that occurring in other body fluids is not because the membranes of the kidney are less permeable to sugar. The kidney glomerulus actually passes a filtrate containing glucose in the same concentration as is present in blood plasma (102). But this filtrate is then subject to the action of the cells of the kidney tubules, which reabsorbs most of the sugar (73). The process by which the kidney tubules reabsorbs glucose depends upon phosphorylating mechanisms (88). Inhibition of the latter by the glucoside phlorhizin prevents the reabsorption of the sugar and results in so-called phlorhizin diabetes (71).

When the body is at rest, the lactic acid content of the blood ranges between 10 mg and 20 mg percent. The lactic acid content of the other tissues is in equilibrium with that of the blood plasma, since lactic acid is freely diffusible across cell membranes (8). In most tissues of the body, lactic acid is not a necessary intermediate of carbohydrate metabolism. It is formed by the reduction of pyruvic acid only when the oxidative removal of pyruvate is relatively or

absolutely deficient.

It has been well established and confirmed many times, that in a fasting animal, the liver is virtually the sole source of blood sugar (92, 93). Some evidence has been presented that indicates that the kidney can contribute sugar to the blood, but in insignificant amounts when compared to the total carbohydrate requirements of the normal intact animal (15, 21, 32, 89).

The work of Claude Bernard was the first to indicate the predominate role of the liver in supplying blood sugar and to demonstrate the existence of liver glycogen. His early works stated that no sugar was present in blood going to the liver. Later, however, Bernard showed that blood going to the liver contained glucose (104) but not as much as that which was leaving the liver. It remained for Soskin (92) to demonstrate that in hepatectomized dog that usual hyperglycemic agents, epinephrine, ether, and asphyxia, no longer had influence upon blood sugar.

Cori (26) has described what is referred to as the lactic acid cycle. This cycle occurs under certain conditions. Significant amounts of lactic acid can readily diffuse from skeletal muscle into the blood stream. This diffused lactic acid may be carried to the liver and converted into liver glycogen, and thus eventually reappear as blood sugar.

In 1957, two separate groups of experimentors reported the

discovery of adenosine-3', 5'-phosphate, (cyclic 3, 5-AMP) (22, 86). Rall and Sutherland (87) described the accumulation of cyclic 3, 5-AMP in hepatic tissue after the introduction of epinephrine. Rall and Sutherland (87) concluded, from their work with liver particles and epinephrine, that epinephrine helped activate an enzyme cyclase. They showed that the cyclase converted ATP to cyclic 3, 5-AMP and this process was expediated in the presence of epinephrine. The cyclizing enzyme has been found to be present in nearly all animal tissues and is apparently activated in all places by epinephrine and related adrenergic amines. The relative potencies to produce an increase in 3, 5-AMP levels reported by Murad and coworkers (70) of certain sympathomimetic amines are: isoproterenol 20, epinephrine 5, and norepinephrine 1. Sutherland and Rall have established that skeletal muscle will produce cyclic 3, 5-AMP in the presence of epinephrine (95).

The formation of cyclic 3, 5-AMP in cardiac muscle preparations has drawn attention from researchers to attempt to associate or disassociate the inotropic action of certain catecholamines upon the heart with elevation of cyclic 3, 5-AMP levels. There are considerable data on production of elevated, cardiac levels of 3, 5-AMP produced by introduction of catecholamines (86, 87). Murad and coworkers (69) reported that isoproterenol was the most potent amine, with regard to elevation of cardiac 3, 5-AMP, while epinephrine and

norepinephrine were equal, and amphetamine was inactive. This order of potency in the heart was considerably different from the order found in liver, where epinephrine was much more active than norepinephrine.

Sutherland and Rall have suggested that the role in increased glycogen breakdown of certain adrenergic amines can be related to the ability of these amines to enhance the formation of cyclic 3, 5-AMP and that this nucleotide increases the conversion rate of phosphorylase b to the active phosphorylase a (95). The activated phosphorylase is then present in a higher than usual concentration and glycogen is more rapidly depolymerized. The exact role of cyclic 3, 5-AMP on enhanced inotropic action of the heart has not positively been shown.

Posternak and co-investigators (84) reported in 1962 that hyperglycemia could be produced in intact dogs, by injection of cyclic 3, 5-AMP. A hyperglycemic response to the injection of the cyclic nucleotide has also been shown in rats by Northrop and Parks (79). These workers also reported, in 1964, that an isolated rat liver perfused with blood containing cyclic 3, 5-AMP will produce hyperglycemia, glycogenolysis, and phosphorylase activation (80).

It has been known for many years that certain responses induced by adrenergic amines can be inhibited. These inhibitors (blocking agents) have proved to be valuable tools for investigations

of the nature of adrenergic receptors.

The studies on agents which are classified as adrenergic blockers are many and conflicting. Dale (31) first showed that ergot could block certain adrenergically induced phenomena, but not all. Since then many different adrenergic blocking agents have been evaluated, with some eventually being used clinically. A review of the literature concerning adrenergic blocking agents immediately gives rise to one obvious fact that no one adrenergic blocker can simultaneously inhibit all the responses of epinephrine, norepinephrine or other adrenergic amines.

The effects of the usual blocking agents are traditionally ascribed to the particular receptor antagonized (alpha or beta). Some physiological responses induced by sympathomimetic amines are antagonized only by the employment of both alpha and beta blocking agents (55). There are many unexplained results concerning the lack of a beta block with a beta antagonist, or the ability of a beta blocking agent to antagonize an alpha adrenergic amine (60). For example, Bennett and coworkers (14) reported that a beta antagonist could not block contraction of the dilator muscle of the iris produced by isoproterenol, a potent beta adrenergic amine. However, phenoxybenzamine, a potent alpha blocker, inhibited the action of isoproterenol.

Nickerson (78) has attempted to classify the adrenergic

blockers. The first class is called competitive antagonists. These antagonists interfere with the reaction of the antagonist with the receptor. Competitive antagonists are in a mass action equilibrium with the receptor and the inhibition produced is a measure of the competition between the agonist and the antagonist for the specific receptor. Found in this class of adrenergic blockers are the ergot alkaloids, imidozolines, benzodioxan, yohimbine, and some synthetic compounds of the isoquinoline group. The second type of blocking agent is called non-equilibrium antagonists. This group is not in a mass action equilibrium with the receptors, and should be considered to be nearly irreversible. These blockers form stable complexes with the receptor and are not overcome by large doses of an agonist. The adrenergic blockers which are considered to be non-equilibrium antagonists are dibenamine and phenoxybenzamine and other beta-haloalkylamines (78). It has been postulated that this poorly reversible antagonism occurs in two separate steps (75, 76). The first is a reversible adsorption of the antagonist onto the receptor. This is followed by a stable chemical reaction of the antagonist with the receptor.

There is a disagreement as to the exact nature of the blockade evoked by the beta-haloalkylamines. Brodie and coworkers (17) showed that phenoxybenzamine, when given intravenously in dogs, was partially metabolized rapidly. They also reported that part of

the blocker was absorbed into fat depots, and slowly diffused into the blood stream. This group of workers suggested that it was unnecessary to assume that phenoxybenzamine or related blockers act irreversibly with cell receptors. Axelrod and coworkers (7) concluded that dibenzamine did not produce an irreversible block and that there was a close correlation between adrenergic blocking activity and the antagonists concentration in fat depots. However, Nickerson (77) using isolated rabbit aorta, which had all existing adipose tissue removed, reported that the duration of phenoxybenzamine was sustained even without fat depots present. He concluded from these results that phenoxybenzamine's long duration could be ascribed to a chemical bond between the receptor and the blocker. He also showed that in intact rats, prior administration of thiosulfate, which chemically inactivates phenoxybenzamine, prevented an adrenergic blockade by phenoxybenzamine. However, if the rats received phenoxybenzamine prior to the administration of thiosulfate, the adrenergic blockade was not altered. Therefore, he concluded from these results that the long duration of action of phenoxybenzamine could be ascribed to a chemical bond formation between the blocker and the receptor, and that the duration was not due to the deposition of the beta-haloalkylamine in fat tissue and its subsequent slow release. Agarival and coworkers (3) showed in cross circulated cats, that phenoxybenzamine's long duration of action

would be better explained with the hypothesis that it formed a chemical bond with receptors, than by assuming it to be slowly released from fat depots.

Nickerson (78) has also suggested a third class of adrenergic antagonists. These are considered to be noncompetitive antagonists. It is proposed that this type of antagonist interferes with the response of the agonist by interrupting at some point between the receptor and the ultimate response. Nickerson places certain antihistamines into this class of blockers.

Komrad and coworkers (49) have described certain antihistamines with the ability to block epinephrine induced hyperglycemia in rabbits. However, the accompanying hyperlactic acidemia was not inhibited by the antihistamines. From this data, the authors concluded that the antihistamines could preferentially inhibit hepatic glycogenolysis. The non-competitive antagonism seen with antihistamines in rabbits does not occur in dogs. In fact, antihistamines often potentiate epinephrine induced hyperglycemia in dogs and exhibit little effect upon lactic acidemia.

The ability of different adrenergic blocking agents to alter the cardiovascular responses of adrenergic amines is controversial. The strain-gauge arch has been of tremendous value in deciphering effects of adrenergic blockers and amines upon the inotropic action of the heart. A number of workers (28) employed the strain-gauge

arch (29) to evaluate the ability of a number of classical adrenergic blockers to inhibit the positive inotropic response of epinephrine, norepinephrine, and other adrenergic amines. These workers, using relatively large doses (up to 32 mg/Kg) of the blockers, concluded that the positive inotropic responses to the challenging adrenergic amines were inhibited. This conclusion was in direct contrast to the studies reported earlier by Acheson (2) and Hunt (46). Acheson (2) had reported that, in a heart-lung preparation, dibenamine lacked the ability to inhibit the positive inotropic action of the heart. Hunt (46), working in vitro preparations of isolated papillary muscles, also pointed out that dibenzamine could not alter epinephrine's positive inotropic action on the muscles. Neither Hunt nor Acheson reported a negative inotropic effect on the heart caused by the blocker by itself.

It is of considerable interest to review data obtained by Cotten (30). He attempted to resolve the conflicting reports on whether or not alpha adrenergic blocking agents had the capacity to inhibit or decrease the positive inotropic and chronotropic responses of adrenergic agents. Cotten showed that alpha adrenergic blocking agents could increase both heart rate and cardiac contractile force in open chest dogs when the blockers were used alone. However, if the blockers were used alone in isolated heart preparations the drugs produced a cardiac depression. Millar (164) had already

described measuring an elevation of catecholamines in plasma after introduction of adrenergic blockers. Brown and coworkers (18) suggested that if one assumes that these adrenergic blocking agents have only an affinity for alpha receptors, then the circulating amine levels would be naturally elevated and could then act upon the heart, which is presumed to be beta orientated. Yet, with in vitro preparations, since there are no natural circulating amines present, the observed depression is produced by the adrenergic blocker's own capability to induce myocardial depression. This depression is most likely unrelated to the blocking ability of the antagonists. This postulation could also explain the observation that stimulation of an isolated rabbit atriasympathetic nerve preparation produced an even greater increase in cardiac rate after the introduction of phenoxybenzamine than before (45).

Nickerson and Chan (78), in a re-evaluation of these apparent conflicting reports, employed several different alpha adrenergic blockers, among which were Hydergine, dibenamine, and phenoxybenzamine. They concluded that no apparent inhibition of either chronotropic or inotropic responses to epinephrine was produced by any of the blocking agents.

The discovery of Powell and Slater (85), that 1-(3', 4' dichlorophenyl)-2-isopropylamine-ethanol (dichloroisoproterenol) selectively blocks some of the inhibitory responses to epinephrine and

isoproterenol, has provided a valuable tool for the investigation of the adrenergic receptor mechanism. These authors found that the depressor action of isoproterenol and the secondary depressor action of epinephrine were inhibited by the dichloro analog. In addition, the inhibitory effect of epinephrine on isolated rabbit intestine was blocked. Accordingly, they proposed that this dichloro analog was combining with certain adrenergic inhibitory receptor sites (beta) without itself producing much physiological effect, and yet competing for these sites with physiologically active agents.

Moran and Perkins (68) reported that this compound selectively blocks the positive cardiac chronotropic and inotropic effects produced adrenergic agents. They also stated that the dichloro analogs of epinephrine and arterenol have similar blocking actions, but are less potent.

These workers showed that the dichloroisoproterenol had definite physiological properties of its own when given in divided doses, in atropinized animals. The actions of dichloroisoproterenol appear not too dissimilar from isoproterenol. Small doses increased the cardiac rate and contractile force and concurrently lowered blood pressure. Subsequent doses decreased the contractile force. From this work these investigators concluded that the adrenergic receptors in mammalian heart are similar, if not identical, with the inhibitory adrenergic receptors found elsewhere.

It is seen in the previous discussion that blockade of the cardiovascular effects of adrenergic amines is in disagreement. Similarly, the inhibition of adrenergically induced glycogenolysis, by adrenergic blocking agents, is also controversial.

The reports on the ability of adrenergic blocking agents to inhibit adrenergically induced hyperglycemia are many and often conflicting. The observation that blocking agents themselves may alter blood sugar is also reported but conflicting.

As far removed as 1912 it had been shown that the ergot alkaloids prevented epinephrine induced hyperglycemia. It has been conceded that the ergot alkaloids are potentially the most potent blockers of epinephrine induced hyperglycemia (74). The inhibiting properties of the dihydro derivatives of the ergot alkaloids have been shown by Freis and substantiated by others (39, 41, 54, 62). The dihydro ergot alkaloids are known to be impotent blockers of epinephrine's induced positive inotropic and chronotropic action upon caridac muscle (74).

The role of haloalkylamines as adrenergic blockers of adrenergically induced hyperglycemia is, at best, undecided. Komrand and coworkers evaluated the ability of a number of closely related haloalkylamines to prevent epinephrine induced hyperglycemia in rabbits (50). They concluded that a characteristic property of several of the proposed blockers was the ability to diminish

epinephrine hyperglycemia at least in some species. It is important to note that these blockers were used in doses somewhat larger than is required to produce an epinephrine reversal of blood pressure responses in dogs.

Species variation to responses of adrenergic agents was described by Harvey and Nickerson (43). These authors determined the degree of inhibition of epinephrine induced hyperglycemia produced by a series of beta-haloalkylamines, among which were dibenamine and phenoxybenzamine. Their data show that the supression of epinephrine induced hyperglycemia was more easily accomplished in rabbits than in cats. The same work produced data which showed that phenoxybenzamine could suppress epinephrine induced glycogenolysis in vitro at a dose level which was also shown to be effective in vivo. They reported that phenoxybenzamine, by itself, slightly decreased blood glucose in both rabbits and cats. However, they failed to determine the intrinsic effect of the blocker on glycogenolysis in the in vitro preparations.

With the discovery of dichloroisoproterenol, a new tool was introduced that could afford a study of the beta receptors and its role in adrenergically induced glycolysis. It has been used successfully to inhibit epinephrine induced hyperglycemia in rabbit, dog, cat, and rat. It, however, cannot prevent epinephrine's glycemic action in the mouse (61). The work of Claassen and Noach

with rats is of considerable interest (20). They showed that dichloroisoproterenol had intrinsic hyperglycemic properties. They also showed that although epinephrine and isoproterenol induced hyperglycemias were inhibited by dichloroisoproterenol, the glycemic action of norepinephrine was unaffected by the beta blocking agents.

STATEMENT OF PROBLEM

The importance of adrenergic amines in both medicine and research has stimulated a large quantity of work concerned with these amines and their respective receptors.

Although adrenergic receptors occur in many tissues, the present work is only concerned with the characterization of the nature of those receptors which are responsible for an adrenergically induced increase in cardiac contractility, a vasodepressor response, and glycogenolysis. The present concept is that all three of these pharmacological responses to adrenergic amines are mediated through the same receptor which differs only in anatomical location. These particular receptors are shown for study because this concept appears to be an over-simplification of complex mechanisms.

To characterize the nature of these receptors, three classical adrenergic blocking agents, phenoxybenzamine, Hydergine, and dichloroisoproterenol are employed in conjunction with four adrenergic amines, phenylephrine, norepinephrine, epinephrine, and isoproterenol. A skeletal muscle relaxing agent, d-tubocurarine, is also employed with the assumption that such an agent could separate adrenergically induced glycogenolysis in liver from glycogenolysis in skeletal muscle. Experiments are designed to measure the effect of the adrenergic amines on cardiac contractility and blood pressure

before and after the blocking agents. In addition, the effect of the amines on glycogenolysis in intact dogs before and after the blocking agent is also determined. Isolated tissues obtained from liver and skeletal muscle are utilized to determine if a separation of glycogenolysis is produced with d-tubocurarine. Finally, the hyperglycemic effect of phenoxybenzamine by itself, and also the controversial effect of phenoxybenzamine on the hyperglycemia induced by epinephrine, are re-examined.

It is hoped that data obtained concerning the interactions of amines and blocking agents will further delineate and also add insight to existing concepts of adrenergic receptors.

GENERAL PROCEDURES

Healthy mongrel dogs of either sex, weighing between eight and 19 kilograms, were employed in this study. All dogs were starved for eight to 12 hours before use. Penobarbital sodium, 35 mg/Kg was injected intraperitoneally to anesthetize the experimental animals. Without exception, the dogs were artificially ventilated with a Harvard Respirometer via a tracheal cannula. The rate of respiration was set at ten cycles per minute, respiratory volume per cycle was 25 cubic centimeters per kilogram of body weight.

In a number of animals, blood pressure and right ventricular cardiac contractile force were measured concurrently. Blood pressure was measured by means of a heparin-containing polyethylene catheter which was inserted approximately seven to nine centimeters into the right cartid artery. The catheter was connected to a Model 266B Sanborn pressure transducer. Cardiac contractile force was measured by a Walton-Brody strane gauge arch (103). The heart was exposed through a right thoracotomy, the pericardium was cut away, and the strain gauge was sutured directly onto the right ventricle wall. The sutures were placed well into the muscle with a non-cutting needle. The feet of the strain gauge were ligated firmly to the muscle with number eight cotton thread. The muscle segment under the arch was stretched to about 50 percent of its normal

diastolic length to produce suitable contractions (103). The blood pressure and contractile force transducers were connected to a Sanborn 150 polygraph and recordings made on heat sensitive paper.

A number of dogs were adrenalectomized by employing a standard technique described by Markowitz (59). Occasionally an animal undergoing adrenalectomy became hyperglycemic (probably due to a release of epinephrine from the manipulated adrenal gland); such animals displaying a hyperglycemic response were discarded. Several dogs were sacrificed to obtain tissue for in vitro experiments. Approximately 50 grams were excised, from liver and skeletal muscle, from the left central lobe of the liver and right semimembranous muscle, respectively. These tissues were utilized to determine if isoproterenol could preferentially increase glucose uptake and lactic acid production by either or both tissues. Two antagonists, dichloroisoproterenol and d-tubocurarine, were employed in an attempt to preferentially inhibit isoproterenol's metabolic action in either liver tissue or skeletal muscle tissue. The intrinsic metabolic actions of these blockers, by themselves, on both types of tissues were also measured and evaluated.

The adrenergic amines were administered to the dogs by two methods. In the first method, they were rapidly injected into the right external jugular vein which had been cannulated with a polyethylene catheter. Following each injection of an amine the catheter

was flushed with two to three milliliters of normal saline. In the second method, the adrenergic amines were slowly infused into the left radial vein. A Harvard infusion apparatus was employed to maintain a constant rate of infusion.

The doses of the adrenergic amines, expressed as the free base, were comparable to those commonly reported in literature (55, 62). By the rapid injection method the doses of the drugs employed were as follows: l-phenylephrine 20 mcg/Kg, and l-norepinephrine, dl-isoproterenol, all 1.5 mcg/Kg. When these amines were infused for 60 minutes, the doses were: l-phenylephrine 20 mcg/Kg/minute, and l-norepinephrine, l-epinephrine, dl-isoproterenol, all 1.5 mcg/Kg/minute. All of the amines used were made up in stock solutions and preserved by addition of 0.1 percent sodium bisulfite and 0.1 percent chlorbutanol. These stock solutions were subsequently diluted with normal saline on the day of use. Preliminary work showed that addition of the preservative substances did not alter any of the measured cardiovascular or metabolic parameters.

The doses of the blocking agents employed were: phenoxybenzamine HCl, 8 mg/Kg; dichloroisoproterenol HCl, 8 mg/Kg; a combination of phenoxybenzamine HCl, 8 mg/Kg and dichloroisoproterenol HCl, 8 mg/Kg; Hydergine 4 mg/Kg; d-tubocurarine chloride 0.2 mg/Kg. The doses of these blocking agents, expressed as the salt, were comparable to those generally reported in literature

(48, 61, 91). The phenoxybenzamine, after dilution with normal saline, was slowly injected over a 15 minute period. The combination of phenoxybenzamine and dichloroisoproterenol was also injected over a 15 minute period. Hydergine was given over a ten minute period. No additional blocking agent was given after the initial dose. However, in the case of d-tubocurarine, subsequent doses of two-tenths the initial dose were given every 20 minutes during the course of an experiment because of the relatively short biological activity of d-tubocurarine (16).

INFLUENCE OF ADRENERGIC AMINES ON CARDIOVASCULAR RESPONSES BEFORE AND AFTER BLOCKING AGENTS

Introduction

There is considerable disagreement concerning the receptors responsible for the blood pressure and cardiac contractile force responses evoked by adrenergic amines. Ahlquist has placed all adrenergic receptors into two separate classes, alpha and beta (4). According to Ahlquist's classification, amines which produce vasoconstriction excite only alpha receptors. Adrenergic amines which produce vasodilation excite only beta receptors and those amines which increase cardiac contractile force also excite the beta receptors in the heart. These beta receptors in the vessels and in the heart are considered to be identical, and differ only in anatomical location. To substantiate his classification Ahlquist showed that adrenergic amines which induced peripheral vasodilation also excited the heart, and that norepinephrine which is a potent vasoconstricting agent, does not increase cardiac contractile force.

Lands, however, took issue with Ahlquist's classification of adrenergic receptors. Lands presented evidence that nearly all adrenergic amines could increase cardiac contractility. It made no difference whether these amines were also capable of producing

vascular dilation (51), such as, norepinephrine and phenylephrine which produce only pressor responses, yet both drugs increase cardiac contractile force. He concluded that the receptors in the heart are neither alpha nor beta but appear to be undifferentiated and were stimulated by substances with a strong affinity for either receptor, alpha or beta.

Experiments were designed in the present section to determine the nature of the cardiac receptor responsible for adrenergically induced increases in cardiac contractile force.

Methods

Four adrenergic amines, phenylephrine, norepinephrine, epinephrine, and isoproterenol, were rapidly injected into the jugular vein and blood pressure and cardiac contractile force responses were simultaneously recorded as described in GENERAL PROCEDURES. Twenty-five dogs were employed in this portion of the work. The blood pressure responses were expressed as average blood pressure in millimeters of mercury and the effect of the drugs expressed as average changes from normal blood pressure. Positive inotropic effects were expressed as percent change from normal contractile force. The alterations in these two parameters produced by the amines were recorded and compared to the alterations induced by the amines after the administration of blocking agents.

The blood pressure and the contractile force of the heart were always allowed to return to the control value before another injection of drug. Usually 15 minutes elapsed between successive injections of amines. After the introduction of a blocking agent, 60 minutes were allowed to permit full blocking effect before readministering the amines. However, because of the rapid detoxification of d-tubocurarine, only 15 minutes were allowed to elapse before the amines were injected. The pre- and post-blockade responses to the amines were tabulated and statistically analyzed. An analysis of variance and least significant difference of means were determined by the procedure described in the APPENDIX, p. 93 and 94.

Results

The normal blood pressure responses to phenylephrine, norepinephrine, epinephrine, and isoproterenol appear in the far left column of TABLE I. The pressor response, at the doses employed, of phenylephrine or norepinephrine was essentially the same, 76.4 and 65.8 millimeters of mercury above the control blood pressure, respectively. The average pressor response of epinephrine at the dose level employed was considerably lower. The final drug in the table, isoproterenol, displayed its characteristic depressor response, a fall of blood pressure of 35 millimeters of mercury. Phenoxybenzamine and Hydergine significantly inhibited the

TABLE I. EFFECT OF ADRENERGIC DRUGS ON THE BLOOD PRESSURE OF DOGS.

Amine	Changes in blood pressure, in mm Hg, produced by amines after:					
	no treatment	PBZ[a]	DCI[b]	PBZ-DCI combination	Hydergine	d-tubocurarine
phenylephrine	76.4	2.0	99.0	6.3	8.8	84.2
norepinephrine	65.8	18.8	57.6	22.7	36.2	62.5
epinephrine	37.6	-29.0	61.0	13.6	-26.6	42.2
isoproterenol	-34.7	-37.5	-6.5	-7.3	-37.5	-31.9

Least significant difference, at five percent level, for treatment means is, 21.2.

a = PBZ-phenoxybenzamine

b = DCI-dichloroisoproterenol

pressor responses of phenylephrine, norepinephrine, and epinephrine, but did not effect the depressor response of isoproterenol. The depressor response seen with epinephrine after phenoxybenzamine and Hydergine is the classic response reported in the literature, commonly referred to as the epinephrine reversal. Dichloroisoproterenol blocked the depressor response of isoproterenol. It failed to reduce the pressor responses of phenylephrine, norepinephrine, and epinephrine. It did, however, augment the usual pressor responses of phenylephrine and epinephrine. It is also shown in the right hand column of TABLE I, that d-tubocurarine, in the dose used, did not alter the blood pressure response of any of the amines. In fact, the control values were duplicated after d-tubocurarine an indication of the reproducibility of the experimental method. When phenoxybenzamine and dichloroisoproterenol were used in combination, the blood pressure responses, of all four amines, were essentially blocked.

In the far left column in TABLE II are shown the normal effects of phenylephrine, norepinephrine, epinephrine and isoproterenol on cardiac contractile force. All amines significantly elevated contractile force. The values shown are expressed as percent increases in contractile force above the control obtained before the administration of any drugs. Phenylephrine elevated the contractile force 22 percent, and norepinephrine and epinephrine increased it 90 percent

TABLE II. EFFECT OF ADRENERGIC DRUGS ON THE CARDIAC CONTRACTILE FORCE OF DOGS.

Amine	Percent changes in cardiac contractile force produced by amines after:					
	no treatment	PBZ[a]	DCI[b]	PBZ-DCI combination	Hydergine	d-tubocurarine
phenylephrine	21.7	26.4	4.3	0.0	5.6	25.5
norepinephrine	90.5	100.0	8.2	2.2	65.0	89.0
epinephrine	80.1	74.0	7.0	1.3	76.0	79.0
isoproterenol	147.7	140.0	9.8	10.6	142.0	142.0

Least significant difference, at five percent level, for treatment means is, 12.6.

a = PBZ-phenoxybenzamine

b = DCI-dichloroisoproterenol

and 80 percent, respectively. Isoproterenol was even more effective as shown by its ability to increase contractile force by 148 percent.

The inotropic effect of the amines was not altered by d-tubocurarine. The abilities of these amines to increase cardiac contractile force were the same before and after d-tubocurarine and demonstrate the reproducibility of the responses. Phenoxybenzamine, a potent alpha adrenergic blocker, also displayed no ability to interfere with these catecholamines' action upon the heart. Hydergine significantly reduced the inotropic responses of norepinephrine and completely blocked that of phenylephrine. However, Hydergine possessed no ability to interfere with the increases in contractile force produced by epinephrine and isoproterenol.

A potent inhibition of all the amines' abilities to excite cardiac muscle was produced by dichloroisoproterenol. The combination of blocking agents, dichloroisoproterenol and phenoxybenzamine, also inhibited the increases in cardiac contractile force produced by the amines. However, there was no significant difference between the blockade produced by this combination and the blockade produced with dichloroisoproterenol, alone.

It is significant to note at this point that the blocking agents themselves had no significant effect on cardiac contractile force or blood pressure at the dose levels employed. In all cases, after

the 60 minute waiting period, following the introduction of the respective blocking agent, both of these physiological parameters were at control levels. It was noted, however, that dichloroisoproterenol did increase cardiac contractile force significantly within 15 minutes after its introduction. This increase subsided to the pre-blockade control value by the time the waiting period was over. The latter observation was also reported by Moran and Perkins (68).

Discussion

It is generally assumed that epinephrine and isoproterenol produce a depressor response due to vascular beta receptor excitation. It has been reported that phenylephrin and norepinephrin have little or no ability to excite these beta receptors (20, 55). It would be expected that if either phenylephrine or norepinephrine had the ability to excite beta receptors, phenoxybenzamine or Hydergine would have evoked a reversal in the blood pressure response in a manner analogous to epinephrine. TABLE I shows that such a reversal was not produced even though the pressor responses to phenylephrine and norepinephrine were blocked by phenoxybenzamine or Hydergine. These results corroborate the conclusion that neither amine intrinsically excited beta vascular receptors. Furthermore, dichloroisoproterenol, a known beta blocking agent, had no blocking action upon phenylephrine and norepinephrine blood

pressure responses. This blocker inhibited the blood pressure response only of isoproterenol, an agent that excites beta receptors. TABLE I shows that in the presence of dichloroisoproterenol, the pressor responses of phenylephrine and epinephrine appear to be augmented. No experiment was designed to explain this potentiation. However, it would be exceedingly interesting to determine if isolated smooth muscles would also show this augmentation. It can be hypothesized that this augmentation occurs between normal circulating catecholamines no longer attach to vascular beta receptors since these receptors are blocked by dichloroisoproterenol. Consequently, endogenous catecholamines, along with the exogenously injected ones will all be directed towards alpha vascular receptors and thus more peripheral vasoconstriction will occur.

Ahlquist suggests that adrenergically induced increases in cardiac contractile force occur because of excitation of beta receptors in the heart and that these receptors are identical with the vascular beta receptors (4). Epinephrine and isoproterenol, according to Ahlquist and Levy, increase cardiac contractile force because of their ability to excite cardiac beta receptors (4, 56). They report that norepinephrine and phenylephrine do not excite beta receptors and also do not increase cardiac contractile force (4, 55, 56). TABLE II shows that both epinephrine and isoproterenol were potent inotropic agents. However, TABLE II shows that

phenylephrine and norepinephrine also significantly increase cardiac contractility. These results are contrary to those of Ahlquist and Levy and raise the question: Do these amines have the ability to stimulate cardiac beta receptors, even though they do not excite vascular beta receptors? When dichloroisoproterenol, a known beta blocker, was used, the cardiac responses of all four amines were blocked, TABLE II. This result, alone would imply that the cardiac responses observed with phenylephrine and norepinephrine were mediated through cardiac beta receptor stimulation. However, TABLE II also shows that Hydergine, a classical alpha adrenergic blocker, produced a significant blockade of the increases in cardiac contractile force produced by phenylephrine and norepinephrine. Yet, when phenoxybenzamine, another known alpha blocking agent, was employed, no blockade of contractility occurred with any of the four amines. The observation that Hydergine inhibits the cardiac responses of phenylephrine and norepinephrine, while phenoxybenzamine could not, posed another question: Does Hydergine have weak beta blocking properties?

The present standard test for beta blocking activity was designed by Levy and Ahlquist (55, 56). This test, which uses ethylnorepinephrine as a test substance, was used in our laboratory to measure the beta blocking properties of Hydergine. Ethylnorepinephrine is an amine which, when rapidly injected (50 mg/Kg), will

display a pressor response followed by a depressor response. The pressor response is produced by excitation of vascular alpha receptors. The depressor response occurs because of excitation of beta vascular receptors. Consequently, any agent that has beta blocking activity will either reduce or block the normal depressor response of ethylnorepinephrine, leaving only the pressor response. Experiments in our laboratory showed that after Hydergine, the pressor response of ethylnorepinephrine was blocked, but the depressor response was the same as the control depressor response (-32.0±4.2 millimeters of mercury, control; -30.8±2.8 millimeters of mercury, after Hydergine). These results show that Hydergine has no beta blocking activity when evaluated by the method of Levy and Ahlquist. Thus we can conclude that the Hydergine blockade of the increases in contractile force produced by phenylephrine and norepinephrine is not due to blockade of beta receptors in the heart.

It appears that cardiac contractile force can be increased by amines that do not excite beta receptors, and that this excitation can be prevented by a blocking agent which does not have beta receptor blocking properties. These results do not fit Ahlquist's stringent requirement that adrenergically induced increases in cardiac contractility, has properties resembling both alpha and beta receptors (Ahlquist's terminology). Lands has previously proposed that the cardiac receptor is undifferentiated (51). We find our data to

be compatible with this proposal. Finally, it appears unlikely that the vascular beta receptor and the cardiac receptor are the same, because phenylephrine or norepinephrine did not evoke a depressor response under any condition.

INFLUENCE OF ADRENERGIC AMINES ON SERUM LEVELS OF GLUCOSE, LACTIC ACID AND POTASSIUM BEFORE AND AFTER BLOCKING AGENTS

Introduction

Injections of catecholamines cause an increase in serum potassium. It is fairly well conceded that the elevation of serum potassium results from a release of potassium from the liver (34, 81). Many other organs, e. g. digestive tract, spleen, kidney, thyroid, pancreas, and adrenals, have been excluded as important sources of potassium serum elevation after epinephrine. When the liver was eliminated from circulation, epinephrine produced only a small increase in serum potassium (53). However, it has also been noted that this hyperkalemic response is transitory and in fact the serum potassium usually returns to normal or sub-normal levels even during epinephrine infusion (33, 90). The peak time for measuring epinephrine induced hyperkalemia is within one or two minutes after the introduction of epinephrine because the potassium concentration begins to fall in four to five minutes (33).

The mechanisms by which adrenergic amines produced a release of potassium from liver, and subsequent hyperkalemia, are not known. Ellis has shown that dibenamine and phenoxybenzamine, alpha adrenergic blocking agents, were capable of blocking

epinephrine's hyperkalemic responses without altering epinephrine's hyperglycemic response (37). Such results suggest that adrenergically induced hyperkalemia is not dependent upon liver glycogenolysis.

The hyperglycemic and hyperlactic acidemic responses produced by catecholamine injections have been relegated predominantly to liver and skeletal muscle glycogenolysis (23, 24, 99). Lactic acid from muscle was shown to be largely responsible for hepatic gluconeogenesis through the lactic acid cycle (26, 27). There are many reports regarding the increase of lactic acid and blood sugar produced by norepinephrine, epinephrine, isoproterenol and other adrenergic amines (6, 9, 25, 65, 66, 67). However, reports as to blockade of these effects have been conflicting. The inhibition of these responses has varied from being relatively effective to relatively ineffective (20, 62, 63, 74), with no reports of a complete block. There are also reports of the blocking agents, alone, elevating blood sugar and lactic acid levels (20, 61, 63).

There is considerable confusion over which particular adrenergic receptor is responsible for glycogenolysis. Much of this confusion arises because many workers tend to stringently adhere to the dual receptor concept of Ahlquist which categorizes all adrenergic receptors into two types of receptors, alpha and beta.

Van der Pol, in 1956, attempted to re-evaluate Ahlquist's dualistic receptor theory. He determined the glycemic responses

of norepinephrine, epinephrine and isoproterenol, in rabbits (96, 97, 98). Van der Pol showed that although epinephrine was the most potent glycemic agent, norepinephrine and isoproterenol, alpha and beta stimulators, respectively, also could elevate blood glucose a small but significant amount. He surmised that the most potent hyperglycemic agent would be one that had an affinity for both alpha and beta receptors, and that dual stimulation was required for a potent response. This of course would explain epinephrine's powerful hyperglycemic effect since it is considered to be both an alpha and a beta stimulating amine. This could also explain the incomplete blockade of adrenergically induced hyperglycemia by either alpha or beta adrenergic blocking agents. The work of Lei and McCutcheon (54) supports the contention that glycogenolysis is mediated through both receptors. These workers showed that a combination of norepinephrine and isoproterenol could elevate blood sugar and lactic acid more than either alone, but not to the extent of epinephrine. They also showed that a combination of Hydergine and dichloroisoproterenol was effective in inhibiting these two metabolic parameters.

Claassen and Noach (20), working with assumptions set forth by Van der Pol, showed in rats that dichloroisoproterenol would completely inhibit isoproterenol induced hyperglycemia, reduce epinephrine induced hyperglycemia, and leave unaffected the hyperglycemic response of norepinephrine. They concluded that with the

beta receptors blocked, the alpha receptors could continue to function and their subsequent stimulation would produce hyperglycemia. Claassen and Noach concluded, after a review of the work of Vrij (100) suggesting that liver glycogenolysis is mainly an alpha function and muscle glycogenolysis is predominantly a beta function, that hyperglycemia is produced by the interaction of catecholamines with both alpha (liver) and beta (muscle) receptors. Yet Mayer and coworkers (61) indicated that this does not occur in dogs because their data showed that norepinephrine (predominantly alpha) does not produce hyperglycemia after dichloroisoproterenol, a beta blocker. Fairly recently, McCutcheon (62) reported data that indicated that the glycemic response to infusions of epinephrine and isoproterenol in dogs is predominantly a beta function or, as Mayer (61) also suggested, possibly a function of an unknown receptor. McCutcheon (62) also reported a potency ratio of epinephrine being greater than isoproterenol in dogs, while Mayer (61) found the two amines to have an equal hyperglycemic response in the same type of animal. Furchgott concluded, after a review of literature, that glycogenolysis is mediated through receptors different from either alpha or beta (42).

Because of these conflicting reports concerning the receptor(s) responsible for adrenergically induced glycogenolysis, it appeared necessary to compare the abilities of several adrenergic blocking agents and a skeletal muscle blocking agent to inhibit adrenergic

amine induced glycogenolysis in an attempt to better delineate the glycogenolytic receptor. The skeletal muscle blocking agent, d-tubocurarine, was included in the study to determine if paralysis of skeletal muscle would alter any glycemic or lactate responses to adrenergic amines. It was assumed that a separation of liver and skeletal muscle glycolysis might be accomplished with such an agent. In addition, the hyperkalemic response of each of the four adrenergic amines before and after the blocking agents was measured. This was done to determine if the separation of the hyperkalemic responses from the hyperglycemic response of the amines could be produced with all of the blocking agents employed.

Methods

The procedure for preparation of the animals is discussed under GENERAL PROCEDURES. Sixty dogs were employed for this portion of the work. Two milliliter blood samples were removed from the right femoral vein with a heparin coated syringe as suggested by Jarpes (47). Determinations of glucose, lactic acid and potassium levels in blood serum were made. The procedure for determining serum glucose was described by Nelson (72). Barker's technique for determining serum lactic acid was utilized (10). Serum potassium was determined in a Beckman model 121 flame photometer, by removing a blood sample exactly two minutes

after the start of the adrenergic amine infusion. Serum glucose and lactic acid were measured in milligrams percent while serum potassium was measured in m Eq/liter. Control serum samples were obtained prior to the start of the infusions. The amines were then infused for 60 minutes and a blood sample was removed at the end of the infusion. The elevation above control value was recorded for each parameter. After blood sugar and lactic acid returned to normal, which usually required 30 minutes, a blocking agent was introduced as described under GENERAL PROCEDURES. A blood sample was removed at the end of the waiting period (60 minutes) to determine the glycogenolytic effect of the blocker alone. (Preliminary work established that, in all cases, the glycogenolytic effect of the blocking agents was also the same after 120 minutes.) Infusion of the amine was then begun again and a blood sample was removed at the end of the 60 minute infusion. Least significant difference and analysis of variance were determined by the procedure described in the APPENDIX, page 93 and 94.

Results

It was found that one of the blockers, phenoxybenzamine, significantly elevated the normal serum glucose level by 38 milligrams percent. Also, the combination of phenoxybenzamine and dichloroisoproterenol significantly increased the normal blood sugar level

by 32 milligrams percent. Dichloroisoproterenol, Hydergine or d-tubocurarine had no effect on the normal blood sugar level. Normal serum potassium and lactic acid levels were unaffected by any of the blockers or combinations of blockers.

In the far left columns of TABLES III, IV and V are shown the control levels of serum glucose, lactic acid, and serum potassium, respectively, to phenylephrine, norepinephrine, epinephrine and isoproterenol infusions. The glycemic and lactate responses of phenylephrine and norepinephrine were not significant, whereas epinephrine and isoproterenol significantly elevated blood sugar and lactic acid. All the amines significantly elevated the serum potassium level.

It should be noted that the changes in blood sugar produced by these amines after phenoxybenzamine, and the combination of phenoxybenzamine and dichloroisoproterenol, have been corrected. The correction was made by subtracting the amount of hyperglycemia produced by the blocker, alone, from the determined hyperglycemia at the end of the amine infusion after the blocker had been introduced. These corrections were important because it was then possible to ascertain the degree of intrinsic hyperglycemia caused by the adrenergic amines after the blockers had been given. No corrections were required in blood lactic acid or potassium because the blockers alone did not alter these parameters.

TABLE III. EFFECT OF ADRENERGIC DRUGS ON THE SERUM GLUCOSE LEVEL OF DOGS.

Changes in serum glucose levels, in milligrams percent, produced by amines after:						
Amine	no treatment	PBZ[a]	DCI[b]	PBZ-DCI combination	Hydergine	d-tubocurarine
phenylephrine	4.8	7.0[c]	4.6	5.2[d]	3.4	5.1
norepinephrine	10.6	7.2[c]	11.6	5.4[d]	4.4	12.1
epinephrine	94.6	77.0[c]	44.8	31.0[d]	16.4	93.4
isoproterenol	109.6	89.0[c]	50.4	37.4[d]	104.0	80.6

Least significant difference, at five percent level, for treatment means, is 22.6.

a = phenoxybenzamine

b = dichloroisoproterenol

c = values less 38 milligrams percent, the elevation produced by blocker alone.

d = values less 32 milligrams percent, the elevation produced by blockers alone.

TABLE IV. EFFECT OF ADRENERGIC DRUGS ON THE SERUM LACTIC ACID LEVEL OF DOGS.

Changes in serum lactic acid levels, in milligrams percent, produced by amines after:						
Amine	no treatment	PBZ[a]	DCI[b]	PBZ-DCI combination	Hydergine	d-tubocurarine
phenylephrine	2.9	2.7	3.2	3.8	2.4	3.3
norepinephrine	6.7	7.0	2.4	2.4	6.4	7.8
epinephrine	22.8	28.1	12.2	7.0	5.0	19.5
isoproterenol	34.9	45.4	10.8	5.5	35.8	17.4

Least significant difference, at five percent level, for treatment means, is 6.81.

a = phenoxybenzamine

b = dichloroisoproterenol

TABLE V. EFFECT OF ADRENERGIC DRUGS ON THE SERUM POTASSIUM LEVEL OF DOGS.

Changes in serum potassium levels, in milliequivalents per liter, produced by amines after:						
Amine	no treatment	PBZ[a]	DCI[b]	PBZ-DCI combination	Hydergine	d-tubocurarine
phenylephrine	1.54	1.47	1.54	1.60	1.76	1.14
norepinephrine	3.60	2.12	3.14	1.96	3.52	3.58
epinephrine	7.63	2.47	4.00	2.40	2.08	8.06
isoproterenol	4.43	3.20	3.12	2.96	4.50	4.21

Least significant difference, at five percent level, for treatment means, is 0.98.

a = phenoxybenzamine

b = dichloroisoproternol

Phenoxybenzamine failed to inhibit amine induced hyperglycemia. It did, however, inhibit the hyperkalemic responses of norepinephrine, epinephrine, and isoproterenol. Phenoxybenzamine was the only adrenergic blocking agent which separated the hyperkalemia and hyperglycemic responses of the amines. Dichloroisoproterenol, and the combination of dichloroisoproterenol and phenoxybenzamine, significantly reduced hyperglycemia, hyperlactic acidemia and hyperkalemia induced by epinephrine and isoproterenol. Hydergine prevented the hyperglycemia, hyperlactic acidemia and hyperkalemia of only epinephrine. Tubocurarine inhibited the hyperglycemia and hyperlactic acidemis of only isoproterenol, but failed to block hyperkalemic response of any of the adrenergic amines.

Discussion

It was proposed by McCutcheon that adrenergically induced glycogenolysis in the dog is a result of beta receptor stimulation (62). It would follow that phenylephrine and norepinephrine (amines which excite only alpha receptors) would not produce hyperglycemia or hyperlactic acidemia. The results in the present study confirm this, as these two alpha adrenergic amines did not elevate either metabolic parameter. However, the results show that epinephrine and isoproterenol (amines which can excite beta receptors) significantly elevated both blood glucose and lactic acid levels. These

results adhere to McCutcheon's proposal that beta receptor stimulation is responsible for adrenergically induced glycogenolysis in dogs.

The hyperglycemia and hyperlactic acidemia produced by epinephrine and isoproterenol were significantly reduced by dichloroisoproterenol, a known beta blocking agent. Furthermore, this proposal is strengthened by phenoxybenzamine, an alpha blocking agent, which did not alter the hyperglycemia or hyperlactic acidemia induced by either epinephrine or isoproterenol. The combination of an alpha blocking agent, phenoxybenzamine, and a beta blocking agent, dichloroisoproterenol, did not produce a more potent blockade of epinephrine or isoproterenol induced hyperglycemia and hyperlactic acidemia, than did dichloroisoproterenol by itself. These results add further support to the proposal that the beta receptor is responsible for adrenergically induced hyperglycemia.

Hydergine however, does not fit into the above mentioned proposal. It is classified as an alpha adrenergic blocking agent. Van der Pol proposed that glycogenolysis occurs because of simultaneous stimulation of both alpha and beta receptors. It would then follow, according to the work of Van der Pol, that if alpha receptors are blocked by Hydergine, thus leaving only beta receptors available for stimulation, epinephrine would no longer produce glycogenolysis. Our results show that Hydergine blocked the hyperglycemia and hyperlactic acidemia induced by epinephrine (an amine which excites

alpha and beta receptors). Yet Hydergine did not alter the hyperglycemic and hyperlactic acidemic responses of isoproterenol. These results complement Van der Pol's work. However, phenoxybenzamine, a potent alpha blocking agent, did not significantly reduce the hyperglycemia or hyperlactic acidemia induced by epinephrine. These results did not follow the reasoning put forth by Van der Pol because his work suggests that if alpha receptors are not available for simultaneous stimulation with the beta receptor, epinephrine should not have produced hyperglycemia.

The skeletal muscle blocking agent, d-tubocurarine, significantly reduced the hyperglycemia and hyperlactic acidemia induced by isoproterenol. However, it did not alter the effect of epinephrine on these metabolic parameters. This observed blockade of beta adrenergic induced glycogenolysis occurred without altering any of the cardiovascular responses of isoproterenol (see Section IV). These data do not indicate whether this blockade is occurring in skeletal muscle, liver, or both. The locus of this blockade is discussed in Section VI.

Phenylephrine and norepinephrine did not significantly elevate serum glucose or lactic acid. Therefore, with these amines, an attempt at separation of glycogenolysis and hyperkalemia by blocking agents was pointless. However, since epinephrine and isoproterenol were potent glycogenolytic and hyperkalemic agents, determining the

ability of blocking agents to separate these responses was of value. The results showed that any blocking agent that inhibited glycogenolysis also reduced the hyperkalemic response to the amines. The results also showed that although phenoxybenzamine did not block glycogenolysis induced by either amine, it did inhibit the hyperkalemic responses of the amines, an apparent separation of adrenergically induced glycogenolysis and hyperkalemia. Phenoxybenzamine is in the same chemical class as dibenamine, a beta-haloalkylamine. Dibenamine and phenoxybenzamine were used by Ellis to produce results similar to those obtained in the present study (37). It appears that the separation of glycogenolysis and hyperkalemia is a property of the beta-haloalkylamines and not a general property of all adrenergic blocking agents.

The results obtained in the present study of metabolic responses produced by adrenergic amines do not completely support the proposal of McCutcheon (beta receptor) and do not support the proposal of Van der Pol (both alpha and beta) or the proposal of Claassen and Noach (either alpha or beta). It appears that adrenergically induced glycogenolysis in the dog occurs by excitation of a receptor that is closely related to the beta receptor, but cannot categorically be identified as either alpha or beta.

INHIBITION OF THE METABOLIC EFFECTS OF ISOPROTERENOL BY DICHLOROISOPROTERENOL AND d-TUBOCURARINE IN ISOLATED SKELETAL MUSCLE AND LIVER TISSUE

Introduction

There are several reports in literature concerning the use of isolated tissues to determine the intrinsic abilities of catecholamines to increase glycogenolysis. The most commonly employed adrenergic amine has understandably been epinephrine. In many cases the decrease in glycogen content in the tissue itself has not been measured. Often a simple determination of glucose reduction in the bathing solution and elevation of lactic acid in the tissue are used as indirect evidence of enhanced glycogenolysis. Quite commonly, the circumstances under which indirect glycogenolysis is measured are anaerobic and at temperatures varying from 0° centigrade to 37.5° centigrade. It is not recommended to leave isolated skeletal muscle, smooth muscle or liver tissues under anaerobic conditions, at temperatures above 25° centigrade, for longer than 45 to 60 minutes if indirect measurements of glycogenolysis are to be utilized because of possible gross damage to cells.

It has been shown in Section V that the hyperglycemia and hyperlactic acidemia produced with a 60 minute infusion of isoproterenol, the strongest beta adrenergic amine employed, could be

significantly inhibited with d-tubocurarine. This metabolic inhibition occurs without any inhibition to the usual cardiovascular responses to rapidly injected isoproterenol. It is also significant that with the dose of d-tubocurarine employed there was no antagonism of the hyperglycemia and hyperlactic acidemia produced by phenylephrine, norepinephrine and epinephrine, all of which are predominantly alpha adrenergic amines.

These results stimulated interest to determine if this apparent metabolic inhibition of isoproterenol by d-tubocurarine occurred in both tissues or predominantly in skeletal muscle or predominantly in liver. In addition, the ability of dichloroisoproterenol to inhibit liver and skeletal muscle glycogenolysis induced by isoproterenol was also evaluated.

Methods

Liver slices from the left central lobe and skeletal muscle slices from the semimembranous muscle were rapidly removed from nine anesthetized dogs. The tissue slices were rinsed continually for 15 minutes in ice-cold 0.01 M phosphate-Ringer's solution, pH 7.4, containing glucose. The concentration of glucose in the solution was determined colorimetrically by the method of Nelson (72). The concentration was usually 130-140 milligrams percent. After washing, the tissues were placed in baths containing the above

mentioned Ringer's solution and incubated at 37° centigrade for 30 minutes. The solutions were not oxygenated. Both types of tissue were subjected to identical treatment with the baths.

A duplicate series of six baths were employed in each experiment. All baths were made up to contain a final volume of 40 milliliters. At the end of the 30 minute test period, determinations of the glucose concentration in the bath and lactic acid concentration in the tissue were determined. The six baths all contained the Ringer's solution. The baths were as follows: (1) control bath; (2) d-tubocurarine bath (0.01 mg/ml); (3) dichloroisoproterenol bath (0.1 mg/ml); (4) isoproterenol bath (1.0 mcg/ml); (5) d-tubocurarine and isoproterenol bath; (6) dichloroisoproterenol and isoproterenol bath. The drugs were always added to the baths just prior to the addition of the tissue. The wet weight of each tissue slice was determined before addition to the bath. The decrease of glucose concentration in the bath was used as an indicator of glucose uptake by the tissue. The method of determining lactic acid in tissue was that described by Walass (101).

The results are expressed in glucose uptake or lactic acid production in milligrams per gram of wet tissue in 30 minutes. Standard error of the means and paired "t" tests were determined for each metabolic parameter on the two types of tissue by the procedure described in APPENDIX, page 93 and 94.

Results

TABLES VI and VII show that the control values for glucose uptake and lactic acid production in skeletal muscle, after 30 minutes incubation, were 4.6 mg/gm and 3.2 mg/gm, respectively. These two tables also show that neither d-tubocurarine nor dichloroisoproterenol by themselves had any significant intrinsic effects on the two metabolic parameters in skeletal muscle. Isoproterenol proved to be a potent agonist of both glucose uptake and lactic acid production; both parameters were doubled. Glucose uptake and lactic acid production were not elevated in skeletal muscle by isoproterenol in baths also containing d-tubocurarine or dichloroisoproterenol.

TABLES VIII and IX show that the control values for glucose uptake and lactic acid production in liver, after 30 minutes incubation, were 13.1 mg/gm and 6.3 mg/gm, respectively. As in skeletal muscle, isoproterenol proved to be a potent agonist which significantly increased glucose uptake and lactic acid production. The agent, d-tubocurarine, had no intrinsic ability to alter either metabolic parameter in liver. However, dichloroisoproterenol, by itself, significantly increased both glucose uptake and lactic acid production in the liver slices, as shown in TABLES VIII and IX. It was therefore necessary to use corrected values when determining the ability of dichloroisoproterenol to antagonize the effects of isoproterenol in

TABLE VI. EFFECT OF ISOPROTERENOL ON SKELETAL MUSCLE UPTAKE OF GLUCOSE AND INHIBITION BY TUBOCURARINE AND DICHLOROISOPROTERENOL (DCI).

Glucose uptake, in milligrams, per gram of wet tissue per thirty minutes.					
Control	d-tubocurarine	DCI	isoproterenol	d-tubocurarine and isoproterenol	DCI and isoproterenol
6.1	6.3	6.2	10.4	5.6	5.2
2.1	2.5	2.6	7.3	4.6	4.5
4.2	4.6	5.0	9.6	5.6	5.0
5.8	4.2	6.4	9.8	5.8	5.2
4.6±0.92	4.9±0.89[a]	5.1±0.87[a]	9.3±0.68[b]	5.4±0.27[c]	5.0±0.17[c]

a = no significant difference from control ($P > 0.2$)

b = significant difference from control ($P < .01$)

c = significant difference from isoproterenol ($P < .01$)

TABLE VII. EFFECT OF ISOPROTERENOL ON SKELETAL MUSCLE PRODUCTION OF LACTIC ACID AND INHIBITION BY TUBOCURARINE AND DICHLOROISOPROTERENOL (DCI).

Extractable lactic acid, in milligrams, per gram of tissue per thirty minutes.					
Control	d-tubocurarine	DCI	isoproterenol	d-tubocurarine and isoproterenol	DCI and isoproterenol
3.0	3.0	3.3	8.1	3.4	3.4
3.0	3.2	3.6	7.7	3.4	3.6
3.5	3.7	3.9	6.8	3.6	3.4
3.2	3.1	3.6	8.2	3.2	3.3
3.2 ± 0.17	3.1 ± 0.06[a]	3.6 ± 0.17[a]	7.7 ± 0.45[b]	3.4 ± 0.035[c]	3.4 ± 0.029[c]

a = no significant difference from control ($P > 0.2$)

b = significant difference from control ($P < .01$)

c = significant difference from isoproterenol ($P < .01$)

TABLE VIII. EFFECT OF ISOPROTERENOL ON LIVER SLICE UPTAKE OF GLUCOSE AND INHIBITION BY TUBOCURARINE AND DICHLOROISOPROTERENOL (DCI).

Glucose uptake, in milligrams, per gram of wet tissue per thirty minutes.					
Control	d-tubocurarine	DCI	isoproterenol	d-tubocurarine and isoproterenol	DCI and isoproterenol*
14.3	15.0	16.4	31.0	32.1	14.5
10.2	10.4	15.2	19.2	19.0	10.6
16.4	16.5	19.3	29.0	28.7	17.0
15.0	15.4	18.5	27.0	27.5	15.4
9.6	10.0	13.0	17.3	17.9	10.0
13.1 ± 0.67	13.4 ± 1.29[a]	16.5 ± 1.06[b]	24.3 ± 2.7[b]	25.0 ± 2.71[c]	13.5 ± 1.37[d]

* = Corrected values (minus 3.4, the elevation produced by DCI, by itself)

a = no significant difference from control ($P > 0.2$)
b = significant difference from control ($P < .01$)
c = no significant difference from isoproterenol ($P > 0.2$)
d = significant difference from isoproterenol ($P < .01$)

TABLE IX. EFFECT OF ISOPROTERENOL ON LIVER SLICE PRODUCTION OF LACTIC ACID AND INHIBITION BY TUBOCURARINE AND DICHLOROISOPROTERENOL (DCI).

Extractable lactic acid, in milligrams, per gram of tissue per thirty minutes.					
Control	d-tubocurarine	DCI	isoproterenol	d-tubocurarine and isoproterenol	DCI and isoproterenol*
6.2	6.2	8.8	10.8	11.4	6.5
6.0	6.4	8.6	11.2	11.0	6.4
6.4	5.9	9.1	10.0	10.0	6.8
6.4	6.6	8.7	11.0	10.8	6.7
6.4± 0.095	6.3± 0.15	8.8± 0.195	10.8± 0.26	10.8± 0.294	6.6± 0.124

* = Corrected values (minus 2.2, the elevation produced by DCI, by itself)

a = no significant difference from control ($P > 0.2$)
b = significant difference from control ($P < .01$)
c = no significant difference from isoproterenol ($P > 0.2$)
d = significant difference from isoproterenol ($P < .01$)

liver. The values in the extreme right column of TABLE VIII were corrected by subtracting 3.4, which was the elevation of glucose uptake produced by dichloroisoproterenol alone. Similarly, the extreme right column in TABLE IX shows the corrected values for lactic acid production induced by isoproterenol in the presence of dichloroisoproterenol. The correction was made by subtracting 2.2 from each result. This value, 2.2, represents the mg/gm of increase in tissue lactic acid produced by dichloroisoproterenol alone.

TABLES VIII and IX show that d-tubocurarine did not prevent glucose uptake or lactic acid production induced by isoproterenol, in liver. These two tables also show the dichloroisoproterenol was capable of antagonizing the increases in glucose uptake and lactic acid production induced by isoproterenol, in liver.

Discussion

It is shown in TABLES VI, VII, VIII, and IX that isoproterenol markedly increases the normal uptake of glucose and also lactic acid production of liver and skeletal muscle slices. It is interesting to note that Claassen and Noach reported that liver glycogenolysis is mainly an alpha receptor function in rats (20). Vrij and coworkers reported that muscle glycogenolysis in rats is a beta receptor function (100). Results of the present study indicate that liver glycogenolysis can be mediated via a beta receptor, because

isoproterenol, a beta adrenergic amine, strongly increased liver uptake of glucose and lactic acid production. This difference in results can be explained by the different species utilized by this laboratory (dogs) and by Claassen or Vrij (rats).

TABLES VI, VII, VIII and IX show that d-tubocurarine has no intrinsic ability to increase glucose uptake or lactic acid production in either type of tissue. However, these tables also show that dichloroisoproterenol does not have an intrinsic effect upon skeletal muscle glycogenolysis, but does have this effect upon isolated liver tissue. This is a difficult observation to reconcile. To date, there are no observations to explain why dichloroisoproterenol would excite glycogenolytic receptors of liver, but not those of skeletal muscle.

It is possible that this difference in induced glycogenolysis between skeletal muscle and liver may be due to the different concentration of glycogen in liver and muscle tissue. Skeletal muscle serves as a larger storehouse for glycogen as compared to liver. However, the liver actually has much more glycogen present per gram than does skeletal muscle. It has been shown in dogs that skeletal muscle contains 0. 55 grams of glycogen per gram of wet weight and that liver contains 6. 10 grams of glycogen per gram of wet weight (94). In our work the mass of skeletal muscle used was equal to the mass of liver used. It is reasonable to suggest that,

Due to the conflictions mentioned above, the following series of experiments were designed to determine:

(1) The magnitude of the hyperglycemic effect of phenoxybenzamine by itself.

(2) The role of the adrenal gland in phenoxybenzamine induced hyperglycemia.

(3) The ability of phenoxybenzamine to reduce epinephrine induced hyperglycemia.

Methods

Thirty dogs were randomly separated into six groups of five each. The animals were anesthetized and control serum glucose levels were obtained before any treatment. All serum glucose levels were determined by the method of Nelson (72) and were expressed as milligrams percent. The experimental groups of dogs were as follows:

(1) Dogs which received phenoxybenzamine, 8 mg/Kg; glucose serum level determined 60 minutes after the blocking agent;

(2) Dogs which received phenoxybenzamine, 8 mg/Kg, 30 minutes after adrenalectomy (method described in GENERAL PROCEDURES); serum glucose determined 60 minutes after phenoxybenzamine;

(3) Dogs which recieved a 60 minute epinephrine infusion (1.5

mcg/Kg/min); serum glucose level determined at the end of the infusion;

(4) Dogs which received phenoxybenzamine, 8 mg/Kg, followed 60 minutes later by a 60 minute infusion of epinephrine (1.5 mcg/Kg/min); serum glucose level determined at the end of infusion;

(5) Dogs which received phenoxybenzamine, 8 mg/Kg, followed 120 minutes later by a 60 minute infusion of epinephrine (1.5 mcg/Kg/min); serum glucose level determined at the end of infusion;

(6) Dogs which were adrenlectomized and injected with phenoxybenzamine, 8 mg/Kg, followed 60 minutes later by a 60 minute infusion of epinephrine (1.5 mcg/Kg/min); serum glucose level determined at end of infusion.

The results were tabulated and statistically analyzed. An analysis of variance, least significant difference and standard error were determined by the procedure described in the APPENDIX, page 93 and 94.

Results

TABLE X shows the mean control level of serum glucose (91 milligrams percent) and the mean serum glucose values obtained in each of the six experimental groups. It is shown that phenoxybenzamine, alone (Group 1), elevated serum glucose 38 milligrams percent above the control after 60 minutes. Group 2 results show that

TABLE X. EFFECT OF PHENOXYBENZAMINE (PBZ) ON THE SERUM GLUCOSE LEVEL OF DOGS.

Serum glucose levels, in milligrams percent, after:						
no treatment	Group (1) PBZ	Group (2) adrenalectomy and PBZ	Group (3) epinephrine infusion	Group (4) PBZ and epinephrine infusion (60 minutes after PBZ)	Group (5) PBZ and epinephrine infusion (120 minutes after PBZ)	Group (6) adrenalectomy, PBZ and epinephrine infusion (60 minutes after PBZ)
94	139	96	181	225	198	200
100	131	107	202	215	202	207
82	120	100	178	182	184	175
88	120	93	188	202	206	177
90	134	96	180	226	215	183
91 ± 3.0	129 ± 5.3	98 ± 2.9	186 ± 4.4	211 ± 8.3	201 ± 6.3	180 ± 6.5

Least significant difference, at five percent level, for treatment means, is 28.

in adrenalectomized dogs, phenoxybenzamine no longer produced the elevation of serum glucose, but had no significant effect on serum glucose. A 60 minute infusion of epinephrine produced an elevated serum glucose level of 95 milligrams percent above the normal. The hyperglycemic response to a 60 minute infusion of epinephrine was not inhibited by an injection of phenoxybenzamine given 60 minutes prior to the infusion (Group 4). The waiting period between the time of injection of the blocking agent and the start of the epinephrine infusion was increased to 120 minutes in Group 5 instead of the 60 minute waiting period used in Group 4. However, it is shown in Group 5 that this increased waiting period does not produce a significant inhibition of epinephrine induced hyperglycemia. In Group 6, the adrenalectomized animals received phenoxybenzamine 60 minutes prior to the epinephrine infusion. However, even with the combination of phenoxybenzamine and adrenalectomy the epinephrine induced hyperglycemia was not significantly reduced.

Discussion

The results show that a phenoxybenzamine injection will elevate serum glucose significantly. This corroborates the previous work of Mayer who also found an elevated serum glucose after phenoxybenzamine (61). Benfey has shown that phenoxybenzamine increases the urinary level of catecholamines (12, 13). Euler

presented evidence that phenoxybenzamine caused a release of epinephrine from adrenal granules (38). The work of Benfey and Euler strongly suggests that the increased glucose level after phenoxybenzamine, observed in the present study, was a result of phenoxybenzamine releasing epinephrine from the adrenals and that the released epinephrine evoked the hyperglycemia. However, the above mentioned results are only indirect evidence that the hyperglycemia seen after phenoxybenzamine was due to a release of epinephrine from the adrenals. Euler's and Benfey's works do not rule out the possibility that the hyperglycemia induced by phenoxybenzamine was because of phenoxybenzamine acting as a direct agonist of glycogenolytic receptors. Therefore it was necessary to establish if the adrenal glands must be present for a hyperglycemic response to phenoxybenzamine to occur. When phenoxybenzamine was injected into adrenalectomized dogs the hyperglycemic response observed in intact dogs was absent.

These data lead to the conclusion that phenoxybenzamine does not have intrinsic glycogenolytic properties. Further, the hyperglycemia evoked by phenoxybenzamine is probably a result of epinephrine release from the adrenal glands, due to a mechanism not yet described.

The results show that an epinephrine infusion produced its customary hyperglycemic response. This response was not

reduced by the alpha blocking agent, phenoxybenzamine, but was instead augmented. Preliminary work in the laboratory suggested that if the waiting period between phenoxybenzamine and the start of the infusion was 120 minutes (as opposed to the usual 60 minute period) the blockade of epinephrine might be significant. However, TABLE X shows that the slight depression that occurred was not significant.

The work of Benfey (13) and Euler (38) and this present study have shown that phenoxybenzamine increases blood glucose by causing a release of epinephrine from the adrenals. It appeared to us that possibly the reason phenoxybenzamine could not block epinephrine induced hyperglycemia in the intact dog was because phenoxybenzamine released epinephrine endogenously. This endogenous epinephrine, alone with the exogenously infused epinephrine could produce an extremely high level of epinephrine and the high level would overcome the phenoxybenzamine blockade of the glycogenolytic receptors. Therefore, dogs were adrenalectomized to remove a major source of epinephrine, injected with phenoxybenzamine and infused with epinephrine. The results show that phenoxybenzamine still failed to inhibit epinephrine induced hyperglycemia.

From the data obtained from this present study, it is concluded that phenoxybenzamine elevates blood sugar by a release of epinephrine from the adrenal gland. It also appears that an alpha adrenergic blockade with phenoxybenzamine cannot inhibit epinephrine induced hyperglycemia.

GENERAL DISCUSSION

It is difficult to determine which worker first conceived that adrenergic receptors existed. Dale received credit for making the first attempt at characterizing the nature of adrenergic receptors. However, Dale's work could not have been done without the earlier endeavors of Langley, Elliot, and Lewandowsky. Several different concepts of the nature of the adrenergic receptor have evolved from the original work of Dale. The concept of Ahlquist was proposed in 1948 and is still generally accepted. Ahlquist proposed two distinct types of adrenergic receptors which he called alpha and beta. He suggested that alpha receptors are concerned with excitatory responses, such as vasoconstriction, stimulation of the uterus, and contraction of the nitatating membrane, and that beta receptors are concerned with inhibitory responses, such as vasodilation, and bronchial relaxation. Ahlquist also proposed that adrenergically induced myocardiac stimulation occurs because of beta receptor excitation in the heart.

The classification of Ahlquist appeared to be an over-simplification of complex mechanisms. Therefore, in the present study, experiments were undertaken to concurrently measure blood pressure and cardiac contractile force to determine the nature of the cardiac contractility. The positive inotropic and blood pressure

responses of four adrenergic amines, phenylephrine, norepinephrine, (alpha amines), epinephrine (alpha-beta adrenergic amine), and isoproterenol (beta adrenergic amine), were determined before and after two alpha adrenergic blocking agents and a beta adrenergic blocking agent. According to the dualistic concept of Ahlquist it would be expected that phenylephrine and norepinephrine would not increase cardiac contractile force, whereas epinephrine and isoproterenol would produce increases. However, in the present study, all four amines significantly increased cardiac contractility. The inability of phenylephrine and norepinephrine to excite beta receptors was shown by their lack of a depressor response after alpha blocking agents.

Ahlquist proposed that the vascular beta receptor and the cardiac beta receptor are identical and differ only by anatomical location. If this is correct, it would follow that phenylephrine and norepinephrine, which was found to increase cardiac contractile force, should also produce a depressor response after an alpha blocking agent (in an analogous manner to epinephrine). The latter response, however, did not occur, and leads to the conclusion that the beta vascular receptor and the proposed cardiac receptor responsible for increases in cardiac contractility cannot be identical.

The alpha blocking agent, phenoxybenzamine, did not inhibit the positive inotropic effects of any of the amines. However, the

alpha blocking agent, Hydergine, significantly inhibited this effect of phenylephrine and norepinephrine. Dichloroisoproterenol, a beta blocking agent, inhibited the increases in cardiac contractility produced by all four amines.

These results lead to the conclusion that the adrenergic receptor responsible for increases in cardiac contractility has properties similar to both the alpha and beta receptors proposed by Ahlquist. It appears that this receptor is undifferentiated and can be excited by either alpha or beta adrenergic amines.

The controversy of adrenergic receptors is not limited to the cardiovascular system but extends into adrenergically induced glycogenolysis and hyperkalemia. Nearly all adrenergic amines will produce an elevation of the serum potassium level. The mechanism for this response is not understood, but the locus is considered to be the liver. Ellis (37) has shown that certain beta-haloalkylamines, (dibenamine and phenoxybenzamine) can separate the glycogenolytic and hyperkalemic responses of injected epinephrine. In the present study phenoxybenzamine, Hydergine, dichloroisoproterenol and d-tubocurarine were used to determine if these blocking agents could prevent the hyperkalemic response of phenylephrine, norepinephrine, epinephrine and isoproterenol. It was shown that only phenoxybenzamine could separate adrenergically induced glycogenolytic and hyperkalemic effects of the amines. It was concluded that this dissociation

of induced glycolysis and hyperkalemia was a property of beta-haloalkylamines and not a general property of all blocking agents.

Although the hyperkalemic response to adrenergic amines is considered to be of hepatic origin, adrenergically induced glycogenolysis is relegated mainly to liver and skeletal muscle. Van der Pol suggested that alpha and beta receptors must be simultaneously stimulated to produce glycogenolysis (96). Claassen and Noach have proposed that alpha adrenergic amines produce liver glycogenolysis and beta adrenergic amines produce skeletal muscle glycogenolysis (20). McCutcheon has proposed that adrenergically induced glycogenolysis occurs because of beta receptor excitation (62).

Because of these conflicting reports on the receptor or receptors responsible for adrenergically induced glycogenolysis, several experiments were undertaken to better delineate the nature of glycogenolytic receptor. Phenylephrine and norepinephrine, alpha adrenergic amines, did not produce hyperglycemia or lactic acidemia, but epinephrine and isoproterenol significantly elevated serum levels of glucose and lactic acid. The glycogenolytic effects of epinephrine and isoproterenol were inhibited by dichloroisoproterenol, a beta blocking agent. This result supports McCutcheon's proposal. However, the glycogenolytic effect of epinephrine was inhibited by Hydergine, an alpha blocking agent, which did not alter the glycogenolytic effect of isoproterenol, a beta adrenergic amine. Consequently,

the proposal that adrenergically induced glycogenolysis occurs entirely because of beta receptor excitation can not be entirely accepted. The results in the present study indicate that although amines which can excite beta receptors do produce glycogenolysis, this cannot be the true glycogenolytic receptor since an alpha blocking agent, Hydergine, can inhibit the elevation of serum levels of glucose and lactic acid induced by epinephrine.

The work of Van der Pol, in rabbits, does not relate to adrenergically induced glycogenolysis in dogs because isoproterenol, a beta adrenergic amine, elevates serum levels of glucose and lactic acid. Claassen and Noach suggested that in rats, either an alpha or a beta adrenergic amine could elevate blood glucose and lactic acid levels because alpha adrenergic amines would excite glycogenolytic receptors in the liver, while beta adrenergic amines would excite these glycogenolytic receptors in skeletal muscle. This proposal must be rejected in the dog because neither phenylephrine nor norepinephrine elicited any significant elevation of serum glucose or lactic acid levels.

These results lead to the conclusion that adrenergically induced glycogenolysis in the dog occurs by excitation of a receptor which is closely related to the beta receptor, but cannot be categorically identified as a beta receptor.

The ability of a skeletal muscle relaxing agent, d-tubocurarine,

to inhibit adrenergically induced glycogenolysis, was determined. This type of agent was employed because it was assumed that a separation of skeletal muscle and liver glycogenolysis might be accomplished. This agent did not alter the glycogenolytic effect of epinephrine, but d-tubocurarine significantly reduced the hyperglycemic and hyperlactic acidemic responses of isoproterenol. These results did not establish whether this observed inhibition was occurring in skeletal muscle, liver, or in both tissues. It appeared necessary to utilize tissue slices of liver and skeletal muscle and measure isoproterenol's glycogenolytic effect, before and after d-tubocurarine, in vitro. In addition, the ability of dichloroisoproterenol to inhibit the glycogenolytic effect of isoproterenol was also determined, in vitro. The results showed that isoproterenol, by itself, increased glycogenolysis in both types of tissues. The results also showed that this induced glycogenolysis could be blocked in both types of tissue by dichloroisoproterenol, but d-tubocurarine inhibited the glycogenolytic effect of isoproterenol only in skeletal muscle. These results present two major points. The first point is that evidence was obtained which clearly shows that a beta adrenergic amine can enhance glycogenolysis in both liver and skeletal muscle slices from dogs. Claassen and Noach (20) have shown that, in rats, liver glycogenolysis is not enhanced with isoproterenol. The results obtained in the present study probably differ from the work of Claassen and Noach

because of the different species used. The second major point presented in the results is that d-tubocurarine produces a clear separation of skeletal muscle and liver glycogenolysis induced by isoproterenol. The inhibition of adrenergically induced glycogenolysis by d-tubocurarine has not been reported prior to the present study. More important, however, is that d-tubocurarine may serve as a valuable tool in determining if adrenergically induced glycogenolysis in liver and skeletal muscle may be occurring via a different biochemical pathway.

These results lead to the conclusions that, in dogs, beta adrenergic amines can induce glycogenolysis in skeletal muscle and in liver. Further, d-tubocurarine can inhibit beta adrenergic amine induced glycogenolysis in skeletal muscle, but does not alter this response in liver.

It is in general agreement that the alpha adrenergic blocking agent, phenoxybenzamine, does not inhibit the hyperglycemia produced by an infusion of epinephrine. Also, there is general agreement that this blocking agent, by itself, will produce a hyperglycemia. The mechanism of this hyperglycemia was examined in this present study. It appeared important to determine if the hyperglycemia induced by phenoxybenzamine was due to a direct agonist effect of the blocker on glycogenolytic receptors or if the hyperglycemia was due to an adrenal medullary release of catecholamines, which then

produced hyperglycemia. In addition, the lack of inhibition, by phenoxybenzamine, of epinephrine's induced hyperglycemia was re-evaluated.

The results showed that phenoxybenzamine did not significantly reduce the hyperglycemic effect of epinephrine. These results corroborate those reported in literature. The results also show that the blocking agent, by itself, is a fairly potent hyperglycemic agent. However, phenoxybenzamine does not produce hyperglycemia in the adrenalectomized dog. These results suggest that the hyperglycemic effect of phenoxybenzamine is most likely due to a release of epinephrine from the adrenal glands. It is interesting to review here the works of Benfey (13) and Euler (38). Benfey, who also reported a hyperglycemic effect with phenoxybenzamine, measured an elevated urinary level of catecholamines after phenoxybenzamine. Von Euler has shown that phenoxybenzamine causes a release of epinephrine from adrenal medullary granules. The works of these two investigators strengthen the results presented in the present study.

These results lead to the conclusion that the hyperglycemia produced by phenoxybenzamine is due to a probable release of epinephrine from the adrenal gland and not because of an interaction of phenoxybenzamine with glycogenolytic receptors.

SUMMARY AND CONCLUSIONS

Presently, the most generally accepted concept of adrenergic receptors is the proposal of Ahlquist. This proposal states that all adrenergically induced responses occur because of excitation of either of two receptors, named alpha and beta. Ahlquist concluded that excitation of alpha receptors produced excitatory responses such as vasoconstriction, contraction of the nictitating membrane and dilation of the pupil. Excitation of beta receptors produced inhibitory responses such as vasodilation, relaxation of the uterus, relaxation of the bronchial muscles and one important excitatory response, increased myocardial contractility.

Periodically, reports appear in literature that do not adhere to the stringent dualistic receptor concept of Ahlquist. The controversial papers generally were concerned with either the cardiac receptor responsible for adrenergically induced increases in cardiac contractility or the receptor responsible for adrenergically induced glycogenolysis. Therefore the nature of these two receptors were studied in this present investigation. Certain conclusions were reached as follows:

(1) The cardiac receptor responsible for adrenergically induced increases in cardiac contractile force is not identical to the beta vascular receptor as proposed by Ahlquist. It appears that this

particular receptor has properties similar to both alpha and beta receptors because the present study demonstrates that either alpha or beta adrenergic amines can significantly increase cardiac contractile force and this response of the alpha or beta adrenergic amines was inhibited by dichloroisoproterenol, while the response of the alpha adrenergic amines was inhibited by Hydergine. Therefore, we propose that the cardiac receptor be classified as an undifferentiated adrenergic receptor.

(2) It was shown that only adrenergic amines, which can excite beta receptors, were capable of inducing glycogenolysis in dogs. The beta adrenergic blocking agent, dichloroisoproterenol, inhibited the glycogenolysis induced by isoproterenol and epinephrine, but the alpha blocking agent, Hydergine, inhibited this response of only epinephrine. It was concluded that adrenergically induced glycogenolysis in the dog occurs by excitation of a receptor that is closely related to the beta receptor, but cannot categorically be identified as a beta receptor.

(3) The proposal that liver and skeletal muscle glycogenolysis are stimulated by alpha or beta adrenergic amines, respectively, cannot be applied to dogs. In vitro studies have shown that isoproterenol, a beta adrenergic amine, stimulates glycogenolysis in both types of tissue. Further, it was shown that d-tubocurarine can inhibit the skeletal muscle glycogenolysis induced by isoproterenol

without affecting this amine's glycogenolytic effect in liver.

(4) It was shown that phenoxybenzamine produces significant hyperglycemia in intact dogs. This effect does not occur in adrenalectomized animals. These results indicate that the hyperglycemia induced by phenoxybenzamine is probably due to a release of epinephrine from the adrenal glands and not because phenoxybenzamine is a glycogenolytic agent.

BIBLIOGRAPHY

1. Abel, John J. On a simple method of isolated epinephrine. Johns Hopkins Hospital Bulletin 13:29-36. 1902.

2. Acheson, G., A. Farah and G. N. French. Some effects of dibenzyl-beta-chlorethylamine on mammalian heart. Journal of Pharmacology and Experimental Therapeutics 97:455-465. 1949.

3. Agarival, S. L., and S. C. Harvey. Mechanism of the long duration of action of dibenzyline. Journal of Pharmacology and Experimental Therapeutics 117:106-111. 1956.

4. Ahlquist, Raymond P. A study of the adrenotropic receptors. American Journal of Physiology 153:586-600. 1948.

5. Ahlquist, Raymond P., and Bernard Levey. Adrenergic receptive mechanism of canine ileum. Journal of Pharmacology and Experimental Therapeutics 127:146-149. 1959.

6. Allwood, M. J., and A. F. Cobbold. Lactic acid release by intraarterial adrenaline infusions before and after dibenyline, and its relationship to blood-flow changes in the human forearm. Journal of Physiology 157:328-334. 1961.

7. Axelrod, J., L. Aronow and B. B. Brodie. The physiological disposition and biotransformation of dibenamine and a method for its estimation in biological tissues. Journal of Pharmacology and Experimental Therapeutics 106:166-179. 1952.

8. Bang, O. The lactate content of the blood during and after muscular exercise. Skandinavisches Archiv für Physiologie 74:51-56. 1936.

9. Barcroft, H., and A. F. Cobbold. The action of adrenaline on muscle blood flow and blood lactate in man. Journal of Physiology 132:372-378. 1956.

10. Barker, S. B., and William H. Summerson. The colorimetric determination of lactic acid in biological material. Journal of Biological Chemistry 138:535-554. 1941.

11. Belleau, B. Relationships between agonists, antagonists, and receptor sites. In: Ciba Foundation Symposium on Adrenergic Mechanisms. Boston, Little and Brown, Co. 1960, p. 223-245.

12. Benfey, B. G., G. Ledoux and K. I. Melville. A hypertensive phase of phenoxybenzamine action. Federation Proceedings 19: 295. 1960.

13. Benfey, B. G., G. Ledoux and K. I. Melville. Increased urinary excretion of adrenaline and noradrenaline after phenoxybenzamine. British Journal of Pharmacology and Chemotherapy 14: 142-142. 1959.

14. Bennett, Donald R., David Reinke and Edward Alpert. The action of intraocularly administered adrenergic drugs on the iris. Journal of Pharmacology and Experimental Therapeutics 134: 190-198. 1961.

15. Bergman, Hyman and D. R. Drury. The relationship of kidney function to the glucose utilization of the extra abdominal tissues. American Journal of Physiology 124:279-284. 1938.

16. Bovet, D., F. Bovet-Mitti and G. B. Bettalo. Curare and curare-like agents. Amsterdam, Elsevier, 1959. 478 p.

17. Brodie, B. B., L. Aronow, and J. Axelrod. The fate of dibenzyline in the body and the role of fat in its duration of action. Journal of Pharmacology and Experimental Therapeutics 111: 21-29. 1954.

18. Brown, G. L., B. N. Davies and J. S. Gillespie. The release of chemical transmitter from the sympathetic nerves of the intestine of the cat. Journal of Physiology 143:41-54. 1958.

19. Cannon, W. B. and A. Rosenblueth. Studies on conditions of activity in endocrine organs. American Journal of Physiology 104:557-573. 1933.

20. Claassen, V. and E. L. Noach. Dichloro-isuprel inhibition of sympathomimetic hyperglycemia. Archives Internationales de pharmacodynamie et de therapie 126:332-340. 1960.

21. Cohn, Clarence and Muriel Kolinsky. Effect of blood sugar levels and insulin lack on gluconeogenesis by the kidney of the dog. American Journal of Physiology 156:345-348. 1949.

22. Cook, W. H., D. Lipkin and R. Markham. The formation of a cyclic dianhydrodiadenylic acid by the alkaline degradation of adenosine -5'- triphosphoric acid. Journal of the American Chemical Society 79:3607-3608. 1957.

23. Cori, Carl F. The influence of insulin and epinephrine on the lactic acid content of blood and tissues. Journal of Biological Chemistry 63:253-268. 1925.

24. Cori, Carl F. and Gerty Cori. The influence of epinephrine on lactic acid production and blood sugar utilization. Journal of Biological Chemistry 84:683-698. 1929.

25. Cori, Carl F. and K. W. Buchwald. Effect of continuous intravenous injection of epinephrine on the carbohydrate metabolism, basal metabolism and vascular system of normal men. American Journal of Physiology 95:71-78. 1930.

26. Cori, Carl F. Mammalian carbohydrate metabolism. Physiological Reviews 11:143-275. 1931.

27. Cori, Carl F. Enzymatic reactions in carbohydrate metabolism. Harvey Lectures 41:253-272. 1945.

28. Cotton, M. deV. and R. P. Walton. Dibenzamine blockade as a method of distinguishing between inotropic action of epinephrine and digitalis. Proceedings of the Society for Experimental Biology 78:810-815. 1951.

29. Cotton, M. deV., N. C. Moran and P. E. Stopp. A comparison of the effectiveness of adrenergic blocking drugs in inhibiting the cardiac actions of sympathomimetic amines. Journal of Pharmacology and Experimental Therapeutics 121:183-190. 1957.

30. Cotton, M. deV. and Neil C. Moran. Cardiovascular pharmacology. Annual Review of Pharmacology 1:261-286. 1961.

31. Dale, H. H. On some physiologic actions of ergot. Journal of Physiology 34:163-206. 1906.

32. Drury, Douglas R., Arne N. Wick and Eaton MacKay. Formation of glucose by the kidney. American Journal of Physiology 163:655-661. 1950.

33. D'Silva, John L. The action of adrenaline on serum potassium. Journal of Physiology 82:393-398. 1934.

34. D'Silva, John L. The action of adrenaline on serum potassium. Journal of Physiology 86:219-228. 1936.

35. Elliot, T. R. On the action of adrenaline. Journal of Physiology 31:20-21. 1904.

36. Elliot, T. R. The action of adrenaline. Journal of Physiology 32:401-467. 1905.

37. Ellis, Sydney and Sibyl B. Beckett. Mechanism of the potassium mobilizing action of epinephrine and glucagon. Journal of Pharmacology and Experimental Therapeutics 143:318-326. 1963.

38. Von Euler, U. S., L. Stjarne and F. Lishajko. Effect of reserpine, segontin and phenoxybenzamine on the catecholamines and ATP of isolated nerve and adrenomedullary storage granules. Life Sciences 3:35-40. 1964.

39. Von Euler, U. S. and F. Lishajko. Effect of some drugs on the release of noradrenaline from isolated nerve granules. Biochemical Pharmacology 9:77-84. 1962.

40. Foster, G. L. Studies on carbohydrate metabolism. Journal of Biological Chemistry 55:291-301. 1923.

41. Freiss, E. D., J. R. Stanton and R. W. Wilkins. The effect of certain dihydrogenated alkaloids of ergot in hypertensive patients. American Journal of Medical Science 216:163-171. 1948.

42. Furchgott, Robert F. The receptors for epinephrine and norepinephrine (adrenergic receptors). Pharmacological Reviews 11: 429-439. 1959.

43. Harvey, Stewart C., C. Y. Wang and Mark Nickerson. Blockade of epinephrine-induced hyperglycemia. Journal of Pharmacology and Experimental Therapeutics 104:363-376. 1952.

44. Hassen, Ali. Glucose in normal urine. Biochemical Journal 22:1332-1340. 1928.

45. Hukovic, S. Isolated rabbit atria with sympathetic nerve supply. British Journal of Pharmacology and Chemotherapy 14:372-376. 1959.

46. Hunt, C. C. The adrenergic blocking effect of certain beta-chloroethyl amines. Journal of Pharmacology and Experimental Therapeutics 95:177-184. 1949.

47. Jarpes, J. A. Heparin in the treatment of thrombosis. 2d ed. New York, Oxford University Press, 1946. 260 p.

48. Kilburn, Kaye H. Effect of adrenergic blockade with hydergine on hyperglycemia accompanying hypothermia in dogs. American Journal of Physiology 199:955-958. 1960.

49. Komrad, E. J. and E. R. Loew. Effect of certain anti-histamines of epinephrine-induced hyperglycemia and lactic acidemia. Journal of Pharmacology and Experimental Therapeutics 103: 115-122. 1951.

50. Komrad, Eugene L. and Earl R. Loew. Inhibition of epinephrine induced hyperglycemia with adrenergic blocking drugs. American Journal of Physiology 165:66-77. 1951.

51. Lands, A. M. Sympathetic receptor action. American Journal of Physiology 169:11-21. 1952.

52. Langley, J. N. Observations on the physiological action of extracts of the supra-renal bodies. Journal of Physiology 27:237-256. 1901.

53. Larson, P. S. and George Brewer. On the relation of potassium to the blood pressure response to epinephrine. Journal of Pharmacology and Experimental Therapeutics 61:213-217. 1937.

54. Lei, Benjamin W. and Rob S. McCutcheon. Influence of adrenergic receptors on blood sugar and lactic acid levels in the rat. Journal of Pharmaceutical Sciences 53:503-506. 1964.

55. Levy, Bernard and Raymond P. Ahlquist. An analysis of adrenergic blocking activity. Journal of Pharmacology and Experimental Therapeutics 133:202-210. 1961.

56. Levy, Bernard and R. P. Ahlquist. Blockade of the beta adrenergic receptors. Journal of Pharmacology and Experimental Therapeutics 130:334-339. 1960.

57. Lewandowsky, M. Ueber die Wirkung des Nebbenierenextracts auf die glotten Muskelm, Besonderen des Auges. Archiv für Anatomie und Physiologie, Physiologische Abteilung 2:360-364. 1902.

58. Loewi, Otto. Ueber humorale Uebertragbarkeit der Herznervewirkung. II. Pflueger's Archiv fur die geste Physiologie des Menschen und der Tiere 193:201-213. 1922.

59. Markowitz, J., J. Archibald and H. G. Downie. Experimental surgery. 4th ed. Baltimore, Williams and Wilkins, 1959. p. 931.

60. de la Mata, R. Castro, Mario Penna and Domingo Aviado. Reversal of sympathomimetic bronchodilation by dichloroisoproterenol. Journal of Pharmacology and Experimental Therapeutics 135:197-203. 1962.

61. Mayer, Steven, Neil Moran and John Fain. The effect of adrenergic blocking agents on some metabolic actions of catecholamines. Journal of Pharmacology and Experimental Therapeutics 134:18-27. 1961.

62. McCutcheon, Rob S. Canine blood sugar and lactic acid responses to adrenergic amines after adrenergic blockade. Journal of Pharmacology and Experimental Therapeutics 136:209-212. 1962.

63. McCutcheon, Rob S. Canine blood sugar and lactic acid responses to adrenergic amines after ganglionic block. Journal of the American Pharmaceutical Association, Scientific 49:714-716. 1960.

64. Millar, R. A., E. B. Keener and B. G. Benfey. Plasma adrenaline and noradrenaline after phenoxybenzamine administration, and during hemorrhagic hypotension, and in normal and adrenalectomized dogs. British Journal of Pharmacology and Chemotherapy 14:9-13. 1959.

65. Mohme-Lundholm, Ella. The mechanism of the relaxing effect of adrenaline on smooth muscle. Acta Physiologica Scandinavica 29:Supplementum 108, 1-63. 1953.

66. Mohme-Lundholm, Ella. Effect of adrenaline, noradrenaline, isopropyl-noradrenaline and ephedrine on tone and lactic acid formation in bovine tracheal muscle. Acta Physiologica Scandinavica 37:1-4. 1956.

67. Mohme-Lundholm, Ella. Effect of calcium ions upon the relaxing and lactic acid forming action of adrenaline on smooth muscle. Acta Physiologica Scandinavica 37:5-7. 1956.

68. Moran, Neil C. and Marjorie E. Perkins. Adrenergic blockade of the mammalian heart by a dichloro analogue of isoproterenol. Journal of Pharmacology and Experimental Therapeutics 124: 223-237. 1958.

69. Murad, F., T. W. Rall and E. W. Sutherland. Formation of adenosine-3', 5'-phosphate by particulate preparations of ventricular muscle. Federation Proceedings 19:192. 1960.

70. Murad, F., et al. The relation of adenosine -3', 5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmacological Reviews 12:265-299. 1960.

71. Nash, Thomas P., Jr. Phlorhizin diabetes. Physiological Reviews 7:385-430. 1927.

72. Nelson, Norton. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry 153:375-380. 1940.

73. Ni, Tsang-G. and Paul Rehberg. On the mechanism of sugar excretion. Biochemical Journal 24:1039-1046. 1930.

74. Nickerson, Mark. The pharmacology of adrenergic blockade. Pharmacological Reviews 1:27-101. 1949.

75. Nickerson, Mark and W. S. Gump. The chemical basis for adrenergic blocking activity in compounds related to dibenamine. Journal of Pharmacology and Experimental Therapeutics 97:25-47. 1949.

76. Nickerson, Mark. Receptor occupancy and tissue response. Nature 178:697-698. 1956.

77. Nickerson, Mark. Mechanism of the prolonged adrenergic blocking action of dibenzyline. Federation Proceedings 15: 463. 1956.

78. Nickerson, Mark. Blockade of the actions of adrenaline and noradrenaline. Pharmacological Reviews 11:443-461. 1959.

79. Northrop, Gretajo and R. E. Parks, Jr. 3', 5'-AMP-induced hyperglycemia in intact rats and in the isolated perfused rat liver. Biochemical Pharmacology 13:120-123. 1964.

80. Northrop, Gretajo and R. E. Parks, Jr. The effects of adrenergic blocking agents and theophylline on 3', 5'-AMP-induced hyperglycemia. Journal of Pharmacology and Experimental Therapeutics 145:87-91. 1964.

81. O'Brien, G. S., et al. Effect of elimination of hepatic circulation on cyclopropane-epinephrine ventricular tachycardiac and arterial plasma potassium in dogs. Journal of Pharmacology and Experimental Therapeutics 112:374-377. 1954.

82. Oliver, George, and E. A. Schaffer. The physiological effects of extracts of the suprarenal capsules. Journal of Physiology 18:230-276. 1895.

83. Patterson, Jocelyn. The carbohydrate of normal urine. Biochemical Journal 20:651-655. 1926.

84. Posternak, T., E. W. Sutherland and W. F. Henson. Derivatives of cyclic 3', 5'-adenosine monophosphate. Biochimica et Biophysica Acta 65:558-560. 1962.

85. Powell, C. E. and I. H. Slater. Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. Journal of Pharmacology and Experimental Therapeutics 122:480-488. 1958.

86. Rall, T. W., Earl W. Sutherland and Jacques Terthet. The relationship of epinephrine and glucogon to liver phosphorylase. Journal of Biological Chemistry 224:463-475. 1957.

87. Rall, T. W. and Earl W. Sutherland. Formation of a cyclic adenine ribonucleotide by tissue particles. Journal of Biological Chemistry 232:1065-1076. 1958.

88. Rapoport, S., et al. The turnover of acid-soluble phosphorus in the kidneys of rats. Science 93:88-89. 1941.

89. Reinecke, Roger M. The kidney as a source of glucose in the eviscerated rat. American Journal of Physiology 140:276-285. 1943.

90. Robertson, William B. and Pincus Peyser. Changes in water and electrolytes of cardiac muscle following epinephrine. American Journal of Physiology 166:277-283. 1951.

91. Smith, C. B. Relaxation of the mictitating membrane of the spinal cat by sympathomimetic amines. Journal of Pharmacology and Experimental Therapeutics 142:163-170. 1963.

92. Soskin, S. Muscle glycogen as a source of blood sugar. American Journal of Physiology 81:382-383. 1927.

93. Soskin, S. The blood sugar: its origin, regulation and utilization. Physiological Reviews 21:140-193. 1941.

94. Soskin, S. and Rachmiel Levine. Carbohydrate metabolism. Chicago, University of Chicago Press, 1952. 346 p.

95. Sutherland, Earl W. and T. W. Rall. The relation of adenosine -3', 5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmacology Reviews 12: 265-299. 1960.

96. Van der Pol, M. C. The effect of some sympathicomimetics in relation to the two receptor-theory. I. Acta Physiologica et Pharmacologica Neerlandica 4:524-531. 1956.

97. Van der Pol, M. C. The effect of some sympathicomimetics in relationship to the two receptor-theory. II. Acta Physiologica et Pharmacologica Neerlandica 4:532-540. 1956.

98. Van der Pol, M. C. The effect of some sympathicomimetics in relationship to the two receptor-theory. III. Acta Physiologica et Pharmacologica Neerlandica 4:541-546. 1956.

99. Vosburgh, C. H. and A. N. Richards. An experimental study of the sugar content and extravascular coagulation of the blood after administration of adrenaline. American Journal of Physiology 9:35-51. 1903.

100. Vrij, C. et al. The effect of isopropyl-nor-adrenaline and nor-adrenaline on the glycogen content of skeletal muscle and liver of the rat. Acta Physiologica et Pharmacologica Neerlandica 4:547-554. 1956.

101. Walass, Otto and Eva Walass. Effect of epinephrine on rat diaphragm. Journal of Biological Chemistry 187:769-776. 1950.

102. Walker, Arthur M. and John Reisinger. Quantitative studies of the composition of glomerular urine. Journal of Biological Chemistry 101:223-237. 1933.

103. Walton, R. P., J. S. Leary and H. P. Jones. Comparative increase in ventricular contractile force produced by several cardiac glycosides. Journal of Pharmacology and Experimental Therapeutics 98:346-357. 1950.

104. Young, Frank G. Claude Bernard and the theory of the glycogenic function of the liver. Annals of Science 2:47-83. 1939.

APPENDIX

APPENDIX

The following procedures were used in the statistical analysis of the data obtained.

A. Explanation of Symbols

where k = the number of amines

m = the number of antagonists

n = the number of observations on each combination of amines and antagonists

σ^2 = the population sampling variance

σ^2_I = the variance due to interaction between the effects of amines and antagonists

$\sigma^2_{A_n}$ = the variance due to differences in the effects of antagonists

$\sigma^2_{A_m}$ = the variance due to differences in the effects of amines

$t_{.025}$ = the 2.5% point of the Student's t-distribution

s^2 = the estimate of the population sampling variance

B. The Least Significant Difference

$$LSD = t_{.025}\sqrt{\frac{s^2}{n}}$$

C. Analysis of Variance

Source of Variation	D. F.	Expected Mean Squares
Amines	k-1	$\sigma^2 + nm\sigma^2_{A_m}$
Antagonists	m-1	$\sigma^2 + nk\sigma^2_{A_n}$
Interaction	(k-1)(m-1)	$\sigma^2 + n\sigma^2_I$
Sampling Error	km(n-1)	σ^2

Analysis of Variance for Table I

Source of Variation	D. F.	Sum of Squares	Mean Square	F
Amines	3	11,433.5	3,811.1667	1,231.08
Antagonists	5	28,428.7	5,685.7400	1,836.60
PBZ vs Control	1	9,030.025		2,916.86
DCI vs Control	1	48.400		15.63
PBZ/ DCI vs Control	1	10,048.900		3,245.98
Hyd. vs Control	1	6,996.025		2,259.84
d-tubo vs Control	1	19.600		6.33
Interaction	15	39,473.9	2,631.5933	850.05
Error	96	297.2	3.0958	---

Analysis of Variance for Table II

Source of Variation	D. F.	Sum of Squares	Mean Square	F
Amines	3	108,691.40	36,230.47	3,527.80
Antagonists	5	156,540.30	31,308.06	3,048.50
PBZ vs Control	1	2.50	2.50	.24
DCI vs Control	1	66,585.60	66,585.60	6,483.51
PBZ/ DCI vs Control	1	60,450.62	60,450.62	5,886.14
Hyd. vs Control	1	1,677.02	1,677.02	163.29
d-tubo vs Control	1	14.40	14.40	1.40
Interaction	15	47,351.50	3,156.77	307.38
Error	96	985.60	10.27	---

Analysis of Variance for Table III

Source of Variation	D.F.	Sum of Squares	Mean Square	F
Amines	3	120,735.10	40,245.03	4,379.22
Antagonists	5	17,600.67	3,520.13	383.04
PBZ vs Control	1		846.40	92.10
DCI vs Control	1		6,969.60	758.39
PBZ/DCI vs Control	1		11,902.50	1,295.16
Hyd. vs Control	1		4,928.40	536.28
d-tubo vs Control	1		422.50	45.97
Interaction	15	30,760.20	2,050.68	223.14
Error	96	882.40	9.1902	---

Analysis of Variance for Table IV

Source of Variation	D.F.	Sum of Squares	Mean Square	F
Amines	3	9,281.20	3,093.73	4,534.93
Antagonists	5	3,551.94	710.39	1,041.32
PBZ vs Control	1	158.00	158.00	231.60
DCI vs Control	1	936.05	936.05	1,372.10
PBZ/DCI vs Control	1	1,476.22	1,476.22	2,163.91
Hyd. vs Control	1	174.31	174.31	255.51
d-tubo vs Control	1	234.26	234.26	343.39
Interaction	15	5,168.81	344.59	505.12
Error	96	65.49	.6822	---

Analysis of Variance for Table V

Source of Variation	D.F.	Sum of Squares	Mean Square	F
Amines	3	142.2498	47.4166	3,020.16
Antagonists	5	84.7761	16.9552	1,079.95
PBZ vs Control	1	39.4022	39.4022	2,509.69
DCI vs Control	1	18.2250	18.2250	1,160.83
PBZ/DCI vs Control	1	44.9440	44.9440	2,862.68
Hyd. vs Control	1	17.8222	17.8222	1,135.17
d-tubo vs Control	1	.0276	.0276	1.76
Interaction	15	130.9306	8.7287	555.97
Error	96	1.5106	.0157	---

Analysis of Variance for Table X

Source of Variation	D. F.	Sum of Squares	Mean Square	F
Among Treatments	6	75,847.6	12,641.2667	96.530
Within Treatments	28	3,666.8	130.9571	
Total	34	79,514.4		