THE INFLUENCE OF ADRENERGIC RECEPTORS ON THE BLOOD SUGAR AND LACTIC ACID LEVELS IN THE RAT

by

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INTRODUCTION

Historical Development of Receptor Theory

To trace the development of the receptor theory, one should begin with Lewandowsky (48) who was the first to note the significant correlation between the actions of sympathetic nerve stimulation, and the actions resulting from the application of supra-renal extract. His observations were later confirmed and extended by Langley (41, p. 256) who stated that "the effects produced by the extract and by electrical stimulation of the sympathetic nerve correspond exactly." His first thought was that the extract had a specific stimulating effect on the effector nerve, but since this activity continued after denervation, Langley concluded that the extract must exert its action directly on the effector organ and that this mode of action was probably true in all cases. He continued, however, by saying, "In such case the difference in action on different autonomic tissue must depend upon their intrinsic differences, and this takes out of our reach any immediate hope of explanation..."

During this time the crude supra-renal extract, which had been used by Langley and others, was purified by J. J. Abel. When it later became commercially available, T. R. Elliott, a Coutts-Trotter research student under Langley, performed these same experiments using the active principle. Elliott not only confirmed the earlier results, but also advanced a possible explanation for the mechanism of action. In a communication to the Physiological Society, Elliott wrote,

> "Therefore it cannot be that adrenalin excites any structure derived from, and dependent for its persistence on, the peripheral neurone. But since adrenalin does not evoke any reaction from muscle that has at no time of its life been innervated by the sympathetic, the point at which the stimulus of the chemical excitant is received, and transformed into what may cause the change of tension of the muscle fibre, is perhaps a mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fibre, the function which is to receive and transform the nerve impulse. Adrenalin might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery." (23).

Discouraged from pursuing this line of thinking by Langley (20), his major professor, Elliott's paper, which came out a year later contained little of what he had postulated earlier (24). Unfortunately, it was not until many years later, that the correctness of this hypothesis was confirmed in the now classical experiment of Otto Loewi (22, p. 353)(51), who demonstrated the theory

of chemical transmission through the use of two frog hearts arranged in the usual manner of recording contractions. One heart was connected for electrical stimulation, while the other heart was free from any external connection. The first heart was perfused with ringer's and this perfusate was then collected and allowed to perfuse the second heart. Loewi demonstrated that when the sympathetic nerve was stimulated in the first heart by electrical impulse, the second heart, whose only connection was the common perfusate, also responded by an accelerated heart beat. Subsequent chemical analysis of the perfusate established the chemical mediator to be epinephrine. Corresponding experiments were performed using the parasympathetic nerves with equivalent results (22, p. 353)(50).

The subsequent papers published by Langley contained the germs which were to become the receptor theory (41)(42)(43). His first reference to a receptor substance was contained in his summarizing remarks when he concluded, "that the poisons [epinephrine] do not act directly on the contractile substance, but on other substance in the muscle which may be called receptive substances." (42, p. 412). He concluded further, that a cell may make either motor or inhibitory receptive substances, or both, and that the

effects of the nervous impulse depends on the proportion of the two kinds of receptive substances which are affected by the impulse.

The following year, Henry H. Dale, a previous Coutts-Trotter research student under Langley, reported the physiological effects of ergot alkaloids on the effects of epinephrine and sympathetic nerve stimulation (19, p. 200). From his studies he was able to distinguish three types of organs: (1) those with an inhibitory nerve supply from the sympathetic system, (2) those in which a normal purely motor sympathetic effect is reduced by ergot to a minimum, or below the limits of perception, and (3) those in which the normal effect is motor, the effect after ergot inhibition. Further, he observed that: (1) not all motor effects are replaced by inhibition, some being abolished, (2) stimulation of the sympathetic nerves and the injection of nicotine produces the same abnormal inhibitory effect as does adrenalin, (3) the normal inhibitory actions of adrenaline and sympathetic excitation not only escaped abolition by ergot, but preserved their normal types unchanged. Confronted with these facts and observations, Dale offered the following explanations to account for the reactions exhibited by these three groups of organs: (1) in the first group, the myoneural junctions (24, p. 435), being predominantly inhibitory, are not perceptibly affected, (2) in the second group, being purely motor, the junctions

are simply paralyzed, (3) in the third group, being mixed, but predominantly motor, the junctions undergo a reversal of function, since the paralysis of the normally preponderant motor elements allow the emergence of a normally masked inhibitory effect. His explanation of the dichotomous nature of epinephrine holds to this day.

From this time to 1948, there was no significant development regarding the nature of the receptors nor their mechanism of action, although in 1933 there was a restatement of Langley's earlier proposal by Cannon and Rosenblueth (16, p. 566). From their experimental results they were lead to conclude that a substance A, from an outside source, or M, from a local source from within the animal, united with another substance H, thus making a combination AH or MH which was then responsible for the reaction which was proportional to the amount formed. The H, however, was to be regarded as either I (inhibitory) or E (excitatory), and the combination after nerve stimulation, for example, would be ME in contracting muscle and MI in relaxing muscle. In their terminology Sympathin is a chemical mediator released from the sympathetic nerve endings which become either Sympathin E or Sympathin I when it combines with the receptive substance in the cell. This Sympathin E or I was then responsible for the reaction. They

postulated that this Sympathin E and I could circulate in the blood and cause their effects elsewhere in organs innervated by the sympathetic. Thus, their substance H appears to be what Langley called receptive substance, and their Sympathin E and I appears to be what Langley called the "chief" substance (42, p. 400). The Sympathin E and I have never been isolated, nor demonstrated experimentally.

Recent Development of Receptor Theory

It was not until 1948, when Ahlquist (1) proposed his alpha and beta receptor theory, that some advancement was made regarding the nature of these receptors. Previously, the adrenotopic receptors had been considered to be of two classes, those whose actions resulted in excitation, and those whose actions resulted in the inhibition of the effector cells. In his experiment 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 (1, p. 586). He was able to demonstrate that one type of receptor was associated with most of the inhibitory responses and the other was associated with most of the excitatory

responses, although there were exceptions in each case. After testing a series of six amines on various organs, he found that all the organs which were stimulated responded in the same relative order and further that all those organs which were inhibited responded to a different order of amines in the same manner. He named those receptors concerned mainly with the excitatory responses alpha receptors. These were most sensitive to epinephrine, which was 2 to 10 times more potent than levarterenol and more than 100 times more potent than isoproterenol. Those receptors which were mainly responsible for the inhibitory responses were called beta. These receptors were most sensitive to isoproterenol, which was approximately 2 to 10 times more potent than epinephrine, and more than 100 times more potent than levarterenol (22, p. 380). It should be understood that it was probable that both receptors are present in every sensitive organ, and that the resultant response was due to the predominance of one type over the other.

Despite the attractiveness of this theory, Lands (40) proposed another concept for the receptors, because he objected to the assumption that the stimulation of either receptors may cause either excitation or inhibition. Further, he objected to the assumption that

the nature of the response was determined by the particular organ after the union of the stimulator with receptor. To avoid these suppositions, Lands proposed the following classification: Ac receptors for the excitatory responses, Ar for the inhibitory, and Acr for the undifferentiated receptors which would respond to stimulation by substances with a strong affinity for either receptor.

In 1959 while summarizing some of the work regarding receptors, Furchgott (32) also proposed a modification of Ahlquist's classification. Alpha would designate those receptors responsible for the contraction of smooth muscle (other than intestinal), beta receptors for the relaxation of smooth muscle (other than intestinal), delta receptors for the inhibition of intestinal smooth muscles, and gamma receptors for glycogenolysis. These distinct and separate classifications were proposed because he felt that it was naive to attribute the many diverse effects of the catecholamines to a common primary metabolic route.

At the recent Ciba Foundation Symposium on Adrenergic Mechanisms, some notable advances appeared to be in the making (5)(6)(10)(33)(71). Perhaps, the most provocative report came from Belleau (10) who suggested that the receptor sites may be a phosphate anion which combined with the sympathetic amines through electrostatic attraction. In his analysis of the chemical

requirements for the excitatory activity, he found strong support for the view that the primary step in the triggering of responses involved the pairing of an ammonium ion with a negative charge on the receptor. Through the conceptual constructs of isosterism, he developed several explanations which appeared to clarify many observations regarding these receptors. He attributed the excitatory responses to the union of the receptor anion with those substances which carried a small cationic head. The small catonic head was the most effective configuration in the neutralization of these sites. He, then, suggested that the steric hindrance of ion pair formation produced through the introduction of substituent groups on the basic nitrogen resulted in inhibitory activity. This he deduced from Coulomb's Law:

$$F = \frac{q_1 q_2}{Dr^2}$$

F equals the force of attraction between the two oppositely charged ions (q), D is the dielectric constant, and r is the radius between the two charges. Thus, the force of attraction was the greatest when the radius was small (those compounds with small cationic heads possessed the greatest excitatory effect), and least when the radius was great (those with large ionic groups, e.g. secondary and tertiary, had inhibitory effects)(10, p. 228-232).

Location of Receptors

Despite the considerable number of papers published regarding these receptors, our understanding concerning their identity and mechanism of action is still somewhat theoretical. Therefore, the location of these inscrutable receptors is necessarily speculative. There is evidence which implicates the cellular membrane as the location. Most of this evidence, largely electro-physiological, has been accumulated by such people as Bozler (13) using wick electrodes, Bülbring (14) using intracellular electrodes, and Burnstock (15) using the "sucrose gap" method. The other site suggested for the location was the intracellular material. When Langley (42, p. 411) first proposed the presence of these receptive substances, it was understood to be within the protoplasmic material. Later workers (25)(53)(60)(64)(66) favored the intracellular location because of the various metabolic activities which were located within the cell.

Antagonism of Receptors

Perhaps the best substantiated characteristic of these receptors is the ability to block or to inhibit their activity with some degree of specificity. The first investigator to note the phenomena

of drug antagonism, or blockade, was Dale in 1905 while studying the physiological actions of ergot (19). His paper contained a thorough and accurate account of the pharmacological activity now called adrenergic blockade. This was the abolition of many, but not all, responses to adrenaline, noradrenaline, and other sympathomimetic amines as well as sympathetic nerve activity. Since that time various types of blockade has been distinguished (66)(67). The first type is called competitive antagonism. These antagonists interfere with the reaction of the agonist with the receptors. These compounds are in a mass action equilibrium with the receptor and the blockade produced is a measure of the competition between agonist and antagonist for the receptors. Included in this class of adrenergic blockers are the ergot alkaloids (e.g. Hydergine ®/1, imidazolines, benzodioxanes, yohimbine, and certain synthetic compounds of the isoquinoline group (66, p. 43-66)(67, p. 444).

The second type of blockade is called a non-equilibrium antagonism. This group is characterized as being irreversible and is not in a mass action equilibrium with the receptors. These form

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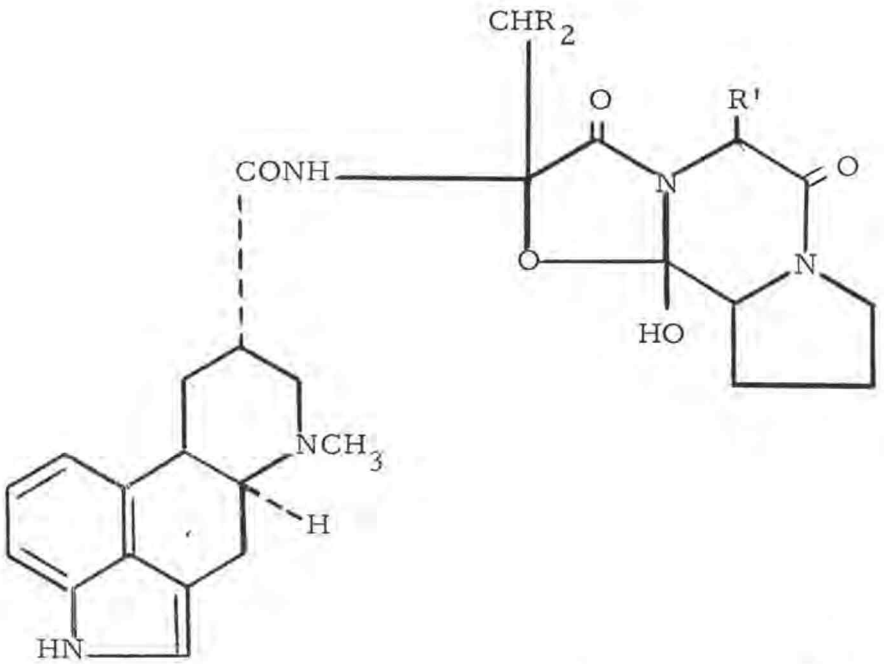
/1 Reg. trade mark of Sandos Pharmaceuticals' for brand of equilproportional mixture of Dihydroergocornine, Dihydroergokryptine, and Dihydroergocristine as methanesulfonate. One cc ampul contains 0.1 mg of each ergot alkaloid. See Figure 1.

Figure 1

General Structure for the three ergot alkaloids of Hydergine ®



	R	R'
Dihydroergocristine	CH_3	$CH_2C_6H_5$
Dihydroergokryptine	CH_3	$CH_2CH(CH_3)_2$
Dihydroergocornine	CH_3	$CH(CH_3)_2$

Hydergine ® (an equiproportional mixture of Dihydroergocornine, Dihydroergocristine, and Dihydroergokryptine as methanesulfonates)

rather stable complexes with the receptors and are not overcome with massive doses of the agonist. At the present time there are only two groups which have been shown to be of this type: the β-haloalkylamines and the phenoxybenzamine cogeners (66, p. 28-43) (67, p. 444).

The third and last type of blockade is called non-competitive antagonism. The blocking agents produce their effects somewhere between the receptors and the ultimate response. Thus, they do not compete for the receptors, nor do they form any complexes, consequently their activity is not too specific and not much attention has been given to this group (67, p. 444).

Physiological Characteristics of Receptors

Adrenergic receptors are also characterized as to the type of physiological response they will or will not elicit as a result of this blockade. Unfortunately, the responses to the various adrenergic blockers are variable, and further, the adrenergic blockers have physiological effects other than their blocking activity (67). Thus, only a general rule may be made regarding the response of various inhibitors. According to the current terminology of Ahlquist (1), the alpha receptors are concerned primarily with the excitatory activities, and it is normally these receptors which are blocked by

the various antagonists discussed under the types of adrenergic blockade. The physiological functions which have been antagonized, or blocked, include the following: nictitating membrane contraction (66, p. 29)(67, p. 449), pressor response (2)(47)(55)(67, p. 449) (81), retractor penis contraction (47), prevention of epinephrine induced cardiac arrhythmias (22, p. 380)(67, p. 449), seminal vesicle contraction (66, p. 45)(67, p. 449), certain uterine contractions (66, p. 55)(67, p. 449)(75), hyperkalemia (29), the inhibition of epinephrine induced lymphocyte depression (76), and mydriasis (66, p. 31).

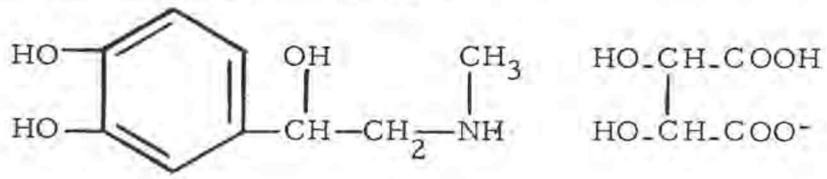
The inhibitory effects were not amenable to adrenergic blockade to any great extent until Powell and Slater (69) introduced a new compound called dichloro isoproterenol (1-(3', 4' dichloro-phenyl)-2-isopropylaminoethanol hydrochloride). This is a dichloro analogue of isoproterenol (DCI). The chemical structures are shown in Figure 2. Since the introduction of this compound many tissues and organs have been reinvestigated. The chronotropic and inotropic contractions of the heart were inhibited by this compound (64). This specific beta blocker was instrumental in demonstrating that the inhibitory actions of the intestine were mediated by both alpha and beta receptors (2)(45).

Figure 2

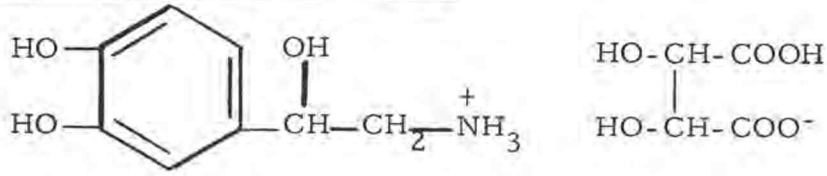
Structure formulas for adrenergic stimulators and blockers

Sympathomimetic Amines

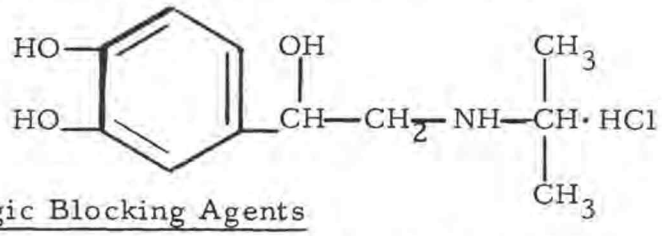
Epinephrine Bitartrate U.S.P.



Levarterenol Bitartrate U.S.P.

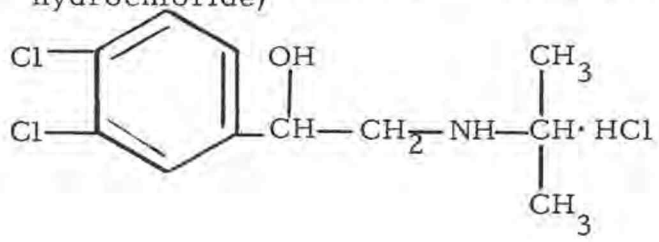


Isoproterenol Hydrochloride U.S.P.



Adrenergic Blocking Agents

DCI (1-(3', 4'-dichlorophenyl)-2-isopropylaminoethanol hydrochloride)



Of the various metabolic effects mediated by the catecholamines, the most prominent are liver glycogenolysis with the subsequent rise in blood sugar, and muscle glycogenolysis with the resultant rise in both blood sugar and lactic acid (58). Although there are many reports regarding the increase in both blood sugar (17)(18)(25)(27)(28)(30)(36)(52, p. 312)(54, p. 45)(56)(57)(58)(77)(80) and lactic acid (4)(8)(21)(28, p. 487)(30)(52, p. 10-19)(53, p. 312) (56)(57)(58)(60, p. 14-19)(61)(62)(63) as a result of epinephrine, levarterenol, isoproterenol and other sympathetic amines, the reports as to their blockade have been somewhat conflicting. The reported inhibition of this response has varied from being relatively effective to relatively ineffective (17)(56)(57)(58)(66, p. 48)(67, p. 454), but there have been no reports as to a complete block. Indeed, some investigators have found that a rise in blood sugar has resulted from some adrenergic blockers (17)(57)(58). The foregoing statements are equally applicable to blood lactate.

In 1956 while re-evaluating Ahlquist's concept of alpha and beta receptors (75)(76)(77), Van der Pol tested the effects of epinephrine, levarterenol, and isoproterenol on the glycemic response in rats. He found that although epinephrine produced the greatest increase in blood sugar, the two other amines, both potent

alpha and beta stimulators respectively, also caused a small but significant increase (77). This was contrary to the then-accepted postulation that the alpha receptors were responsible for the mediation of excitatory responses and the beta for the mediation of inhibitory responses. In order to preserve Ahlquist's concept, Van der Pol suggested that both receptors were responsible for the hyperglycemic effect. He also suggested that the maximum rise in blood sugar would occur from the synergistic interaction of these two receptors. This would explain the potent glycemic effect of epinephrine and the rather mild response from levarterenol and isoproterenol. This would also resolve the conflict regarding the observations pertaining to the indifferent hyperglycemic blockades as well as the varied hyperglycemic intensities.

To confirm Van der Pol's contention, Claasen and Noach (17), with the aid of DCI, the potent and specific beta blocker, tested the hyperglycemic effect of these same three amines with and without beta blockade. Their data shows that the hyperglycemic response to isoproterenol was completely inhibited, while the hyperglycemic response to epinephrine was only reduced, and the hyperglycemic activity of levarterenol was not affected at all. Thus, it appears that when the beta component was blocked, the

alpha receptors were still able to continue mediating their effects.

Mayer et al. (58), using these same amines with DCI and ergotamine on dogs, reported blood sugar values which were not in accord with Claasen and Noach. Mayer reported the hyperglycemic potency of epinephrine and of isoproterenol to be equally effective and that the effect of levarterenol was considerably less. They also found that the increase in blood sugar caused by levarterenol, an alpha stimulator, was prevented by DCI, a supposedly specific and potent beta blocker. Claasen, however, was unable to demonstrate a blockade with this combination. Indeed, this was the only combination which showed no adrenergic blocking activity (17). Mayer et al., reported, further, that they were able to inhibit the hyperglycemic response of epinephrine with DCI as well as with ergotamine, whereas the DCI blockade reported by Claasen et al. was only partial. Since DCI blocked epinephrine induced augmentation of cardiac contraction, heart phosphorylase, and hyperglycemia, Mayer et al. suggested that the actions must be mediated through the beta receptors.

McCutcheon (57), using various amines which included epinephrine and isoproterenol with the blockers DCI, dibenzyline, and ergotamine on dogs, reported observations which tended to support

the contention that the glycemic response was due to the beta receptors. He found that DCI was effective in preventing both hyperglycemia as well as lacticacidemia. Thus, he concluded, that the responsible receptors must be of the beta type. He reported, further, that the alpha adrenergic blocker, dibenzyline, was ineffective in preventing the increased production of blood sugar and lactic acid. This, then, was further support that the receptors mediating these responses were of the beta type, or as Mayer et al. (58) also suggested, they may be due to some unknown receptor(s).

Thus, it is clear that some conflict remains concerning the receptors, if any, responsible for the mediation of blood sugar and lactic acid. It is clear that if Ahlquist's classification is to be maintained, the order of potency for alpha stimulation must be levarterenol (lev) > epinephrine (epi) > isoproterenol (iso), and for the beta receptors the potency order must be iso > epi > lev. Mayer et al. (58) found that the hyperglycemic potency of epinephrine and isoproterenol was about equal, and that that of levarterenol was considerably less. McCutcheon (57) reported a potency order of epi > iso. Neither of the hyperglycemic potencies as reported by Mayer et al. nor McCutcheon (57) fit the above classification for beta stimulation. Further, a survey of the literature shows that

the potency of isoproterenol, a beta stimulator, for hyperglycemic activity has been the lowest (17)(25)(27)(56)(80) while epinephrine, an alpha and beta stimulator, has had the greatest hyperglycemic potency. The glycemic potency, according to the Claasen and Noach (17) and Ellis (25), for the three amines is approximately epi>lev> iso. Since epinephrine is a potent stimulator for both alpha and beta receptors, this would be the relative order expected. It appears, then, that the contention as stated by Van der Pol (77), that the full glycogenolytic effect requires the interaction of both receptors, has the greatest merit. Claasen and Noach's (17) work has provided substantial support for this hypothesis.

EXPERIMENTAL PROCEDURE

Introduction

This experiment is to furnish what Claude Bernard (11, p. 55) calls counter-proof for the hypothesis that the rise in blood sugar and lactic acid is the result of the synergistic interactions of both alpha and beta receptors. In the following experiment both alpha and beta stimulators as well as blockers were used. For if the glycemic response was due to both receptors, the conjoint administration of both alpha and beta stimulators should produce a rise in blood sugar and lactic acid greater than either alone. Further, if both alpha and beta adrenergic receptors were blocked, the subsequent administration of epinephrine or levarterenol-isoproterenol combined should produce no effects. A complete adrenergic blockade would then support the contention that the alpha and beta receptors were responsible for the mediation of hyperglycemia as well as lacticacidemia. The first premise of hyperglycemic potentiation has been demonstrated by Van der Pol (77); the second premise of complete adrenergic blockade was demonstrated in the present experiment.

Experimental Design

This experiment was composed of six treatment combinations and were numbered as follows: (I) simultaneous control (34): saline against saline (II) epinephrine against saline, (III) levarterenol-isoproterenol combined against saline, (IV) saline against DCI-Hydergine combined, (V) epinephrine against DCI-Hydergine combined, and (VI) levarterenol-isoproterenol combined against DCI-Hydergine combined. Statistically, this is a 2 x 3 factorial design with randomized complete blocks. The adrenergic blockers with saline constituted one factor. And the adrenergic stimulators with saline, epinephrine, and levarterenol-isoproterenol combined constituted the other factor. Thirteen replications were made with each replication representing an individual experiment composed of six treatment combinations with one rat per treatment. The random numbers table (49, p. 487) was used to group the rats into blocks, to determine the order of treatment, and to determine the assignment of treatment to each rat.

Experimental Methods

Adult female rats weighing approximately 230 Gm were used. Treatments were started after 16-18 hours of fasting. In the

pretreatment three rats each received two injections of 0.1 ml saline, and the three other rats received DCI 10 mg/Kg and Hydergine 0.2 mg/Kg conjointly. Thirty minutes after this pretreatment, the three rats receiving saline without adrenergic blockers were given: (I) saline (simultaneous control) 0.1 ml, (II) epinephrine 0.217 mg/Kg, and (III) levarterenol 0.217 mg/Kg and isoproterenol 0.217 mg/Kg combined. The three rats pretreated with the combined alpha and beta adrenergic blockers were treated in the same manner: (IV) saline 0.1 ml, (V) epinephrine 0.217 mg/Kg, and (VI) levarterenol 0.217 mg/Kg and isoproterenol 0.217 mg/Kg combined. Injections of all amines were made from a 0.001 M solution with 0.1 % each of chlorobutanol and sodium bisulfite as preservatives. All animals were routinely anesthetized with sodium pentobarbital (IP) 30 mg/Kg before any treatment. Since all doses were given intraperitoneally the doses found in Van der Pol (77) and Claasen (17) were reduced by one-half to give approximately the same activity according to Ellis (25).

Blood Sugar and Lactic Acid Analysis

One hour after treatment (17)(25)(77), blood was collected by cardiac puncture, with a 1 ml tuberculin (precision) syringe coated with heparin (38, p. 93)(54)(58), and was ejected directly into the

precipitating solution (31). After deproteinization by the method of Van Slyke and Hawkins (78, p. 767), blood sugar was determined according to the method of Nelson (65) and lactic acid by the method of Barker and Summerson (9).

RESULTS

Blood Sugar

The individual observations for the blood sugar values in mg % are shown in Table 1. Treatment (I) the simultaneous control, have values which range from 76 to 127 mg %. This range, as well as the average, are in accordance with those values found in the literature (17)(25)(36)(77). Treatment (II) epinephrine against saline have values which range from 146 to 247 mg % which are also within the range cited above. Treatment (III) the combination levarterenol-isoproterenol against saline, included values which are both higher and lower than those reported by Van der Pol (77) for the same treatment combination. The low value of 102 mg % may be due to the inadvertant omission of the challenging drug, for the corresponding lactate value was also low. The high value of 206 mg % appears in a replication which, with the exception of one treatment, are all generally higher. This is possibly due to the variation of day to day experimental technique.

The last three treatment combinations are composed of the same three challenging drugs, but these are tested against the adrenergic blocking combination of DCI-Hydergine. The values for

TABLE 1

Individual observations of blood sugar values in mg % as affected by (I) saline, (II) epinephrine, (III) levarterenol-isoproterenol after pretreatment with saline and (IV) saline, (V) epinephrine, (VI) levarterenol-isoproterenol after pretreatment with DCI-Hydergine.

Treatments	Replications													Standard Error
	1	2	3	4	5	6	7	8	9	10	11	12	13	of the Mean
I	76	127	92	85	112	83	118	88	82	81	114	85	89	± 4.64
II	210	247	216	285	173	146	176	198	232	217	266	146	207	± 11.75
III	143	156	115	150	117	120	124	142	102	148	206	136	135	± 7.17
IV	153	124	107	98	112	104	102	113	126	89	93	106	87	± 4.95
V	98	175	176	107	133	131	142	126	116	100	120	102	113	± 7.08
VI	99	92	107	87	112	106	98	120	112	98	114	115	111	± 2.75

$$\text{Standard error of the mean} = \text{S. E.} = S\bar{y} = \pm \sqrt{\frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{(n-1)(n)}}$$

these treatments are slightly higher than that of the control. The difference between treatment (I), the simultaneous control with saline against saline, and treatment (IV), saline against DCI-Hydergine, reflects the hyperglycemic effect of the adrenergic blockers (17)(57)(58). Treatment (V), however, included values which are higher than can be attributed to the effect of the two antagonists. This may be due to an incomplete blockade.

From Table 2, the six pretreatments with the corresponding treatments are summarized with the number of replications, mean body weight of the rats, the mean blood sugar value in mg % and the deviation between each treatment and the control. It can be seen that the adrenergic blocking agents are responsible for a 14 mg % rise in blood sugar. The subtraction of this value from values obtained in treatments (V) and (VI) would show the effectiveness of the adrenergic blockade.

Blood Lactate

The individual blood lactate values can be seen in Table 3. Despite the range of some of these values found in treatments (I) and (II), they are well within the range according to the values reported in the literature (4)(8)(21)(56)(57)(58)(59)(61)(62)(63). No

TABLE 2

A summary of the six treatment combinations for blood sugar.

Pretreatment	Treatment	No. of replicates	Mean body weight	Mean blood sugar values in mg %	Mean deviation from control
Saline	Saline	13	230 Gms	95	0
Saline	Epinephrine	13	226	210	115
Saline	Levarterenol-isoproterenol	13	237	138	43
DCI-Hydergine	Saline	13	232	109	14
DCI-Hydergine	Epinephrine	13	251	126	31
DCI-Hydergine	Levarterenol-isoproterenol	13	247	105	10

TABLE 3

Individual observations of blood lactate values in mg % as affected by (I) saline, (II) epinephrine, (III) levarterenol-isoproterenol after pretreatment with saline and (IV) saline, (V) epinephrine, (VI) levarterenol-isoproterenol after pretreatment with DCI-Hydergine.

Treatments	Replications												Standard Error of the Mean
	1	2	3	4	5	6	7	8	9	10	11	12	
I	10.5	11.2	8.9	12.6	17.2	7.1	5.5	7.4	2.2	8.2	14.2	6.5	± 1.19
II	21.7	26.4	27.3	31.2	12.2	6.7	53.0	42.8	16.1	24.4	36.0	22.1	± 3.72
III	23.0	17.5	14.9	28.2	6.1	5.2	21.6	6.6	15.3	15.1	23.0	33.7	± 2.57
IV	13.6	14.7	9.4	15.6	8.0	5.2	36.0	20.8	9.6	12.2	11.4	13.9	± 2.30
V	10.2	16.2	22.0	39.0	11.0	4.4	17.1	18.6	8.5	14.1	12.0	7.9	± 2.60
VI	7.1	9.1	10.1	67.0	13.7	7.3	9.2	17.2	7.5	19.5	12.0	13.9	± 4.75

$$\text{Standard error of the mean} = \text{S. E.} = S\bar{y} = \pm \sqrt{\frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{(n-1)(n)}}$$

values were found in the literature which correspond to the remaining treatments. The direction and magnitude of lactacidemia produced by treatment combination (III) are the same as the blood sugar for the same treatment. In each of treatments (IV), (V), and (VI), there are values which are considerably above the others e.g. (treatment 4, replication 7; treatments 5 and 6, replication 4). At the time of sampling it was suspected that these values would not be normal, but they were included for maintaining the completeness of each block as well as to determine the direction and magnitude of these abnormal samples. For the statistical analysis they should have been dropped with the insertion of dummy values (49, p. 210), or they should have been Winsorized (74, p. 17) to minimize the effects of these values. Rather than run the risk of criticism for manipulating data, these values were included as such. It was interesting to note, however, that had these values been modified by either method, the values for treatments (IV), (V), and (VI) would have all been of the same magnitude ($11.7 \pm .3$). Further, the standard error of the mean for treatments (IV) and (V) would have been reduced by one-half, and for treatment (VI) by three-quarters.

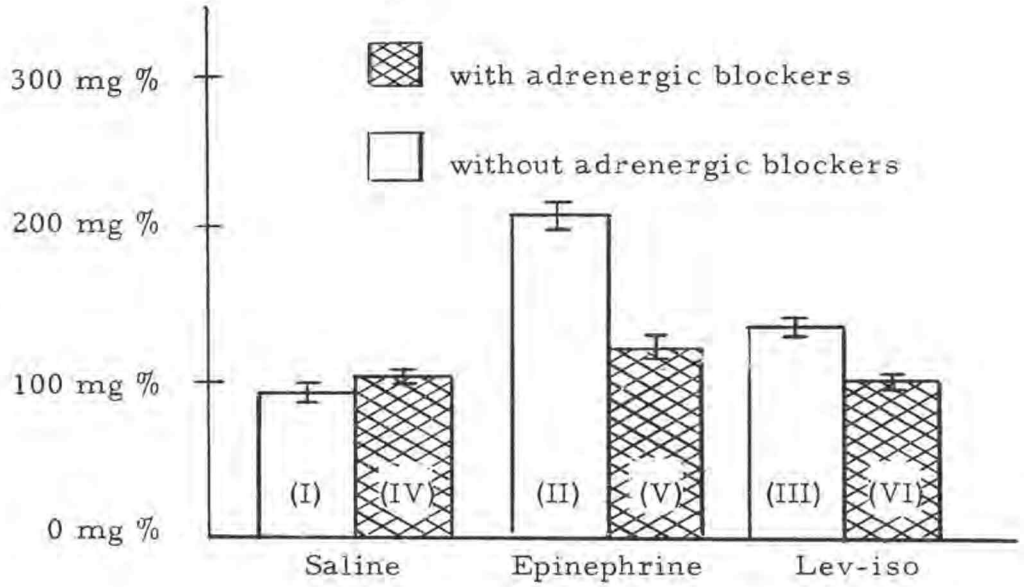
Table 4 provides a summary of the lactate results, showing the six pretreatments with the corresponding treatments, the number of replications, the mean weight of the rats, the mean lactate values, and the deviation between each treatment and the control. The difference between treatments (I) and (IV) is 5 mg %. This increase can be attributed to the blocking agents (57)(58). The subtraction of this value from the values found in treatments (V) and (VI) would show the effectiveness of the adrenergic blockade. The effectiveness of the blockade for both blood sugar and lactic acid can be seen in Figures 3 and 4 by a comparison of the bar graphs.

TABLE 4

A summary of the six treatment combinations for the blood lactate.

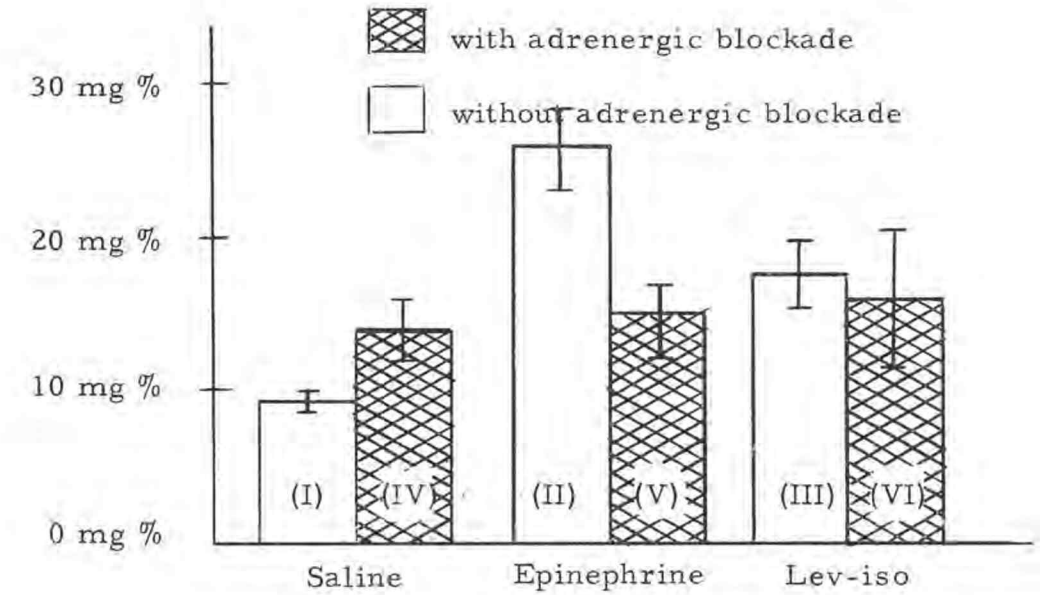
Pretreatment	Treatment	No. of replicates	Mean body weight	Mean blood lactate values in mg %	Mean deviation from control
Saline	Saline	12	230 Gms	9.2	0.0
Saline	Epinephrine	12	226	26.6	17.4
Saline	Levarterenol-isoproterenol	12	237	17.5	8.3
DCI-Hydergine	Saline	12	232	14.2	5.0
DCI-Hydergine	Epinephrine	12	251	15.1	5.9
DCI-Hydergine	Levarterenol-isoproterenol	12	247	16.1	6.9

Figure 3. Mean blood sugar values in response to challenging drugs before and after alpha and beta blockade.



Numbers in parentheses refer to the treatment combination. The values for S. E. are in Table 1.

Figure 4. Mean blood lactate values in response to challenging drugs before and after alpha and beta blockade.



Numbers in parentheses refer to the treatment combination. The values for S. E. are in Table 3.

STATISTICAL ANALYSIS AND DISCUSSION

Blood Sugar

The treatment totals shown in Table 5 in conjunction with the individual observations in Table 1, are used for the calculations of the analysis of variance of blood sugar and are found in Table 6 under that heading.

Replication, the first source of variation, gives a measure of the differences between the 13 individual experiments. This difference will be significant if the computed F value in the analysis either of variance or of indivudual degree of freedom falls within a certain critical region as determined by the significance level and the degrees of freedom. The critical region for F at the 5% significance level with 12 and 60 degrees of freedom occurs where F is greater than 1.9174. The F value obtained from the analysis of variance calculations is 1.4071, which is outside the critical region. This means that the difference in experimental technique as well as animal variation from day to day did not significantly influence the blood sugar values for the six treatments.

The differences between treatments constitute the second source of variation. The critical region for F at the 5%

TABLE 5

Statistical layout with treatment totals
for blood sugar and lactic acid

BLOCKERS / STIMULATORS	SALINE	DCI-HYDERGINE	STIMULATOR SUM
	B. sugar B. lactate	B. sugar B. lactate	B. sugar B. lactate
SALINE	Treatment (I)	Treatment (IV)	SUM S^1
Blood sugar	1232	1414	2646
Blood lactate	111.5	111.5	223.0
EPINEPHRINE	Treatment (II)	Treatment (V)	SUM S^2
Blood sugar	2719	1639	4358
Blood lactate	319.9	122.2	442.1
LEVARTERENOL-ISOPROTERENOL	Treatment (III)	Treatment (VI)	SUM S^3
Blood sugar	1794	1371	4358
Blood lactate	210.2	134.8	345.0
BLOCKER SUM	SUM B^1	SUM B^2	GRAND TOTAL
Blood sugar	5745	4424	10,169
Blood lactate	641.6	368.5	1,010.1

TABLE 6

Analysis of variance calculations
for blood sugar

Preliminary Calculations

Type of total	Total of Squares	No. of Items Squared	Observations per Squared Item	Total of Squares per Observations
Grand	103,408,561	1	78	1,325,750.784
Replication	8,014,967	13	6	1,335,827.833
Treatment	18,694,579	6	13	1,438,044.538
Adrenergic Blockers	52,576,801	2	39	1,348,123.102
Adrenergic Stimulators	36,010,705	3	26	1,385,027.115
Observations	1,483,927	78	1	1,483,927.000

Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Replications	10,077.049	12	839.754	1.4071
Treatment	112,293.754	5	22,458.750	37.6340
Adrenergic Blocker	22,372.318	1	22,372.318	37.4898
Adrenergic Stimulators	59,276.331	2	29,638.165	49.6654
Interaction	30,645.105	2	15,322.552	25.6764
Error	35,805.413	60	596.756	
Total	158,176.216	77		

Significance level at 5% with 12 and 60 degrees of freedom=1.9174
5 and 60 degrees of freedom=2.3683
1 and 60 degrees of freedom=4.0012
2 and 60 degrees of freedom=3.1504

significance level with 5 and 60 degrees of freedom occurs where F is greater than 2.3683. The computed F value 37.6340 is inside the critical region. This means the blood sugar values vary according to the stimulators used as well as according to the blockers.

Because all treatments were analyzed collectively, no information can be obtained regarding the differences within a particular level without a more specific statistical analysis. Therefore, treatment was partitioned into three components: adrenergic blockers, adrenergic stimulators, and interaction. The source of variation in adrenergic blockers is from the two pretreatment combinations, with and without adrenergic blockers. The critical region for F at the 5% level with 1 and 60 degrees of freedom occurs where F is greater than 4.0012. The computed F value 37.4898 is inside the critical region. This means that the difference in hyperglycemic activity between the pretreatments was great. The conclusion is that the combined adrenergic blockers have inhibited a rise in blood sugar. The next source of variation, the adrenergic stimulators, gives a measure of the differences between the three challenging drugs. The critical region for F at the 5% level with 2 and 60 degrees of freedom occurs where F is greater than 3.1504. The computed F value 49.6654 is inside the

critical region which means that the three challenging drugs produced increases in blood sugar which were significantly different. The change in blood sugar after saline is taken as zero (0); thus the change after levarterenol-isoproterenol is 43 mg % and after epinephrine is 115 mg %. This analysis of adrenergic stimulators, however, gives no information regarding the differences between the treatments after these amines for the adrenergic blockers; therefore, treatment must be further partitioned to provide this information. This analysis will be considered later. The last source of variation, interaction, gives a measure of the differences between the corresponding treatments. That is, did these treatments vary in a constant manner? The critical region for F at the 5% level with 2 and 60 degrees of freedom occurs where F is greater than 3.1504. The computed F value 25.6764 is inside the critical region. This means that the treatments varied independently and according to the treatment combination received.

For more and specific information regarding these treatments, the source of variation treatment was again partitioned, this time into individual degrees of freedom. Analysis of this partition reveals the relative activity or inactivity between individual treatment combinations. Multipliers were selected so that a certain

hypothesis might be tested. These multipliers, as well as the computed Q^2 and F values, are shown in Table 7 with the corresponding F value at the 5% significance level with 1 and 60 degrees of freedom. The combination of the first set of miltipliers tests the hypothesis that the effect of the treatment receiving saline against saline is the same as the effect of the treatment receiving saline against DCI-Hydergine. The critical region for this and the following tests occurs where F is greater than 4.0012. The computed F value is 2.1349 which is outside the critical region and the hypothesis is accepted. Thus, the slight hyperglycemic effect of the adrenergic blockers was not statistically significant.

The second hypothesis formed by the multipliers tests the contention that the effect of the treatment receiving epinephrine against saline is the same as the effect of the treatment receiving epinephrine against DCI-Hydergine. The computed F value 751.7568 is inside the critical region and the hypothesis is rejected. This means that the adrenergic blockade had prevented epinephrine induced hyperglycemia.

The next hypothesis formed by the multipliers tests the contention that the treatment receiving levarterenol-isoproterenol against saline is the same as the treatment effect of levarterenol-isoproterenol against DCI-Hydergine. The computed F value

TABLE 7

The partitioning of treatment SS into individual degrees of freedom for blood sugar

Multipliers						Computed Q^2 Values	Computed F Values	F Value at 5% significance level with 1 and 55 d. f.
M_1	M_2	M_3	M_4	M_5	M_6			
1	0	0	-1	0	0	1,274.000	2.1349	4.0012 n. s. *
0	1	0	0	-1	0	448,615.384	751.7568	4.0012 s. **
0	0	1	0	0	-1	6,881.884	11.5321	4.0012 s.
0	0	0	1	-1	0	1,947.115	3.2628	4.0012 n. s.
0	0	0	1	0	-1	71.115	0.1191	4.0012 n. s.
1	-1	0	0	0	0	85,044.961	142.5121	4.0012 s.
1	0	-1	0	0	0	12,147.846	20.3564	4.0012 s.

* n. s. Not significant

**s. significant

11.5321 is inside the critical region and the hypothesis is rejected. This means that the combined adrenergic blocking agents were able to inhibit the levarterenol-isoproterenol induced hyperglycemia.

The fourth and fifth hypotheses formed by the next two sets of multipliers test the contention that the effects of the two treatments receiving DCI-Hydergine were the same regardless of the sympathetic amines used. The computed F values, 3.2628 and 0.1191, are both outside the critical region and the hypotheses are accepted. This means that the degree of inhibition was the same for either treatment of adrenergic stimulators.

The six and seventh hypotheses formed by the last two sets of multipliers test the contention that the treatment effects for the saline pretreatments were the same regardless of the sympathetic amines used. Both of the computed F values, 142.5121 and 20.3564, are inside the critical region. Therefore the hypotheses are both rejected. The size of the computed F values for epinephrine and levarterenol-isoproterenol is indicative of their hyperglycemic potencies.

To be more precise, the glycemic component of the adrenergic blockers should be subtracted from each individual observation before the statistical analysis is begun, but since a preliminary

calculation indicated that these values were significant as such, no adjustments were made. The results, as well as the statistical analysis of the results, support the hypothesis that glycogenolysis is mediated through the alpha and beta adrenotropic receptors.

Blood Lactate

A preliminary calculation of the results indicated that some of the hypotheses to be tested would not be significant. To increase the differences between treatments, the rise in blood lactate as a result of the adrenergic blocking agents (57)(58) was subtracted from treatment totals (IV), (V), and (VI) to give the true blocking potency. Each individual observation was also adjusted before the analysis of variance and the test of the individual degree of freedom. Since one value, as a result of this adjustment, would have been negative, the adjustment for that value was made by Winsorization (74, p. 17). These adjusted values can be seen in Table 8. The preliminary calculations for the analysis of variance of blood lactate can be seen in the top of Table 9 .

Replication, the first source of variation considered, gives a measure of the differences between the 12 individual experiments. The critical region for F at the 5% significance level with 11 and

TABLE 8

Individual observations of blood lactate which have been adjusted by subtracting the effect of the blocking agents for computing the various F values for the analysis of variance.

Treatments	Replications											
	1	2	3	4	5	6	7	8	9	10	11	12
I	10.5	11.2	8.9	12.6	17.2	7.1	5.5	7.4	2.2	8.2	14.2	6.5
II	21.7	26.4	27.3	31.2	12.2	6.7	53.0	42.8	16.1	24.4	36.0	22.1
III	23.0	17.5	14.9	28.2	6.1	5.2	21.6	6.6	15.3	15.1	23.0	33.7
IV	8.7	9.8	4.5	10.7	3.1	.3	31.1	15.9	4.7	7.3	6.5	9.0
V	5.3	11.3	17.1	34.1	6.1	.3*	12.2	13.7	3.6	9.2	7.1	3.0
VI	2.2	4.2	5.2	62.1	8.8	2.4	4.3	12.3	2.6	14.6	7.1	9.0

* This value has been Winsorized (34).

TABLE 9

Analysis of variance calculations
for blood lactate

Preliminary Calculations

Type of total	Total of Squares	No. of Items Squared	Observations per Squared Item	Total of Squares per Observations
Grand	1,020,504.04	1	72	14, 173.667
Replication	102,942.20	12	6	17, 157.033
Treatment	204,510.74	6	12	17, 042.561
Adrenergic Blockers	547,442.81	2	36	15, 206.744
Adrenergic Stimulators	364,206.41	3	24	15, 175.267
Observations	24,521.58	72	1	24, 521.580

Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Replication	2,983.366	11	271.215	3.3180
Treatment	2,868.894	5	573.778	7.0196
Adrenergic Blocker	1,033.077	1	1, 033.077	12.6387
Adrenergic Stimulators	1,001.600	2	500.800	6.1268
Interaction	834.217	2	417.108	5.1029
Error	4,495.653	55	81.739	
Total	10,347.913	71		

5 % significance level 1.9763 with 11 and 55 d.f.
2.3886 with 5 and 55 d.f.
4.0221 with 1 and 55 d.f.
3.1707 with 2 and 55 d.f.

55 degrees of freedom occurs where F is greater than 1.9763. The F value computed by the analysis of variance is 3.3180 which is inside the critical region. This means that the differences between experimental technique and animal variation from day to day did significantly influence the lactate values for the six treatment combinations. Since it was known that the blood lactic acid varied when normal (4)(8)(18)(21)(52, p. 14-19)(53, p. 312)(59)(60, p. 35)(61)(62)(63), this small but significant F value was expected. During the course of the experiment, it was found that the unusual handling of the animals resulted in an increase in lactic acid, but that the blood sugar was not significantly altered. This may be because the blockade for the blood sugar was much more stable, or because the blood lactate equilibrium was more sensitive, and therefore more susceptible to change even in the presence of adrenergic blockade.

Treatment, the next source of variation, gives a measure of the differences between the six treatment combinations. The critical region for the F value at the 5% significance level with 5 and 55 degrees of freedom occurs where F is greater than 2.3886. The computed F value 7.0196 is inside the critical region. This means that the lactate values varied according to the stimulators

used as well as according to the blockers.

Since all treatment combinations were analyzed collectively, no information can be obtained regarding the significance of the differences between particular levels without a more specific statistical method. Therefore, treatment was partitioned into three components: adrenergic blockers, adrenergic stimulators, and interaction. The source of variation in adrenergic blockers is from the two pretreatment combinations, with and without DCI and Hydergine. The critical region for F at the 5% significance level with 1 and 55 degrees of freedom occurs where F is greater than 4.0221. The computed F value 12.6387 is inside the critical region. This means that the alpha and beta receptors were blocked and that the sympathomimetic amine induced hyperlacticacidemia has been inhibited. This supports the contention of this thesis.

The next source of variation, adrenergic stimulators, gives a measure of the differences between the three challenging drugs. The critical region at the 5% significance level with 2 and 55 degrees of freedom occurs where F is greater than 3.1707. The computed F value 6.1268 is inside the critical region. This means that the three challenging amines produced increases in blood lactate which were significantly different. The change in blood lactate after

saline is taken as zero (0); thus, the change after levarterenol-isoproterenol is 8.3 mg %, and after epinephrine is 17.4 mg %. This, however, provides no information regarding the variations between the treatment combinations of these amines against DCI and Hydergine; therefore, treatment must be further partitioned for this information. This analysis will be considered later.

Interaction, the last source of variation, gives a measure of the differences between corresponding treatments. That is, did these treatment combinations vary in a constant manner? The critical region for F at the 5% significance level with 2 and 55 degrees of freedom occurs where F is greater than 3.1707. The computed F value 5.1029 is inside the critical region. This means that the treatments varied independently and according to the treatment combination received.

For more and specific information regarding these treatments, the source of variation treatment was again partitioned, this time into individual degrees of freedom. Analysis of these components will reveal the relative activity, or inactivity, between the individual treatment combinations. Multipliers were selected so that a certain hypothesis might be tested. These multipliers, as well as the computed Q^2 and F values, are shown in Table 10 with the

TABLE 10

The partitioning of treatment SS into individual degrees of freedom for blood lactate

Multipliers						Computed Q^2 Values	Computed F Values	F Value at 5% Significance Level With 1 and 55 d.f.
M_1	M_2	M_3	M_4	M_5	M_6			
1	0	0	-1	0	0	0.000	0.000	4.0221 n.s. *
0	1	0	0	-1	0	1,638.553	20.0461	4.0221 s. **
0	0	1	0	0	-1	236.881	2.8980	4.0221 n.s.
0	0	0	1	-1	0	4.770	0.0583	4.0221 n.s.
0	0	0	1	0	-1	22.620	0.2767	4.0221 n.s.
1	-1	0	0	0	0	1,809.606	22.1388	4.0221 s.
1	0	-1	0	0	0	405.903	4.9658	4.0221 s.

* n.s. Not significant

** s. significant

corresponding F value 4.0221 at the 5% significance level with 1 and 55 degrees of freedom. Since the treatment totals were adjusted, there is no need to test the first hypothesis as shown by the first set of multipliers.

The second hypothesis formed by the multipliers tests the contention that the treatment effect of epinephrine against saline is the same as the treatment effect of epinephrine against DCI-Hydergine. The computed F value 20.0461 is inside the critical region and the hypothesis is rejected. This means that the combined alpha and beta adrenergic blockers have prevented epinephrine induced lacticacidemia.

The third hypothesis formed by the multipliers tests the contention that the treatment effect of leverterenol-isoproterenol against saline is the same as the treatment effect of levarterenol-isoproterenol against DCI-Hydergine. The computed F value 2.8980 is outside the critical region and the hypothesis is accepted. This means that the adrenergic blocking combination has failed to inhibit the rise of lactic acid induced by the combined levarterenol-isoproterenol. The acceptance of this hypothesis has lead to a Type II error (49, p. 45). A comparison of the lactate values for treatments (III)and (IV)indicates that the similarity in values is due

to the slight hyperlacticacidemic effect of the levarterenol-isoproterenol combination, rather than to a lack of blocking action.

The fourth hypothesis formed by the multipliers tests the contention that the treatment effect of saline against the adrenergic blockers is the same as the treatment effect of epinephrine against the adrenergic blockers. The computed F value 0.0583 is outside the critical region and the hypothesis is accepted. This means that the effectiveness of the adrenergic blockade was the same for saline and epinephrine.

The fifth hypothesis formed by the multipliers tests the contention that the treatment effect of saline against the adrenergic blockers is the same as the treatment effect of levarterenol-isoproterenol against the adrenergic blockers. The computed F value 0.2767 is outside the critical region and the hypothesis is accepted. This also means that the effectiveness of the adrenergic blockade was the same for saline and levarterenol-isoproterenol. This justifies the conclusion that a Type II error had been made in accepting the third hypothesis.

The sixth hypothesis formed by the multipliers tests the contention that the treatment effect of saline against saline is the same as the treatment effect of epinephrine against saline. The computed F value 22.1388 is inside the critical region and the

hypothesis is rejected. The large F value is an index of the increase in blood lactate after epinephrine.

The last hypothesis formed by the last set of multipliers tests the contention that the treatment effect of saline against saline is the same as the treatment of levarterenol-isoproterenol against saline. The computed F value 4.9658 is just inside the critical region and the hypothesis is rejected. This F value means that the increase in blood lactate after levarterenol-isoproterenol was just significant at the 5% level.

The results, as well as the statistical analysis of these results, show that epinephrine and levarterenol-isoproterenol produced significant increases in both blood sugar and lactic acid. Further, the results show that neither hyperglycemia nor hyperlacticacidemia was demonstrable in the presence of the adrenergic blockers.

If the contention that epinephrine has a greater glycogenolytic effect than either levarterenol or isoproterenol because it stimulates both alpha and beta receptors, then it is reasonable to assume that the combined treatment of levarterenol (1 unit) and isoproterenol (1 unit) would produce an effect greater than the treatment of epinephrine (1 unit). This experiment has shown that this is not the case. The effect was neither greater nor equal, as Van der Pol (77) has also reported. To preserve the first contention, the

following possibilities are offered in explanation.

(1) That both levarterenol and isoproterenol are more susceptible to degradation. Von Euler (79, p. 41) using isotopes, studied the decay time of epinephrine and levarterenol in vivo and found that this time was shorter for levarterenol. Also, Blaschko and Burn (12), while studying the ubiquitous amine oxidase, found that it had a preference for levarterenol. Thus it appears that epinephrine has a greater duration of action in which to cause its effects.

(2) That the degradation product(s) of epinephrine, possibly levarterenol (7)(37, p. 320), is (are) also capable of producing hyperglycemia and hyperlacticacidemia. Thus, after the action of epinephrine has terminated, the degradation product could continue stimulating the receptors (alpha).

(3) That the greatest increase in blood sugar is mediated through the alpha receptors located in the liver. This has been reported by Vrij et al. (80). Their studies with these three amines have shown that glycogen depletion from skeletal muscle was greater with isoproterenol than with levarterenol, and that glycogen depletion from the liver was greater with levarterenol. This would explain why the hyperglycemic potency of levarterenol was greater.

This study has failed to confirm the first contention that the combined administration of levarterenol and isoproterenol would produce an increase in blood sugar and lactic acid greater than epinephrine, and the above possibilities were offered in explanation. The complete adrenergic blockade, however, was clearly demonstrated by its ability to inhibit both blood sugar and lactic acid. This supports the contention that glycogenolysis is mediated through the alpha and beta adrenotropic receptors.

SUMMARY AND CONCLUSIONS

(1) Six treatment combinations were compared for their production of blood sugar and lactic acid. The challenging drugs-saline, epinephrine, and levaterenol-isoproterenol combined-were tested against saline (without adrenergic blockers) and against DCI and Hydergine.

(2) Epinephrine and levarterenol-isoproterenol, the challenging amines, were both effective in increasing blood sugar and lactic acid, although epinephrine was more potent.

(3) The adrenergic blockade produced by DCI combined with Hydergine was effective in inhibiting hyperglycemia and hyper-lacticacidemia.

(4) Since a specific blockade of both alpha and beta adrenergic receptors prevented the glycogenolytic effects of epinephrine and levarterenol-isoproterenol, it is concluded that this effect is mediated through these receptors.

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