I.C.
AKW
B44

AWPP
B44
1973.

BETA-ADRENERGIC RECEPTORS MEDIATING CHANGES IN MECHANICAL RESPONSES AND CYCLIC AMP LEVELS IN RAT ATRIUM AND UTERUS: DIFFERENTIATION USING ENANTIOMERS OF ISOPROTERENOL

BY JAY EDWARD BIRNBAUM

Under the supervision of Assistant Professor Carl Kenneth Buckner

Beta adrenergic receptors of rat atria and uteri have been evaluated by measuring changes in mechanical responses and cyclic AMP levels produced by the enantiomers of isoproterenol. The quantitative difference in potency between the isomers was used to characterize the beta receptors of each tissue. This value, the isomeric potency difference, was calculated as: [(negative log molar ED_{50} (-)-isomer) - (negative log molar ED_{50} (+)-isomer)]. Under proper experimental conditions to control non-receptor processes, dissimilar isomeric potency differences are expected to provide a sensitive indication of receptor differences.

In experiments on atria, inotropic responses were determined from electrically stimulated left atria, while chronotropic responses and cyclic AMP levels were determined from spontaneously beating right atria. While the (+)-isomer was less potent than the (-)-isomer by a factor of over 1000, both isomers were capable of producing equal maximum responses. Increases in rate and force in response to an effective concentration of either isomer were apparent within 10 - 15

seconds and were completely developed in 3 - 6 minutes. In contrast, cyclic AMP levels were elevated as early as 5 seconds after exposure to isoproterenol and attained maximum by 15 seconds. Approximately 100 times the concentration of each isomer was required to elicit a 50 per cent of maximum increase in cyclic AMP than to produce an equal degree of increase in rate or force. However, under similar experimental conditions, isomeric potency differences for mechanical responses and increases in cyclic AMP were nearly identical. These values are 3.31, 3.51, and 3.48 log units for rate, force, and cyclic AMP, respectively. Mechanical responses and increases in cyclic AMP induced by both isomers of isoproterenol were blocked by the beta receptor antagonist practolol.

In experiments on uteri, decreases in contractile amplitude and increases in cyclic AMP levels were measured in spontaneously contracting uterine segments. Maximum effects on the mechanical response usually occurred within 2 - 4 minutes after addition of an effective concentration of isoproterenol. Elevations in cyclic AMP were observed as early as 15 seconds after addition and reached maximum within 120 seconds. As in atria, the concentrations of the isomers of isoproterenol required to produce increases in cyclic AMP were approximately 400 times those to produce mechanical effects. Under similar experimental conditions, the isomeric potency differences were 2.90 log units for uterine relaxation and 2.79 log units for increases in uterine cyclic AMP.

Dissimilar isomeric potency differences, obtained from atria and uteri, are consistent with the existence of different beta receptors in the two tissues. These differences are apparent at the levels of both cyclic AMP formation and mechanical responses. The results indicate that both cyclic AMP formation and mechanical responses induced by isoproterenol may be manifestations of the same receptor event. A role for cyclic AMP in the mediation of mechanical responses is suggested, and this is supported by the observed potentiation of mechanical responses by theophylline. Possible explanations for the differences in effective concentrations of isoproterenol for producing mechanical responses and increases in cyclic AMP levels are considered.

APPROVED: Carl K. Buckner

Carl K. Buckner, Ph.D.
Assistant Professor

DATE: 10/1/73

BETA-ADRENERGIC RECEPTORS MEDIATING CHANGES IN MECHANICAL RESPONSES AND CYCLIC AMP LEVELS IN RAT ATRIUM AND UTERUS: DIFFERENTIATION USING ENANTIOMERS OF ISOPROTERENOL

BY

JAY EDWARD BIRNBAUM

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(Pharmacy)

at the

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

1973

ACKNOWLEDGEMENTS

I wish to thank

Dr. Carl K. Buckner

for his instruction, guidance and support throughout my graduate training and this research project

Mr. Peter W. Abel

for his technical assistance

TO

WENDY, BERNIE and WILLIE

TABLE OF CONTENTS

	Page
INTRODUCTION.	1
Development of the Adrenergic Receptor Concept . .	1
Alpha and Beta Adrenergic Receptors.	8
Metabolic Receptors.	13
Beta Receptor Subclassification.	17
STATEMENT OF THE PROBLEM.	33
MATERIALS AND METHODS	41
Pretreatment of Experimental Animals	41
Preparation of Isolated Tissues.	42
General Considerations.	42
Mechanical Responses from Atria	44
Mechanical Responses from Uterine Segments. .	45
Increases in Cyclic AMP Levels in Atria and Uterine Segments	46
Dose-Response Curves	46
Mechanical Responses.	46
Cyclic AMP Increases.	47
Cyclic AMP Determinations.	48
Isolation of Cyclic AMP-Binding Protein . . .	49
Preparation of Tissue Samples	51
Cyclic AMP Assay.	53
Protein Determinations	55
Drugs and Solutions.	58

Page

RESULTS. 60

Atrial Mechanical Responses to Enantiomers of Isoproterenol. 60

Increases in Atrial Cyclic AMP Levels in Response to Enantiomers of Isoproterenol . . 72

Uterine Mechanical Responses to Enantiomers of Isoproterenol 86

Increases in Uterine Cyclic AMP Levels in Response to Enantiomers of Isoproterenol . . 86

Effect of DES on the Increases in Atrial Rate in Response to Isoproterenol 105

Increases in Atrial Rate in Response to Desoxyisoproterenol. 106

DISCUSSION 111

SUMMARY. 131

APPENDIX 132

BIBLIOGRAPHY 135

LIST OF TABLES

Page

1. Effect of AG 1-X8 Formate Separation on the Assay of Cyclic AMP Levels in Tissue Extracts 54
2. Mechanical Responses to Isoproterenol in Rat Atria. 61
3. Increases in Cyclic AMP Levels in Response to Isoproterenol in Rat Atria. 79
4. Increases in Cyclic AMP in Response to Isoproterenol in Rat Atria 83
5. Mechanical Responses to Isoproterenol in Rat Uteri. 87
6. Time Response of Isoproterenol-Induced Cyclic AMP Increases in Rat Uteri 95
7. Increases in Cyclic AMP Levels in Response to Isoproterenol in Rat Uteri 96
8. Increases in Cyclic AMP in Response to Isoproterenol in Rat Uteri 102
9. Summary of Isomeric Potency Differences for Isoproterenol in Rat Atria and Uteri 112

LIST OF FIGURES

Page

1. Cyclic AMP assay standard curve. 56
2. Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat atria in the presence of tropolone. 63
3. Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat atria in the presence of phentolamine and tropolone 66
4. Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat atria in the presence of theophylline (10^{-4}M), phentolamine, and tropolone 69
5. Effects of theophylline, 10^{-3}M, on the increase in atrial rate in response to (-)-isoproterenol in the absence and presence of practolol, 10^{-3}M. 73
6. Time-response graph for increases in cyclic AMP levels in response to (-)-isoproterenol, 10^{-5}M, obtained from rat atria. 75
7. Log dose-response curves for increases in cyclic AMP levels in response to (-)- and (+)-isoproterenol, obtained from rat atria 77

Page

8. Effect of practolol, 10^{-4}M, on the increase in cyclic AMP levels in response to isoproterenol in rat atria. 84

9. Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat uteri in the presence of phentolamine and tropolone 88

10. Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat uteri in the presence of theophylline (10^{-4}M), phentolamine, and tropolone 90

11. Time-response curves for increases in cyclic AMP levels in response to (-)- and (+)-isoproterenol obtained from rat uteri 93

12. Log dose-response curves for increases in cyclic AMP levels in response to (-)- and (+)-isoproterenol obtained from rat uteri 99

13. Effect of practolol, 10^{-4}M, on the increase in cyclic AMP levels in response to isoproterenol in rat uteri. 103

14. Log dose-response curves for increases in atrial rate in response to (-)-, (+)-, and desoxy-isoproterenol. 109

Page

15. Log dose-response curves for mechanical responses and increases in cyclic AMP induced by (-)- and (+)-isoproterenol in rat atria. 121

INTRODUCTION

A drug receptor has been defined by Furchgott (1964) as the:

> Postulated specific molecular site or structure in (or on) an effector cell, with which molecules of a specific agonist must react in order to elicit the characteristic response of the cell to the agonist.

A specific receptor type, therefore, is known to exist by virtue of the substances with which it interacts. The basis for identification as an adrenergic receptor in particular, is that these are the sites in (or on) the effector cell through which epinephrine (adrenaline) is supposed to exert its various effects. All such sites have in common the ability to interact with epinephrine, and if one wishes to distinguish them from one another, it can best be done on the basis of their differential sensitivity to other drugs. Thus, pharmacological differentiation is the initial process by which information has been obtained about adrenergic receptors, and it is basic to more rigorous methods of characterization and isolation which have been attempted (DePlazas and DeRobertis, 1972; Lefkowitz and Haber, 1971; Moran and Triggle, 1970; Ochoa et al., 1972).

Development of the Adrenergic Receptor Concept

Our present concepts about adrenergic receptors have their origin at the turn of the century with the observations of Langley (1901):

> Adrenalin might then be the chemical stimulant liberated on each occasion when the impulse arrived at the periphery.

The nature of the "mechanism" to which Elliot alluded is unclear in its context. Langley (1905) disagreed with the postulated trophic influence of the sympathetic nerve fiber. With respect to the"mechanism", he considered it to involve two distinct components of the muscle cell itself: one, the "chief substances" which carried out the primary functions of the cell such as contraction, relaxation, and the formation of metabolic products; two, the "receptive substances" which were capable of setting the "chief substances" into action.

> In general an increase or decrease in function in a cell brought about by chemical or nervous stimulation depends upon the presence in the cell of different receptive bodies...A cell may make motor or inhibitory receptive substances or both, and that the effect of a nervous impulse depends on the proportion of the two kinds of receptive substance which is affected by the impulse.

The functional distinctions made between "chief" and "receptive substances" and between "motor" and "inhibitory" receptive substances were soon substantiated by the studies of Dale (1906) on the physiological actions of ergot alkaloids. He found that the pressor effects of $BaCl_2$ or pituitary extract were unaltered by doses of ergot preparations which eliminated the pressor effects of adrenaline or sympathetic nerve stimulation. Moreover, Dale demonstrated that:

> ...ergot contains a principle which has a paralytic action on the motor elements of that myotrophic structure or substance which is excited by adrenaline and by impulses in fibers of the true sympathetic system; the inhibitor elements of the same being relatively or absolutely unaffected.

On the basis of the functional relationship between the supra-renal extracts, epinephrine, and sympathetic nerve stimulation, as well as "morphological evidence that their [supra-renal] medulla and sympathetic ganglia have a common parentage" (Elliot, 1904), it was erroneously believed that epinephrine was the mediator of sympathetic nerve effects. After studying the "sympathomimetic actions" of several series of synthetic analogs of epinephrine, Barger and Dale (1910) found such a conception untenable. Teleologically, demethylated epinephrine (norepinephrine) would more appropriately serve the mediator role, since it was a chemically simpler molecule that had activity comparable to epinephrine. Functionally, primary amines (including norepinephrine) more closely "mimicked" the effects of sympathetic nerve stimulation than did secondary amines analogs (including epinephrine) of the same series. Epinephrine weakly or doubtfully reproduced the motor effects of nerve stimulation while exaggerating inhibitory effects. Similarly, certain motor effects of epinephrine were reversed by smaller doses of ergotine than were needed to reverse sympathetic nerve effects. Most ostensible was the fact that in cats pretreated with ergot,

epinephrine produced a decrease in blood pressure whereas sympathetic nerve stimulation (or norepinephrine) still produced an increase. They generalized from their studies that:

> Motor and inhibitor sympathomimetic activity vary to some extent independently. Of the catechol bases those with a methylamino group, including adrenine, reproduce inhibitor sympathetic effects more powerfully than motor effects. The opposite is true of the primary amines of the same series.

Unfortunately, the impetus of such evidence of a mediator role for norepinephrine and for the existence of separate receptors was lost. Following the experimental lapse caused by World War I, the discoveries from the laboratories of Otto Loewi and of Walter Cannon had firmly established the existence of chemical neurotransmission (see Cannon and Rosenblueth, 1937). To explain the effects of ergot, theories of differential activity based on chemical structure of the transmitter began to arise. Most prominent was that proposed by Cannon and Rosenblueth (1933). These workers were impressed by Loewi's demonstration that stimulation of the sympathetic fibers distributed to the heart of frogs and turtles produced a substance which was identified chemically and pharmacologically as epinephrine (as it was later learned, amphibians are an exception in containing only epinephrine in their sympathetic nervous system). According to their theory, epinephrine or an epinephrine-like substance, M, is liberated from the sympathetic nerve and combines

inside the muscle cell with a differentiating agent. Depending upon the particular cell, the differentiating agent exists as either E (excitatory) or I (inhibitory) or both. The combination products ME, called sympathin E, or MI, called sympathin I, are the actual chemical mediators of contraction and relaxation, respectively. The combination products might also escape from the cell into the blood stream and produce their characteristic responses in other organs of sympathetic innervation. Thus, while epinephrine was probably the substance released from the nerve, it was not itself the neurotransmitter. As to the nature of E and I, Cannon and Rosenblueth (1937) postulated that:

> This differentiating factor is Langley's receptor. No other satisfactory hypothesis has come forth to explain contraction and "inhibition" produced by the same substance.

Bacq (1934) who had worked in Cannon's laboratory considered that sympathin was not a product of the combination between the transmitter and the muscle cell receptor, but the transmitter itself. In this case it seemed less likely that the transmitter was epinephrine than a closely related substance, perhaps norepinephrine.

Greer and coworkers (1938) suggested that two substances, Sc and Sr, are released from stimulated nerves, and <u>without</u> further differentiation, affect cells either locally or distally via the blood stream. Sc was probably norepinephrine, and Sr was probably epinephrine. Furthermore, the experimental evidence indicated:

> ...while either agent may cause relaxation or contraction, depending upon the effector smooth muscle cell concerned, one of them [Sc] has a greater intrinsic power of inducing contraction but less intrinsic power of inducing relaxation than the other [Sr].

In addition to the dualistic nature of a given agent, however, it was apparent to these investigators that:

> ...there are marked differences in effector cells themselves. In considering the responses of effector cells to various substances, it is quite necessary to distinguish between these differences in the cells and any intrinsic differences in the substances in question.

Consistent with the observations of both Greer and Langley were those of Youmans and coworkers (1939). After studying various analogs of epinephrine to determine the relative importance of the different functional groups, they concluded:

> It is clear that no consistent relationship exists between the smooth-muscle-relaxing (intestine) and the smooth-muscle-contracting (nictitating membrane) properties of these amines. Such a result is in accord with the postulation of at least two types of smooth muscle with regard to "receptive mechanisms."...If there are only two physiological types of smooth muscle, it seems that the agents should show the same order of potency for one of these types regardless of the organ in which it is located.

They also found that cardiac effects, while stimulatory in nature, were similar to intestinal effects in their requirement for functional groups on the epinephrine molecule

(Youmans et al., 1940). Using various N-alkyl substituted homologues of epinephrine, Lands et al. (1947) pursued further the structural requirements of sympathomimetic substances for producing either stimulatory or inhibitory effects. They designed the N-isopropyl (isoproterenol) and N-sec-butyl homologues as sympathin-I mimetic on the basis of their ability to decrease blood pressure, dilate bronchioles, and relax the uterus, small intestine, and colon. The one stimulatory action of these substances on the heart was attributed to liberation of excitatory sympathin E.

Alpha and Beta Adrenergic Receptors

In a classic study of "adrenotropic receptors", Ahlquist (1948) refuted Cannon's and Rosenblueth's "law of physiology" concerning the existence of two mediator substances. On a variety of different sympathetic functions he evaluated the relative potencies of six different catecholamines: 1) (±)-norepinephrine, 2) (±)-alpha-methyl-norepinephrine, 3) (±)-epinephrine, 4) (-)-epinephrine, 5) (±)-alpha-methyl-epinephrine, and 6) (±)-isoproterenol. The relative potency in decreasing order was 4, 3, 1, 2, 5, 6, for producing contraction of the smooth muscles of the vasculature (vasoconstriction), uterus, ureter, nictitating membrane, and pupillary dilator, and also for relaxation of the smooth muscle of the intestine. A second order (6, 4, 5, 3, 2, 1) was observed for inhibition of the smooth muscles of the vasculature (vasodilation) and uterus and also for excitation

of cardiac muscle (positive inotropism and chronotropism). The distinct orders of potency were attributed to the involvement of two distinct kinds of receptors which Ahlquist designated alpha and beta, respectively. Clearly, neither excitation nor inhibition was the exclusive domain of either receptor. For example, the cardiac receptor was more closely related to the vasodilator receptor than to the vasoconstrictor receptor, while the intestinal inhibitory receptor appeared to be more closely related to the vasoconstrictor receptor. In support of this "mixed" classification was the fact that all of the excitatory responses produced by epinephrine, only those on the heart could not be inhibited by the adrenergic blocking agent Dibenamine (Nickerson and Goodman, 1947; Nickerson, 1949).

Ahlquist noted that of all the catecholamines tested, epinephrine was the most potent on the alpha receptor and nearly the most potent on the beta receptor. As such, it was the most suitable substance to serve as the sympathetic neurotransmitter. His comparisons had been made with racemic mixtures of all compounds except epinephrine, however. Using the levo-isomers of both epinephrine and norepinephrine, Luduena et al. (1949) found that (-)-norepinephrine was as potent as (-)-epinephrine on the rabbit ileum, more potent than (-)-epinephrine in elevating blood pressure and stimulating dog heart, but less potent on dog retractor penis and cat nictitating membrane. The question of suitability

was really a moot point. By the late forties, Euler (1946; 1948) demonstrated rather conclusively that the pressor activity in extracts of various sympathetic nerves could be attributed to a substance which corresponded in its chemical and pharmacological properties to (-)-norepinephrine, and Peart (1949) showed that this substance was released after stimulation of intact sympathetic nerves. As Barger and Dale (1910) had predicted forty years earlier, the sympathetic neurotransmitter in mammals was indeed different from the active principle of supra-renal extracts.

For several reasons, Ahlquist's classification scheme was not readily accepted. Firstly, it was difficult to accept the tacit assumption that the receptor was responsible only for the combination with the drug and not for the subsequent type of response of the tissue. Langley had suggested this "separation of powers" when he made the distinction between receptive and chief substances. Secondly, no agent was available to demonstrate selective blockade of <u>beta</u> receptor responses in the manner that Dibenamine and ergotamine could produce blockade of <u>alpha</u> receptor responses. Lands (1952) argued that:

> If vasoconstriction and intestinal relaxation involve the same type of adrenotropic receptor (alpha receptor) as proposed by Ahlquist, epinephrine reversal after an adrenolytic agent should be associated with blockade of the intestinal response.

Perhaps then, receptors for intestinal muscle might better

be placed in the same class with other smooth muscle inhibitory receptors, which were also resistant to the blocking effects of adrenolytic agents.

With respect to the "anomalous" excitatory receptors of the heart belonging in the same class with inhibitory receptors, Lands also took issue. After evaluating a number of sympathomimetic compounds, he found that cardiac stimulation was produced by a far wider range of structurally varied compounds than were smooth muscle stimulation or inhibition. He felt that these considerations justified a reclassification of adrenergic receptors into three types: Ac, Ar, and Acr. The Ac receptors, mediating smooth muscle contraction, and the Ar receptors, mediating smooth muscle (including intestine) relaxation, each had different structural requirements for activation. The meta-phenolic hydroxyl seemed most important for compounds which interacted with the Ac receptor, while the alcoholic hydroxyl was most important for the interaction with the Ar receptor. The adrenergic receptor of the heart, Acr, was undifferentiated and could be stimulated by compounds which had a strong affinity for either Ac or Ar.

Ahlquist's theory was considerably strengthened by the finding that dichloroisoproterenol (DCI) was a compound which selectively blocked <u>beta</u> receptors in both smooth muscle (Powell and Slater, 1958) and the heart (Moran and Perkins, 1958) without affecting <u>alpha</u> receptors. Differentiation of

adrenergic receptors could now be made on the basis of both the relative agonistic potency of sympathomimetic amines in producing various responses as well as the ability of receptor selective antagonists to block those responses. The intestinal receptors which Ahlquist had classified as alpha on the basis of agonist orders of potency, however, did not seem to be susceptible to blockade by either alpha or beta receptor blocking agents. Several reports had been made concerning the ability of ergot alkaloids to diminish the inhibitory response of the isolated rabbit intestine to epinephrine (Thienes, 1929; Rothlin et al., 1954). Furchgott (1959) reexamined this effect more extensively and found that dihydroergotamine did in fact block the inhibitory action of low concentrations of epinephrine. However, this blockade could be overcome by a twenty-fold increase in the concentration of epinephrine and an equal increase in the concentration of dihydroergotamine was now ineffective. Furchgott concluded:

> This latter finding throws some doubt on whether dihydroergotamine in blocking the inhibitory action of low concentrations of epinephrine on the intestine is actually competing for adrenergic receptors in that structure.

The troublesome intestinal receptors were finally put in their place by Ahlquist and Levy (1959). They made the following observations on the intact dog ileum. The alpha blocking agents Dibenamine, Dibenzaline, and Dibozane completely blocked the response of the tissue to phenylephrine, an

agonist whose actions were restricted primarily to alpha receptors. High concentrations of these blocking agents partially blocked the response to epinephrine, an agonist with actions on both alpha and beta receptors, but were without effect on the response to isoproterenol, which acts primarily on beta receptors. The beta receptor blocker DCI had no effect on the response of the tissue to either phenylephrine or epinephrine, but consistently blocked the response to isoproterenol. Combination of DCI and an alpha receptor blocking agent completely abolished the response to epinephrine, phenylephrine, or isoporterenol. It was clear that intestinal inhibition was mediated by both alpha and beta receptors.

Metabolic Receptors

During this period of intensive research on adrenergic receptors, various investigators were attempting to "fit" the metabolic actions of epinephrine into the framework of receptor theory. One of the earliest implications of epinephrine in a metabolic role was its ability to produce glycogenolysis in liver, skeletal muscle, cardiac muscle, and certain types of smooth muscle. The effect was first observed in rabbit liver slices and was shown to occur through the activation of glycogen phosphorylase (Sutherland, 1950; Sutherland and Cori, 1951). The potency of (-)-epinephrine was six times that of (-)-norepinephrine in activating the enzyme in this preparation while amphetamine was totally inactive. Moreover, the ergot alkaloids could block the

glycogenolytic response in these liver slices (Ellis, 1953) just as they could block the epinephrine induced hyperglycemia in intact rabbits (Harvey et al., 1952). In an attempt to relate glycogenolysis to other adrenergic effects, Furchgott (1959) reasoned:

> Since glycogenolysis depends on the ability of intracellular enzymes, it appears most likely that its stimulation is initiated by a reaction of epinephrine with receptors located intracellularly.

Furchgott suggested that a separate set of receptors, different from the alpha or beta class, was involved, and he tentatively designated these as gamma receptors. He was aware that the distinction

> ...implies that stimulation of glycogenolysis is not responsible for the effects of catecholamines on the contractile activity of heart and smooth muscle.

That there might be a relationship between the metabolic and contractile responses was first indicated by the observation that epinephrine-induced phosphorylase activation in skeletal muscle was accompanied by an increase in muscle work (Cori, 1956). Hess, Haugaard, and coworkers later showed that in perfused rat heart, enzyme activation and positive inotropism were associated both temporally and with respect to the orders of potency of sympathomimetic compounds in producing them (Hess and Haugaard, 1958; Haugaard et al., 1959; Kukovetz et al., 1959). Only those sympathomimetic amines which increased inotropic actions were capable of

stimulating myocardial phosphorylase. For both responses, (±)-isoproterenol was more potent than either (-)-epinephrine or (-)-norepinephrine, the latter two being equipotent. Epinine, synephrine, and phenylephrine produced only moderate effects and mephentermine, methoxamine, and metanephrine had no effect on either response. Comparable results were obtained by Mayer and Moran (1960) on open-chest preparations of dog heart. For both increases in cardiac contractility and phosphorylase activity, (-)-epinephrine and (-)-norepinephrine were equipotent but about one-seventh as potent as (±)-isoproterenol. Quite significant was the fact that DCI, but not phenoxybenzamine (an alpha blocking agent), blocked both contractile and enzyme response produced by either sympathetic nerve stimulation or sympathomimetic compounds (Mayer and Moran, 1960; Mayer et al., 1961). While this indicated that the receptors for glycogenolysis in heart are of the beta type, the question remained open as to whether or not the stimulation of heart by catecholamines resulted directly from activation of the phosphorylase system.

The epinephrine-induced activation of phosphorylase could also be shown to occur in homogenate preparations of liver, heart, and skeletal muscle by a process separable into two stages (Rall et al., 1957). In the first stage, the particulate fraction of the homogenate produced a heat-stable factor in the presence of epinephrine. In the second stage, this factor activated phosphorylase in the supernatant fractions in which epinephrine itself was inactive. This factor which mediated

the action of epinephrine was subsequently isolated and characterized as adenosine 3',5'-cyclic-monophosphoric acid or cyclic AMP (Rall and Sutherland, 1958; Sutherland and Rall, 1958; Cook et al., 1957). It was now apparent that epinephrine did not directly "act on" the enzyme phosphorylase, but promoted the formation of cyclic AMP which in turn activated phosphorylase.

Shortly thereafter, catecholamine-induced cyclic AMP formation in particulate preparations of both liver and heart was found to correspond nearly identically to phosphorylase activation in the same tissue. This was true for both the potency ratios of agonists as well as susceptibility to blockade by antagonists (Murad et al., 1960, 1962). With respect to catecholamine-induced changes in the heart, Sutherland and Rall made the following prophetic statement in 1960:

> While future work may accumulate evidence that cardiac inotropic effects of sympathomimetics are not the result of these [glycogenolytic] effects, it may be more difficult to exclude cyclic 3,5-AMP from a role as a mediator of both effects.

The enzyme activity responsible for cyclic AMP formation was intensively studied and named adenyl cyclase (Sutherland et al., 1962; Rall and Sutherland, 1962). As already indicated, in broken cell preparations it was associated with particulate material which was believed to originate from cell membranes or nuclei rather than from microsomes or mitochrondria. Sutherland and coworkers demonstrated that

in such particulate preparations, adenyl cyclase catalyzed the formation of cyclic AMP and inorganic pyrophosphate from ATP. It was quite likely that the adrenergic receptor mediating glycogenolysis was not an "intracellular receptor" after all.

Beta Receptor Subclassification

While there was general acceptance of Ahlquist's receptor classification after the introduction of DCI, several workers obtained results which were not entirely consistent with it. As already indicated, Luduena *et al.* (1949) found (-)-norepinephrine equally potent and more potent than (-)-epinephrine on rabbit ileum and dog heart, respectively. Lands (1949) also reported (-)-norepinephrine to be more potent than (-)-epinephrine in heart, noting that the cardiac receptors had a different profile than other *beta* receptors with respect to the potency of agonists in activating them. He also pointed out that the relative potency of different sympathomimetic amines on one type of effector organ often varied considerably with the species of animal and the particular experimental conditions used (Lands, 1952). Furchgott (1959) addressed himself to this criticism in a series of experiments comparing the relative potencies of the (-)-isomers of epinephrine, norepinephrine, and isoproterenol on isolated effector organs all taken from the same animal (rabbit) and all suspended in the same medium (Krebs-bicarbonate). For excitatory responses of the aorta, stomach,

and uterus the potency ratios of these three compounds were similar with the order of potency being epinephrine > norepinephrine >> isoproterenol, and all responses were blocked by dibenamine--a characteristic alpha receptor profile. In the case of inhibitory responses in aorta and stomach, and the two excitatory responses in atria, the order of potency was isoproterenol > epinephrine > norepinephrine and all responses could be blocked out by DCI. However the actual ratios varied considerably from one response to the next, and in the case of the beta receptors of the duodenum (observed in the presence of phentolamine or dibenamine) norepinephrine was actually more potent than epinephrine.

Furchgott (1967) later extended these studies with particular attention to experimental conditions (see below). He analyzed the adrenergic receptors of various isolated tissues on the basis of the relative potencies of four agonists (isoproterenol: ISO; norepinephrine: NE; epinephrine: EPI; phenylephrine: PE) and on the basis of K_B values (apparent equilibrium dissociation constant for the antagonist-receptor complex) for phentolamine and pronethalol. It was assumed that under proper experimental conditions, similar K_B values should be obtained in all tissues that contained similar receptors types. While the alpha receptors for various tissues appeared to be quite similar (similar K_B values and orders of potency of EPI $\simeq$ NE > PE >> ISO) such was not the case for beta receptors. The latter were divided into three types. In one group were placed rabbit

aorta and guinea-pig trachea with an order of potency ISO > EPI >> NE > PE; in a second group, rabbit atria, guinea-pig atria, and guinea-pig duodenum with an order of potency ISO > NE > EPI >> PE; and in a third group, rabbit duodenum and stomach with an order of potency ISO > NE > or ≈ EPI >> PE. Each group was also characterized by a different K_B value for pronethalol: 3×10^{-8}M, 7.5×10^{-8}M, and 50×10^{-8}M, respectively.

Miller (1967) also noted there were considerable differences reported in the literature in the relative potencies of these four amines on rat and rabbit hearts versus rat uterus. Depending upon the particular study, epinephrine was 17-160 times more potent than norepinephrine in relaxing the uterus while in stimulating the heart, the two amines were about equally potent, differing by a factor of less than 2 in either direction. Isoproterenol was 100-200 times more potent than epinephrine in heart, yet less than 10 times more potent in uterus.

While the quantitative differences between the potencies of isoproterenol, epinephrine and norepinephrine might be subtle from one tissue to another, the differences are often magnified by alteration of the functional groups in these molecules. For example, Lands and Brown (1964) evaluated the relative potency of several compounds with various alkyl substitutions on the α-carbon and nitrogen atoms; two such compounds tested were α-methyl- and α-ethylisoproterenol. Relative to isoproterenol they were approximately 1000 times

less potent on the chronotropic response of perfused rabbit heart yet only 2-3 times less potent on the bronchodilator response in isolated perfused guinea-pig lung. The unequal reductions in potency were quite impressive to Lands and his coworkers who went on to test this series of drugs on a variety of beta receptor responses in isolated tissues and intact animals (Lands et al., 1966; 1967a; 1967b). Their results were consistent with the existence of two different sets of receptors which they designated $beta_1$ and $beta_2$. Those responses mediated by $beta_1$ receptors were increased rate and force of contraction of perfused rabbit heart, inhibition of rabbit small intestine, and lipolysis in rat epididymal adipose tissue. Those responses mediated by $beta_2$ receptors were vasodepression in the anesthetized dog, bronchodilation in the perfused guinea-pig lung, inhibition of isolated rat uterus, and facilitation of contraction of rat diaphragm. Subsequently, lipolysis in guinea-pig epididymal adipose tissue (Arnold and McAuliff, 1968) and calorigenesis in the intact rat (Arnold and McAuliff, 1968) were added to $beta_1$ receptor responses, and lactacidemia in the rat (Arnold and Selberis, 1968) and dog (Arnold et al., 1968) were added to $beta_2$ receptor responses. The structural requirements for $beta_1$ versus $beta_2$ receptor activation were not well defined, but several generalizations were apparent. Norepinephrine was usually equal or more potent than epinephrine in stimulating $beta_1$ receptors, yet as much as several hundred fold

less potent in stimulating $\underline{\text{beta}}_2$ receptors. Isoproterenol was considerably more potent than norepinephrine in stimulating both $\underline{\text{beta}}_1$ and $\underline{\text{beta}}_2$ receptors, but the difference in potency was much less on $\underline{\text{beta}}_1$ receptors. Alkyl substitution on the amine nitrogen or on the α-carbon resulted in greater potency for $\underline{\text{beta}}_2$ than $\underline{\text{beta}}_1$ receptors.

Concurrent with this work on agonists, various investigators were reporting diverse spectrums of action for new antagonists. After the introduction of DCI, Moran and Perkins (1958) had suggested that, just as antagonists could be used to identify receptor types, new antagonists should be classified on the basis of their selectivity in blocking responses mediated by either alpha or beta receptors. Thus pronethalol (Black and Stephenson, 1962), propranolol (Black et al., 1964) and MJ1999 or sotalol (Lish et al., 1965) were readily labeled beta blocking agents since they could block beta receptor-mediated responses in all tissues in the same dose range. However, a more selective pattern of blockade was revealed by two derivatives of methoxamine, N-isopropylmethoxamine (IMA) and N-tertbutylmethoxamine or butoxamine (TMA). Like other beta receptor blocking agents, both IMA and TMA antagonized catecholamine-induced increases in plasma free fatty acids, glucose and lactic acid in conscious and anesthetized dogs (Burns et al., 1964; 1967; Burns and Lamberger, 1965; Salvador et al., 1964) as well as antagonizing the catecholamine-induced relaxation of the isolated rat uterus (Levy, 1964; 1966). In addition, TMA (Levy, 1966; Colville et al.,

1965) but not IMA (Levy, 1964) blocked the vasodilator response to isoproterenol. Unlike other beta receptor blocking agents, however, neither IMA nor TMA could block cardiac stimulation in anesthetized dogs (Levy 1964; 1966) or inhibition of anesthetized dog ileum (Levy, 1964; 1966) or isolated rat duodenum (Levy, 1967) produced by catecholamines.

Moran and VanDeripe had obtained a similar profile of activity for the α-methyl derivative of DCI (VanDeripe et al., 1964; VanDeripe and Moran, 1965; Moran, 1966). Whereas DCI and α-methyl DCI were equipotent in blocking isoproternol-induced vasodilation in anesthetized dogs, DCI was at least 15 times more potent than its α-methyl analog in blocking the positive inotropic effect of isoproterenol. Thus, the beta adrenergic antagonists α-methyl DCI, TMA, and IMA all manifested at least some degree of selectivity for responses which Lands and coworkers had designated as $beta_2$ receptor-mediated on the basis of the selectivity of agonists. Quite interestingly all three antagonists had an α-methyl group. Just as the introduction of an alkyl substituent on the α-carbon atom of isoproterenol, norepinephrine, or epinephrine conferred agonist selectivity for $beta_2$ receptors (Lands and Brown, 1964; Lands et al., 1966; 1967a; 1967b), likewise antagonists which contained an α-methyl group in the ethanolamine side chain were more potent at $beta_2$ receptor sites.

The selective $beta_2$ receptor spectrum of α-methyl DCI,

IMA, and TMA was complemented by that of another beta receptor antagonist, practolol (Barrett et al., 1968; Brick et al., 1968; Dunlop and Shanks, 1968). Unlike the former three, practolol was capable of selectively blocking beta receptors mediating positive inotropic and chronotropic responses, as well as intestinal (Levy and Wilkenfeld, 1969), lipolytic (Barrett et al., 1968), and calorigenic (Arnold and McAuliff, 1969) responses to catecholamines. Practolol might thus be referred to as a $beta_1$ receptor antagonist.

The dual beta receptor classification of Lands and co-workers has received further support with the introduction of several new "selective" agonists discovered in the search for clinically effective bronchodilators with a minimal component of cardiac stimulation. Among these are soterenol (Larsen et al., 1967; Dungan et al., 1968), salbutamol (Brittain et al., 1968; Farmer and Levy, 1968; Cullum et al., 1969) and trimetoquinol (Yamato et al., 1966; Iwasawa and Kiyomoto, 1967; Sato et al., 1967). Selective effects on both tracheal and uterine smooth muscle have been reported for all three agents (Brittain et al., 1970; Farmer et al., 1970), and they are regarded as $beta_2$ receptor agonists.

While there is considerable evidence to support the $beta_1$/$beta_2$ receptor concept, the results of other workers have suggested different or more extensive classification (Bristow et al., 1970; Boissier et al., 1971; Farmer and Levy, 1970; Furchgott, 1967; 1972; Hedges and Turner,

1969; Wasserman and Levy, 1972). Arnold and McAuliff (1971) have pointed out that the differentiation of $\underline{\text{beta}}_1$ from $\underline{\text{beta}}_2$ receptors with agonists manifests selectivity that is not uniformly seen with antagonists. The question can always be raised as to what quantitative differences are required in order to conclude that responses are mediated by different types of receptors. The question really remains an open one, since at the present time there are no drugs which have absolute specificity for any of the proposed types of beta receptors. It is possible that the $\underline{\text{beta}}_1$ and $\underline{\text{beta}}_2$ receptors represent two extremes of an entire population of slightly varying receptors. In this light, Robison et al. (1970) have suggested the extension of the concept of isozymes to receptors, i.e. "isoreceptors", to account for tissue specific differences observed in beta receptors. Others have been duely reticent to make this theoretical leap on the basis of the available data.

Moran (1967) foresaw the tendency to overclassify, stating that:

> As more compounds become more available we are in danger of creating more complex schemes of classification unless we maintain a skeptical attitude and insist upon rigorous analysis.

More often than not receptor analysis has not been rigorous. The fact that certain drugs exhibit selective potency in one or another tissue supports but does not prove the existence of multiple beta receptors. The possibility must be considered that "selectivity" is more a reflection of the

differential concentrations which these drugs attain in the biophase, or receptor region, of different tissues. This concentration is a function of a number of variables including: absorption, distribution and disposition in in vivo experiments; and accessibility to and removal from the biophase both in vivo and in vitro. These variables and the response itself may be complicated further by species differences, prandial and hormonal status, conscious versus anesthetized animals, and the particular experimental design. Much of the data on which the notion of multiple beta receptors is based was obtained from experiments in which such variables were not controlled.

Furchgott (1967; 1970; 1972) has emphasized the need for proper experimental precautions and for more quantitative measurements with agonists and antagonists, and he outlined what he considered to be idealized conditions for the evaluation of receptors. Some of these are as follows:

1. The use of simple isolated tissue preparation offers considerable advantages over the in vivo preparation. In in vitro experiments, complicating factors of absorption, distribution, and metabolism of drugs and the influence of feedback regulatory processes encountered in vivo are greatly minimized.

2. The response of an agonist should be the result of its own interaction with the receptor, and not the result of the release of endogenous transmitter. It is well recognized that the sympathomimetic activity of many agents can be attributed primarily or partially to the release of norepinephrine which in turn interacts with the receptor (Burn and Rand, 1958).

Appropriate pre-treatment of an animal with reserpine can deplete endogenous norepinephrine stores and minimize any indirect component of action which may exist.

3. The response to an agonist should be due solely to the activation of one receptor type. Agents such as norepinephrine and epinephrine which act on both alpha and beta receptors, complicate the study of tissues where both receptor types are present. Thus, in those tissues where alpha and beta receptors mediate responses in opposite directions, agonist potency on either may be underestimated. On the other hand, in the intestine, where both receptors mediate relaxation, agonist potency may be overestimated. To study one receptor type exclusive of the other, an appropriate antagonist of the other must be applied.

4. The concentration of drug in the biophase should be in diffusion equilibrium with that in the bathing or perfusing medium at the time of the response. However, if there are mechanisms which remove free drug at a sufficiently rapid rate, then even at equilibrium, the actual concentration achieved in the biophase may be significantly lower than that in the medium. In most tissues where adrenergic receptors are present there exist four primary mechanisms, other than diffusion, which remove free drug from the biophase. These are: uptake into the adrenergic nerve terminals (Iversen, 1967), uptake into extraneuronal sites (Iverson, 1967), oxidative deamination by the enzyme monoamine oxidase or MAO (Blaschko, 1952) and methylation of the phenolic hydroxyl groups by the enzyme catechol-O-methyl transferase or COMT (Axelrod et al., 1958). The relative importance of each of these mechanisms is dependent upon the particular tissue (density of innervation, enzyme activity, etc.) and the particular drug being tested. Appropriate inhibitors are available to limit any or all of these mechanisms (Furchgott, 1972).

In most tissues, norepinephrine and epinephrine are removed primarily by neuronal uptake followed by deamination within the neuron. Isoproterenol, on the other hand, is not a substrate for either neuronal uptake or MAO (Hertting, 1964), but is taken up extraneuronally and inactivated by COMT. Other sympathomimetic amines are also affected by these mechanisms.

Furchgott (1967) has reported how important the control of these factors can be in properly assessing orders of potency of sympathomimetic drugs at the receptor level. For example, in isolated guinea-pig atria prior to any pretreatment, isoproterenol was 70 times more potent than norepinephrine, and epinephrine was 112 times more potent than norepinephrine. After pretreatment with an agent which blocked neuronal membrane uptake, isoproterenol was now only 3 times more potent than norepinephrine, and epinephrine was only 1/2 as potent as norepinephrine. Each drug had been potentiated to a degree proportional to the extent of uptake (prior to pretreatment), resulting in a reversal of the "classical" order of potency of epinephrine and norepinephrine (Ahlquist, 1948).

On a more speculative level, one might suggest that the bronchial selectivity exhibited by a compound like soterenol might be related to a greater activity of COMT in the bronchus than in the heart. Since soterenol is not a substrate for COMT (Dungan et al., 1968), its potency relative to isoproterenol might appear greater in the bronchus than in the heart, independent of any differential action at the beta

receptors in those tissues.

A significant problem which remains even when proper experimental precautions are taken is that of variations in the accessibility of different agonists and antagonists to the biophase in different tissues. Accessibility is a function of both the anatomical characteristics of the tissue and the physico-chemical characteristics of the drug. The operation of both of these factors is probably reflected in different rates of onset of action for the same drug in different tissues (Buckner and Patil, 1971a) and for different drugs in the same tissue (Buckner, 1972).

An experimental tool for minimizing the accessibility problem in receptor studies is the use of optical isomers of adrenergic drugs. One of the basic properties of the adrenergic receptor is its ability to interact stereoselectively with the enantiomers of adrenergic drugs (Easson and Stedman, 1933; Ariens, 1967). With respect to the asymmetry around the β-carbon atom of the phenylethylamine nucleus of these agents, the levorotatory- or (-)-isomer is considerably more potent than the corresponding dextrorotatory- or (+)-isomer (Patil et al., 1970). Stereoselectivity is assumed to reflect the unique asymmetry of the particular receptor (Pfeiffer, 1956). Patil (1969) suggested that the quantitative difference in potency between the isomers of adrenergic agonists and antagonists would provide a sensitive criterion for differentiating adrenergic receptor types. If the receptors in various tissues were of a single type, then

under proper experimental conditions, the isomeric potency differences would be the same in each tissue. Conversely, if the receptors were not the same in various tissues, then the isomeric potency differences would be dissimilar. Of course it is imperative in using such a procedure that any stereoselective mechanism unrelated to the receptor be carefully controlled.

The advantage of using optical isomers of a single agent in lieu of separate agents is that since the isomers have identical physico-chemical properties, their use controls factors which influence the access of drugs to the biophase. These factors may differ from tissue to tissue for 2 or more structurally dissimilar drugs in an unequal manner. When the isomers of the same compound are employed, however, these factors differ in an equal manner for both, and any change in the potency difference between them should be a reflection of a change in the nature of the stereoselective receptor. One isomer thus serves as a reference standard for the other.

Patil et al. (1971) subsequently tested this hypothesis using the isomers of norepinephrine to activate the alpha receptors of rabbit aorta, vena cava, ileum, and spleen, and rat vas deferens and seminal vesicle. While the actual potency of a single isomer varied as much as 100-fold in the different tissues, the potency differences between (-)- and (+)-norepinephrine were the same for all tissues. In contrast, the potency differences obtained from beta receptor activation

in several different tissues were markedly different. Their results indicated 3 subgroups of receptors in the following tissues: 1) rat atria; 2) rabbit and guinea-pig atria, guinea-pig trachea, and rabbit aorta; and 3) bovine iris sphincter. The presence of multiple beta receptors was in agreement with the results of Furchgott (1967), Lands et al. (1967a;1967b) and others, but the similarity indicated for the receptors of the guinea pig-atria and trachea was not. This observed similarity was studied in greater detail and confirmed by Buckner and Patil (1971b) using isomer potency differences of both agonists and antagonists. Although the compounds possessed different absolute potencies in the two tissues, similar isomeric potency differences indicated that atrial and tracheal beta receptors possessed identical abilities to interact with adrenergic agonists and antagonists. Perhaps the "selectivity" which had been observed by other workers for various drugs in these tissues was related more to the physico-chemical properties of the drugs themselves, than to the properties of the receptors with which they interacted.

Compliance with proper experimental conditions to minimize the influence of non-receptor processes and exploitation of the isomeric potency difference to control accessibility factors may provide information which more accurately reflects the nature of the events occurring at the receptor. Yet the investigator does not observe receptor occupation per se, but rather its effect. Depending upon the parameter chosen as an

effect, a different number of steps may be involved in the sequence of events initiated by the agonist-receptor interaction. Obviously the more steps involved, the more complex the relation between receptor interaction and effect and the greater the opportunity in different tissues for divergence from the same receptor event. From these implications, it would appear advantageous to measure the earliest (closest to the receptor) effect known to occur if the nature of the receptor is in question. In the case of beta receptor activation, elevation of tissue cyclic AMP levels may represent just such an effect (Robison et al., 1967; 1970; Sutherland et al., 1968).

Subsequent to the discovery that cyclic AMP was the mediator for catecholamine-induced liver glycogenolysis, essentially every effect of beta receptor activation has been found to be associated with an elevation of this nucleotide (Robison et al., 1967; 1971). Further recognition that adenylate cyclase in particulate preparations retained most of the characteristics attributable to beta receptors in intact tissues, suggested that these receptors might constitute an integral part of the adenylate cyclase system (Robison et al., 1967; 1970; Sutherland et al., 1968). Evidence for this hypothesis came from a number of different observations. The orders of potency of adrenergic agonists in stimulating adenylate cyclase in homogenates and in increasing cyclic AMP levels in intact tissues and the orders of potency

of antagonists in blocking the stimulation corresponds well to those for the physiological response in a given tissue. In a number of cases, the effects of exogenously administered cyclic AMP or one of its derivatives simulate those produced by beta receptor agonists. The level of cyclic AMP can also be affected by altering the activity of the enzyme which is responsible for its inactivation, phosphodiesterase (Butcher and Sutherland, 1962). Both the effects of exogenous cyclic AMP and those of beta adrenergic agonists can be potentiated by phosphodiesterase inhibitors as the xanthines (Rall and West, 1963) and antagonized by phosphodiesterase stimulants as the imidazoles (Butcher and Sutherland, 1962; Kukovetz and Pöch, 1967).

The "intimate relationship" between adenylate cyclase and the beta receptor was further indicated by the observation (Sutherland et al., 1968) that in those tissues which responded to catecholamines with an increase in cyclic AMP,

> ...the stimulation of adenyl cyclase is the earliest event known to occur, and attempts to measure an earlier event have been uniformly unsuccessful.

STATEMENT OF THE PROBLEM

Some initial observations regarding catecholamine-induced cyclic AMP formation have suggested a close relationship of this process to beta receptor activation and the resulting mechanical events in a number of tissues. The relationship appears to be particularly strong for the heart. For example, the positive inotropic response to catecholamines in isolated perfused rat heart is preceded by a rapid and large increase in cyclic AMP (Robison et al., 1965; Cheung and Williamson, 1965; Drummond et al., 1966). In this preparation, the smallest dose of drug which produces a positive inotropic response is associated with an increase in the level of this nucleotide (Robison et al., 1967). A similar profile has been reported for rat heart in situ (Namm and Mayer, 1968) and perfused rabbit (Hammermeister et al., 1965) and guinea-pig heart (Kukovetz and Pöch, 1967). Both the increase in cyclic AMP and the positive inotropic response can be blocked specifically by beta receptor blocking agents such as pronethalol (Robison et al., 1965; Namm and Mayer, 1968).

In washed particulate preparations from dog heart, the orders of potency of beta receptor agonists for stimulating adenylate cyclase activity is the same as that exhibited for the positive inotropic response in vivo, (Murad et al., 1962) and both responses can be blocked by DCI (Murad et al., 1962), pronethalol (Robison et al., 1967), and propranolol (Levey and Epstein, 1969). The adenylate cyclase stimulation

(Levey and Epstein, 1969; Murad and Vaughan, 1969), accumulation of cyclic AMP (Mayer et al., 1970) and positive inotropism (Glick et al., 1968; Lucchesi, 1968; Mayer et al., 1970) produced by glucagon, on the other hand, are unaffected by beta blocking agents.

Lefkowitz and coworkers (Lefkowitz and Haber, 1971; Lefkowitz et al., 1973) have isolated a subcellular fraction of canine myocardium with many characteristics of the beta receptor. Beta adrenergic agonists and antagonists compete with ^{3}H-norepinephrine for binding in a potency series paralleling their effectiveness in producing or blocking the inotropic responses in the intact heart. Adenylate cyclase activity is present in the same tissue fraction, and the same order of potency of agonists is obtained for stimulating cyclic AMP formation.

The mechanical effects of catecholamines can be mimicked by exogenously administered cyclic AMP under some conditions (Levine and Vogel, 1965; Levine et al., 1968). However, more consistent reproduction has been observed with dibutyryl derivatives of cyclic AMP (Posternak et al., 1962) which are more lipid soluble and may penetrate cell membranes more readily. For example, the dibutyryl derivative increases the rate and force of contraction in isolated perfused guinea-pig, rabbit and rat heart (Kukovetz and Pöch, 1970) and the force of contraction in isolated electrically stimulated cat ventricular papillary muscle (Skelton et al., 1970). In the

ventricular preparation, it was further noted that propranolol was ineffective in blocking the positive inotropic effect.

Theophylline, which inhibits the hydrolysis of cyclic AMP by phosphodiesterase (Butcher and Sutherland, 1962), potentiates the positive inotropic response to norepinephrine in isolated rabbit atrium (Rall and West, 1963), to epinephrine in isolated perfused rat heart (Hess et al., 1963), and to both norepinephrine and dibutyryl cyclic AMP in isolated perfused hearts of guinea-pigs, rabbits, and rats (Kukovetz and Pöch, 1970) as well as in cat ventricular muscle (Skelton et al., 1971). Conversely, stimulation of phosphodiesterase with imidazole results in an inhibition of the catecholamine-induced inotropic response in isolated perfused guinea-pig heart (Kukovetz and Pöch, 1967).

The relationship of cyclic AMP to cardiac contractility is further suggested by the recent observation that the levels of this nucleotide vary in different phases of the contraction cycle (Brooker, 1973). Using a quick-freeze technique, Brooker has demonstrated that myocardial cyclic AMP levels are elevated 30% during the systolic portion of the cycle of electrically-stimulated, frog ventricular strips.

The chronotropic response has not been studied as extensively as the inotropic response with respect to the involvement of cyclic AMP. This is primarily due to the small amount of S-A nodal tissue thought to be involved in this response relative to the total atrial tissue mass. However, Levine

and Vogel (1965) reported a positive chronotropic response in humans following injection of cyclic AMP and Kukovetz and Pöch (1970) found a similar effect of dibutyryl cyclic AMP on isolated perfused guinea-pig, rabbit, and rat heart. Perhaps more impressive is the study by Krause et al. (1970). Using a relatively homogeneous preparation of cultured spontaneously beating rat heart cells, these workers demonstrated a positive chronotropic effect of dibutyryl cyclic AMP which could be potentiated by the phosphodiesterase inhibitor caffeine. Since automaticity is a property not restricted only to nodal cells, it might be anticipated that cyclic AMP changes in non-nodal tissue is also involved in the chronotropic response. Danilo et al. (1973) have demonstrated the ability of isolated canine purkinje fiber bundles to increase their rate of spontaneous discharge after exposure to epinephrine. In homogenates of these bundles, stimulation of adenylate cyclase was greatest by isoproterenol, less so by epinephrine and not at all by phenylephrine. Both the increased rate of firing and the adenylate cyclase stimulation were blocked by propranolol.

There is an increasing body of evidence suggesting a similar relationship of catecholamine-induced cyclic AMP accumulation and mechanical events in smooth muscle, particularly in uterus. Isoproterenol, epinephrine and norepinephrine increase the formation of cyclic AMP in isolated rat uterus (Dobbs and Robison, 1968; Mitznegg et al., 1970b; Polacek

and Daniel, 1971) and stimulate adenylate cyclase activity in uterine homogenates (Triner et al., 1970a; 1970b; Harbon and Clauser, 1971). The order of potency of these agents in producing these effects is similar to that for producing uterine relaxation (Dobbs and Robison, 1968; Triner et al., 1970a). The elevation of nucleotide in intact tissue occurs at least as rapidly as relaxation can be measured and can be blocked by propranolol and butoxamine (Dobbs and Robison, 1968; Polacek and Daniel, 1971). Propranolol also blocks the catecholamine-induced stimulation of adenylate cyclase in homogenates (Triner et al., 1970a).

Dibutyryl cyclic AMP relaxes the isolated rat uterus under tension (Dobbs and Robison, 1968; Polacek et al., 1971; Marshall and Kroeger, 1973) as well as uterus contracted with acetylcholine, oxytocin or calcium (Triner et al., 1970a; 1970b; 1971; Mitznegg, 1970a; Dobbs and Robison, 1968). The effects of dibutyryl cyclic AMP are potentiated by theophylline but are not affected by propranolol (Dobbs and Robison, 1968; Polacek et al., 1971). Theophylline itself can reduce uterine tone in humans in vivo (Coutinho and Lopes, 1971) and inhibit acetylcholine-induced contractions in isolated rat uterus (Levy and Wilkenfeld, 1968). A more prominent and consistent effect of theophylline, however, is its ability to potentiate the inhibitory effects of catecholamines on spontaneous (Dobbs and Robison, 1968), acetylcholine-induced (Levy and Wilkenfeld,1968) or oxytocin-induced (Mitznegg et al., 1970b) contractions of isolated rat uterus.

Robison et al. (1970) noted that the level of cyclic AMP rises and falls just as spontaneous contractions of rat uterus disappear and recur. On a similar preparation, Marshall and Kroeger (1973) have measured cyclic AMP levels at various time points in the course of relaxation in response to a single dose of isoproterenol. They found an excellent correlation in the two parameters; a significant increase (2-fold) in tissue cyclic AMP concentration occurred at a time when relaxation was just beginning and both cyclic AMP and relaxation continued to increase thereafter. Similar results were also obtained with papaverine, a phosphodiesterase inhibitor and smooth muscle relaxant.

Catecholamine-induced stimulation of heart and inhibition of uterus are responses which have been attributed to activation of $\underline{\text{beta}}_1$ and $\underline{\text{beta}}_2$ receptors, respectively (Lands et al., 1967a; 1967b). These conclusions are based on the potencies of agonists and antagonists in eliciting or preventing these mechanical responses. The preceding evidence suggests that cyclic AMP formation may be a more immediate consequence of beta receptor activation in these tissues and that this nucleotide may be responsible for mediating subsequent mechanical responses. Accordingly, if differences exist between beta receptors of heart and uterus, it should be possible to discriminate them on the basis of differences in the effects of agonists or antagonists on cyclic AMP formation in the two tissues. Moreover, since this metabolic response appears to precede mechanical responses both temporally and functionally,

it may reflect more accurately the nature of the beta receptors involved.

The present studies have been undertaken to evaluate possible differences in beta receptors of rat atrium and rat uterus at the levels of cyclic AMP formation and/or mechanical response. The quantitative difference in potency between the optical isomers of isoproterenol will be used to characterize the beta receptors of each tissue. Under proper experimental conditions (Furchgott, 1967; 1970; 1972), dissimilar isomeric potency differences in each tissue for a particular response should be a sensitive indication of different receptors (Patil, 1969). In addition, the use of the isomers of a single agonist in lieu of separate agonists provides a control measure for accessibility factors which may complicate comparisons of structurally dissimilar compounds in different tissues. Isoproterenol has been selected as the agonist for these studies on the basis of its relatively "pure" beta receptor spectrum of activity and for its relative resistance to non-receptor influences as adrenergic neuronal membrane uptake and enzymatic inactivation by MAO.

Experiments have been designed to answer the following questions:

1. Does a similar quantitative relationship between the isomers of isoproterenol, expressed as a potency difference, exist for both mechanical responses and changes in cyclic AMP levels in the same tissue? More specifically, do

mechanical responses and changes in cyclic AMP levels simply represent different manifestations of the same receptor event or are different receptors involved in each response?

2. Are the isomeric potency differences for either response similar in both atrium and uterus? In other words, can the "$\underline{\text{beta}}_1$" receptors of rat atrium and the "$\underline{\text{beta}}_2$" receptors of rat uterus be distinguished on the basis of isomeric potency differences for either or both mechanical responses or changes in cyclic AMP levels?

The answers to these questions will be sought by determining isomeric potency differences under several experimental conditions which are thought to influence mechanical responses and cyclic AMP levels in tissues.

MATERIALS AND METHODS

Pretreatment of Experimental Animals

Female albino rats (Holtzman Company; Madison, Wis.), weighing 200-250 g, were used in all experiments. The animals were pretreated with reserpine, 5 mg per kg intraperitoneally, 16-24 hours prior to sacrifice. The effectiveness of such treatment in depleting endogenous catecholamine stores in rat heart and uterus has been well studied (Paasonen and Krayer, 1958; Waud et al., 1958; Rudzik and Miller, 1962; Wurtman et al., 1964).

For uterine experiments, it was desirable to use only animals in estrus. Restricting the experimental animals to a particular stage of the sexual cycle creates a population more homogeneous with respect to endocrine status, and in estrus in particular, the uterus manifests increased drug sensitivity to both mechanical effects (Genell, 1939; Hawkins, 1964; Rudzik and Miller, 1962) and effects on cyclic AMP (Coutinho and Lopes, 1971; Sim and Chantharaksri, 1973). The stages of the rat estrus cycle were readily assessed by vaginal smearing. An eye-dropper containing saline was used to flush the vaginal contents. Estrus was clearly indicated by the presence in the flush of cornified cells with degenerate nuclei. The corresponding uterine changes included enlargement, vascularization, and distension due to the accumulation of luminal fluid (Genell, 1939).

The natural estrus cycle of our animals was found to

be disrupted by reserpine, such that rats in proestrus at the time of treatment with this agent were arrested in that stage of the cycle. If reserpine was given to animals already in estrus, they proceeded to metestrus. However, the cycle could be arrested in estrus by injecting animals who had already reached this stage with both reserpine, 5 mg per kg intraperitoneally, and diethylstibestrol, 100 ug per kg subcutaneously (Gaddum and Hameed, 1954), 16-24 hours prior to experiments. On the basis of vaginal cytology and uterine morphology, this procedure was nearly always successful. Several experiments were also conducted on right atria from animals so treated to ascertain whether diethylstilbestrol affected the response of this tissue to isoproterenol.

It was not possible to use reserpine pretreated animals for experiments measuring uterine mechanical changes, since this treatment led to the cessation of spontaneous contractions in uteri soon after tension was exerted on them in vitro. Therefore, for these experiments, animals untreated with either reserpine or diethylstilbestrol, but in a state of natural estrus, were selected just prior to their use.

Preparation of Isolated Tissues

General Considerations

Animals were killed by cervical dislocation. Right and left atria or uterine horns were rapidly removed, cleaned of excess connective tissue, and transferred to tissue baths containing physiological salt solution (PSS) of the following

composition: NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2 \cdot 2H_2O$, 2.5 mM; $MgCl_2 \cdot 6H_2O$, 0.5 mM; $NaH_2PO_4 \cdot H_2O$, 1.0 mM; $NaHCO_3$, 25 mM; glucose, 11 mM. The chemicals were dissolved in deionized water. The PSS in the tissue baths was aerated with a mixture of oxygen (95 per cent) and carbon dioxide (5 per cent) and was maintained at a temperature of 37.5°C. Tissues were then used for experiments measuring either mechanical responses or changes in cyclic AMP.

Following an appropriate equilibration period, tissues were pretreated with various agents to control non-beta receptor influences which may alter the observed potency of isoproterenol. Tropolone, 10^{-4}M, was used to inhibit the enzyme catechol-o-methyltransferase or COMT (Belleau and Burba, 1961; Kalsner and Nickerson, 1969). O-methylation is a primary mechanism for the inactivation of isoproterenol, which is a substrate for neither monoamine oxidase (Hertting, 1964) nor the adrenergic neuronal membrane uptake mechanism (Iverson, 1967). Theophylline, 10^{-4} or 10^{-3}M, was used to inhibit phosphodiesterase (Butcher and Sutherland, 1962; Kukovetz and Pöch, 1967). Phentolamine, 10^{-5}M, was used to block alpha-adrenergic receptors. This concentration of phentolamine is approximately 100 times the pA_2 values which have been reported for this antagonist (Furchgott, 1972) and should theoretically occupy 99 per cent of the available alpha receptors (Paton, 1961). The implication of alpha receptors in the atria is based on a limited number of observations (see discussion), and this drug was employed

in atrial experiments primarily to facilitate comparisons with responses from uterus, where there may exist a more prominent alpha receptor population (Ahlquist, 1948; Miller, 1967).

Mechanical Responses from Atria

In order to avoid the influence of rate of beating on the force of contraction, chronotropic responses were determined from spontaneously beating right atria, and inotropic responses were determined from electrically stimulated left atria (Koch-Weser and Blinks, 1963). The two atria from a single animal were suspended in separate 10 ml, water-jacketed tissue baths. The amount of tension exerted on each right atrium was the minimum needed to obtain a pen deflection on the polygraph of about 0.5 cm per beat at the highest preamplifier sensitivity which did not produce background noise (see below). The left atrium was supported by bipolar platinum electrodes. A tension of 0.5 g was exerted on the tissue and the following stimulation parameters used: 0.75-1.50 volts (2.5 times threshold voltage); 1 pulse per second; 5 millisecond duration (Grass S44 stimulator).

Changes in rate and force were recorded on a Grass model 79B polygraph via force displacement transducers (Grass, FT03). Right atrial rate was also monitored with a tachygraph (Grass, 7P44A) to help determine when the maximum response had occurred to a given dose of isoproterenol.

Following a 1 hour equilibration period with changes of PSS at 15 minute intervals, tissues were exposed to one or

more agents previously mentioned. The order of their addition was tropolone, theophylline, and phentolamine, and their tissue contact times prior to the addition of isoproterenol were 50 minutes, 45 minutes, and 40 minutes, respectively. In those experiments in which the beta receptor antagonist, practolol, was used, the drug was added 10 minutes prior to the addition of tropolone. The tissue contact time for practolol was 60 minutes. All of these drugs were added in a volume of 0.1 ml.

Mechanical Responses from Uterine Segments

A single uterine horn was randomly selected from each animal. The ovarian and cervical ends were removed, and a 1.5-2.0 cm segment of the remaining portion was cut in half longitudinally. Each half was placed in a 10 ml water-jacketed tissue bath and allowed to equilibrate for 40 minutes with washings at 10 minute intervals. After the first 20 minutes, a resting tension of 0.25 g was exerted on each segment and maintained throughout the remaining 20 minutes of equilibration.

Spontaneous contractions were isometrically recorded (Csapó, 1954) on a two-channel Grass model 5D polygraph via force displacement transducers (Grass, FT03). The parameter chosen for measurement was contraction amplitude, since it was found to be more stable with time than contraction rate, which decreased continually even in the absence of drugs. Usually within 10-15 minutes after the initial application

of tension, the amplitude of the spontaneous contractions was regular and the baseline stable. When tropolone, theophylline, or phentolamine were used they were included in the PSS and remained in contact with the tissues for the entire equilibration period.

Increases in Cyclic AMP Levels in Atria and Uterine Segments

For experiments measuring cyclic AMP levels, spontaneously beating right atria and uterine segments were used. Four to six segments, 50-100 mg wet weight each, were obtained from the two uterine horns. However, each of the segments from a single animal received a different treatment.

Tissues were blotted, weighed, and then placed in tissue vessels containing 1 ml of PSS. Following a 30 minute equilibration period with washings at 10 minute intervals, theophylline, 10^{-3}M, was added to the vessel in a volume of 10 ul. It remained in contact with the tissue for 15 minutes preceding the addition of isoproterenol. When phentolamine, tropolone, or practolol (10^{-4}M), were used, they were included in the PSS and their tissue contact time was 45 minutes.

Dose-Response Curves

Mechanical Responses

From each tissue a single cumulative dose-response curve was obtained for either isomer of isoproterenol or for desoxyisoproterenol. Recordings were made of positive inotropic and chronotropic responses in atria or decreases in contraction amplitude in uteri. A given dose was allowed to produce its maximum effect prior to the addition of a 3-fold higher

dose (Van Rossum, 1963). The maximum effect obtained for each preparation was taken as 100 per cent, and the effect of any given dose of isoproterenol was expressed as a per cent of that maximum.

Potency differences between the (-)- and (+)-isomers of isoproterenol were determined as the difference between their negative log molar ED_{50} values. Standard errors of the mean (S.E.M.) were calculated for ED_{50} values along with 95 per cent confidence intervals (C.I.) for isomeric potency differences.

In experiments on uterus, a different isomer was used on each of the two segments of the same uterine horn. It was thus possible to obtain an isomer potency difference for each animal. The resulting average potency difference did not differ from that obtained when the two segments were considered unpaired, but there was considerably less variability in the former.

Cyclic AMP Increases

For the measurement of cyclic AMP increases, a single dose of one isomer of isoproterenol was used on each preparation. After a fixed period of time, the incubation with isoproterenol was terminated by the addition of 0.1 ml of 50 per cent trichloroacetic acid (TCA) and the tissue was homogenized directly in the incubation vessel which had been fitted with ground glass pestles. Time from addition of TCA to complete homogenization was approximately 3 seconds.

The homogenates were centrifuged at 5000 x g for 30 minutes, and the resulting supernatants and precipitates were used for the determination of cyclic AMP and protein, respectively. Cyclic AMP levels were considered on the basis of both wet weight and tissue protein. In several preliminary experiments, tissues were frozen in liquid nitrogen and powdered while frozen before the addition of TCA. The levels of cyclic AMP were the same as when tissues were homogenized directly in TCA.

A single dose-response curve was constructed for each isomer using values obtained from single doses of isoproterenol. The average cyclic AMP level obtained at a particular dose was expressed as a per cent of the average cyclic AMP level obtained at the maximally effective dose. Curves were then evaluated for ED_{50} values and isomeric potency differences in a similar manner as curves for mechanical responses. The 95 per cent confidence intervals were not calculated, since with single dose-response curves, variations about an extrapolated ED_{50} cannot be validly estimated when the number of observations at any given dose is fewer than 20 (Dr. J. H. Torrie, personal communication).

Cyclic AMP Determinations

Measurements of tissue cyclic AMP levels were made by the method of Gilman (1970). The assay utilizes a cyclic AMP-binding protein, presumably a cyclic AMP-dependent kinase (Walsh *et al.*, 1968), and is based on a competition between ^{3}H-cyclic AMP and tissue cyclic AMP for that protein. The

nucleotide-protein complex is quantitatively adsorbed on cellulose ester filters which are counted for radioactivity.

Isolation of Cyclic AMP-Binding Protein

Fresh rabbit skeletal muscle was prepared as described by Miyamoto et al. (1969) and Gilman (1970). All procedures were performed at 4°C. Approximately 250 g of muscle from back and hind-quarters was homogenized with 3 volumes of 4 mM Na_2-EDTA for 2 minutes in a Waring blender. Buffers used in succeeding purification steps contained 2 mM Na_2EDTA. The homogenate was centrifuged at 27,000 x g for 30 minutes.

The resultant supernatant solution, approximately 650 ml at pH 6.3, was adjusted to pH 4.8 by the dropwise addition of 1N acetic acid with stirring. After 10 minutes, the precipitate was removed by centrifugation at 27,000 x g. The pH of the supernatant solution was then readjusted from 4.95 to 6.50 with 1M potassium phosphate buffer, pH 7.2.

Solid ammonium sulfate (32.5 g per 100 ml) was added to the resultant solution. After 45 minutes of stirring, the precipitate was collected by centrifugation at 16,000 x g for 20 minutes and dissolved in 6 per cent of the crude extract volume (36 ml) of 5 mM potassium phosphate buffer, pH 7.0. This yielded approximately 52 ml of solution which was then dialyzed against 20 volumes of the same buffer with two changes over a period of 18 hours. The dialyzed solution was centrifuged at 27,000 x g for 30 minutes, and the resulting precipitate was discarded.

A DEAE cellulose (medium mesh, 0.89 meq per g, Sigma Chemical Company) column was prepared in the following manner. The powdered material was subjected to successive washings with 1N NaOH, deionized H_2O, 1N HCl, deionized H_2O, 1N NaOH, and finally with deionized H_2O until the washings were of neutral pH. During the washing procedure, the finer particles of DEAE which were slow to settle were poured off and discarded. The cellulose was equilibrated in 5 mM potassium phosphate buffer, pH 7.0,and loaded into a chromatographic tube to form a column 3.2 x 26 cm. The flow rate was 2.5 ml per minute.

The dialysis supernatant was applied to this column, which was then washed with 2 bed volumes of 100 mM potassium phosphate buffer, pH 7.0. Fractions of 20 ml were collected and assayed for binding capacity. The assay mixture consisted of 20 ul from each 20 ml DEAE fraction and 20 ul of ^{3}H-cyclic AMP (1.82 picomoles) in 100 mM sodium acetate, pH 4.0. Other conditions were identical to those to be described for the cyclic AMP assay.

Active fractions were pooled and dialyzed for 15 hours against 20 volumes of 5 mM potassium phosphate buffer, pH 7.0. The buffer was changed at 5 hour intervals. The dialyzed solution was stored in 1 ml aliquots in sterile vials at -70°C. and used in subsequent measurements of tissue cyclic AMP levels. The preparation bound .015 picomoles of cyclic AMP per ug protein under standard assay conditions, and there were only negligible losses in binding capacity over

an 8 month period under these storage conditions.

Preparation of Tissue Samples

TCA supernatants from tissue samples were extracted 6 times with 4 volumes of H_2O-saturated, acidified ether. Residual ether was boiled off by heating the sample at 80°C for 10 minutes. To the aqueous samples was added an equal volume of 300 mM sodium acetate buffer, pH 4.0. Aliquots of these mixtures were either assayed directly for cyclic AMP or first subjected to a purification step (see below).

According to Gilman (1970), the binding protein is not entirely specific for cyclic AMP. Other cyclic 3':5'-nucleotides have relatively high binding affinity, but of these, the only one known to occur naturally is cyclic GMP. This nucleotide is capable of inhibiting the binding of ^3H-cyclic AMP by 50 per cent when present in 125-fold molar excess. The actual cyclic GMP to cyclic AMP ratios in several preparations of rat heart and uterus have been reported: 0.119 (Kuo et al., 1972) and 0.029 (Ishikawa et al., 1969) in whole heart; 0.110 in ventricular slices (Kuo et al., 1972); 0.244 in perfused heart (George et al., 1970); and 0.037 in uterus (Ishikawa et al., 1969). The effect of isoproterenol is to further decrease the ratio by greatly elevating the levels of cyclic AMP (Kuo et al., 1972; George et al., 1970) and, at least in perfused heart, by simultaneously decreasing the levels of cyclic GMP (George et al., 1970). Interference in the cyclic AMP assay from cyclic GMP thus seems unlikely.

Other nucleotides show insignificant inhibition of cyclic AMP binding, and ATP, the most abundant, must be present in 25,000-fold molar excess of cyclic AMP for 50 per cent inhibition. In most tissues, ATP is present 1000-10,000-fold molar excess (Robison et al., 1971; Daniel and Robinson, 1971). Isoproterenol tends to decrease this gradient in rat heart and uterus by elevating the levels of cyclic AMP and additionally, in rat heart, by decreasing the levels of ATP (Weston, 1970). In contrast, the levels of ATP in smooth muscle preparations are increased by beta-adrenergic agonists. This has been demonstrated for isoproterenol and epinephrine in rabbit duodenum and taenia coli (Weston, 1970; Bueding et al., 1967) and for epinephrine in rat uterus pretreated with phenoxybenzamine (Polacek and Daniel, 1971).

To confirm that neither cyclic GMP nor ATP levels in rat atria and uteri, either under control conditions or after treatment with isoproterenol, interfered with the determination of cyclic AMP, the following purification (Murad et al., 1971) was made on several samples. Aliquots of the tissue sample-sodium acetate mixtures were applied to 0.5 x 3 cm columns of AG 1-X8 formate anion exchange resin (200-400 mesh, Biorad Laboratories). After washing the columns with 10 ml of deionized H_2O, cyclic AMP was eluted with 10 ml of 2N formic acid. Cyclic GMP and ATP are retained on the columns during this procedure. Column eluates were lyophilized, dissolved in 150 mM sodium acetate, pH 4.0, and then

assayed for cyclic AMP. As shown in Table 1, similar pre- and post-purification values for cyclic AMP indicate that interference by cyclic GMP or ATP is not a problem, and that cyclic AMP in rat atria and uteri may be assayed by the method of Gilman (1970) without prior purification.

Cyclic AMP Assay

The binding reaction was conducted in a total volume of 60 ul, in 50 mM sodium acetate buffer, pH 4.0, at 0°C. Into disposable 3 inch culture tubes 20 ul of tissue extract or standard solution of unlabeled cyclic AMP and 20 ul of ^{3}H-cyclic AMP (containing either 0.4 or 0.8 picomoles of cyclic AMP) were mixed. The reaction was initiated by the addition of 20 ul of binding protein solution, sufficient to bind 0.22 picomoles of cyclic AMP. Under these saturation conditions, there is sufficient protein to bind a maximum of one-half to one-quarter of the ^{3}H-cyclic AMP present. The effect of added tissue cyclic AMP or standard cyclic AMP solutions may thus be evaluated by a linear (log-log) decrease in total bound ^{3}H-cyclic AMP. After 1 hour, the reaction mixture was diluted with 1 ml of 20 mM potassium phosphate buffer, pH 6.0, at 0°C. Five minutes later, the mixture was passed through a cellulose ester filter (0.45 um, Millipore) which had been presoaked with the same buffer. The filter was then washed with 10 ml of this buffer, placed in a counting vial, and dried in an oven. To the vial was then added 15 ml of scintillation solution of the following composition:

TABLE 1

Effect of AG 1-X8 Formate Separation on the Assay of Cyclic AMP Levels in Tissue[a] Extracts

Concentration of (-)-Isoproterenol	Cyclic AMP (picomoles per mg protein)			
	Rat Atria		Rat Uteri	
	Before Separation	After Separation	Before Separation	After Separation
none	11.6	15.7	5.0	3.5
	10.4	13.8	4.8	3.6
10^{-7}M	38.3	42.4	20.3	21.5
	38.6	39.8	9.7	11.7
10^{-6}M	59.4	52.6	12.1	13.5
	63.0	76.6	10.8	10.4
10^{-5}M	85.9	87.1	16.2	16.9
	64.1	69.1	18.9	17.1

[a]All tissues incubated in the presence of theophylline, tropolone, and phentolamine.

6.0 g PPO and 0.2 g POPOP per liter of toluene. All samples were counted for radioactivity in a liquid scintillation spectrometer (Packard Model 2002).

Cellulose ester filters have a refractive index similar to that of the toluene based scintillation solution. They are thus rendered transparent and may be counted without dissolution at relatively high efficiency (25-30 per cent, as determined by an internal standard). Blanks run in the absence of binding protein were generally identical to samples of scintillation solution counted for background. Standard solutions containing known amounts of unlabeled cyclic AMP were prepared in PSS containing appropriate drugs and were treated in the same manner as tissue samples. This precaution is quite important since high concentrations of isoproterenol caused a reduction in the protein-binding of ^{3}H-cyclic AMP. A typical standard curve is shown in Figure 1.

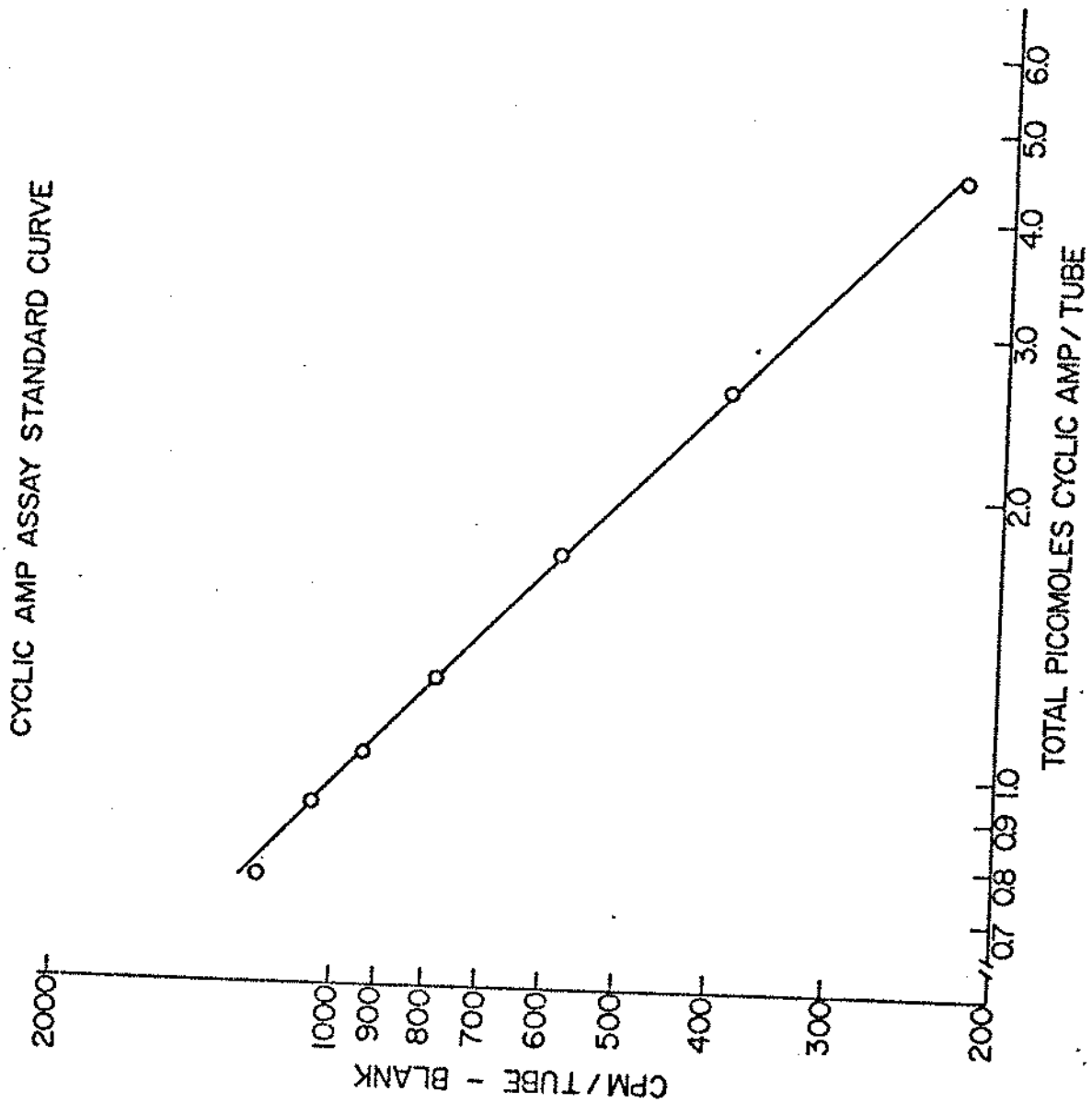
Protein Determinations

The protein content of tissues was determined by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as a standard. The following reagents were prepared just prior to protein assays: reagent A, 2 per cent Na_2CO_3; reagent B, 0.5 per cent $CuSO_4 \cdot 5H_2O$ in 1 per cent sodium tartrate; reagent C, 50 ml reagent A mixed with 1 ml reagent B; reagent D, Folin-Ciocalteu phenol reagent diluted to 1N.

TCA precipitated tissue protein was solubilized by boiling in 1 ml of 1N NaOH for 1 hour and then allowed to

FIGURE 1

Cyclic AMP assay standard curve. Binding reactions were conducted in a total volume of 60 ul, in 50 mM sodium acetate buffer, pH 4.0, at 0°C. Total picomoles of cyclic AMP represents the sum of ^{3}H-cyclic AMP (0.80 picomoles) and unlabeled standard.


CYCLIC AMP ASSAY STANDARD CURVE
CPM / TUBE - BLANK
2000
1000
900
800
700
600
500
400
300
200
TOTAL PICOMOLES CYCLIC AMP / TUBE
0.7
0.8
0.9
1.0
2.0
3.0
4.0
5.0
6.0

stand at room temperature for 24 hours. Protein standards were precipitated and solubilized in the same manner. Twenty ul of tissue samples or standards were pipetted into matched colorimeter tubes containing 0.8 ml deionized water. Four ml of reagent C was added to the tubes, which were mixed well and allowed to stand at room temperature. After 10 minutes, 0.4 ml of reagent D was added rapidly and mixed well within a second or two. After 30 minutes, the samples were read in a Bausch and Lomb Spectronic 20 colorimeter at 500 mu.

Drugs and Solutions

Fresh solutions of drugs were prepared on the day of each experiment and kept refrigerated until 5 minutes before use. Dilutions of levorotatory-, dextrorotatory-, and desoxy-isoproterenol were made from 10^{-2}M or 10^{-1}M refrigerated stock solutions prepared in 0.9 per cent saline containing 0.05 per cent sodium metabisulfite to minimize spontaneous oxidation. Other drugs were prepared in 0.9 per cent saline, with a small amount of dilute HCl required to dissolve (±)-practolol. Theophylline was used in the form of aminophylline (theophylline ethylenediamine), and doses are expressed in terms of theophylline content.

The following drugs were used: (-)-isoproterenol-(+)-bitartate (Sigma Chemical Company); β-desoxy-isoproterenol (Sterling-Winthrop Research Institute); tropolone (Aldrich Chemical Company); phentolamine (Regitine^{R}, Ciba Pharmaceutical Company); aminophylline (Merck Company); practolol (Ayerst Laboratories); reserpine USP (Serpasil^{R}, Ciba Pharmaceutical

Company); diethylstilbestrol (Stilbestrol, Sigma Chemical Company); (+)-isoproterenol-(+)-bitartrate (the procedure for the purification of this isomer from (±)-isoproterenol is described on page 132 of the appendix).

For the cyclic AMP assay and protein determinations, the following chemicals were used: adenosine 3':5'-cyclic monophosphoric acid (cyclic AMP, Sigma Chemical Company); adenosine 3':5'-cyclic monophosphoric acid-[8-^{3}H] (^{3}H-cyclic AMP; specific activity, 14.3 and 28 curies per mmole; Schwarz/Mann); crystalline bovine serum albumin (Sigma Chemical Company); Folin-Ciocalteu phenol reagent (Fisher Scientific Company); PPO-2,5-Diphenyloxazole (Packard); POPOP-1,4-bis-2-(5 phenyloxazolyl)-Benzene (Packard).

RESULTS

Atrial Mechanical Responses to Enantiomers of Isoproterenol

Data obtained for mechanical responses to isoproterenol in atria under various experimental conditions are summarized in Table 2. In all experiments, increases in both rate and force in response to a given dose of either isomer were apparent within 10-15 seconds and were completely developed in 3-6 minutes. While the (+)-isomer was less potent than the (-)-isomer by a factor of over 1000, maximally effective doses of either isomer produced equal responses. Thus, similar increases in rate, in beats per minute, were obtained with both isomers. Increases in force in response to isoproterenol were extremely variable in terms of absolute tension developed from one atrium to another and were not compared in this fashion. However, for both rate and force, after the response to a maximally effective dose of (+)-isomer had occurred in a given atrium, addition of a high dose of (-)-isomer (10^{-4}M) did not produce a further increase.

Average cumulative log dose-response curves to isoproterenol, obtained in the presence of tropolone, are presented in Figure 2. The negative log molar ED_{50} values for rate are 9.09 for the (-)-isomer and 6.02 for the (+)-isomer. The resulting isomeric potency difference is 3.07 log units. Curves for force for both isomers are situated approximately 0.5 log units to the left of those for rate. Negative log molar ED_{50} values are 8.66 and 5.51 for (-)- and (+)-isomers,

TABLE 2

Mechanical Responses to Isoproterenol in Rat Atria

Treatment	Response	Isomer	n[b]	Initial Rate	Maximum Rate	Negative log Molar ED_{50} with S.E.M.	Isomeric potency difference[a] (95% C.I.)
Tropolone	Rate	(-)	7	315 ± 14	461 ± 12	9.09 ± .05	3.07
		(+)	7	311 ± 6	476 ± 5	6.02 ± .07	(2.90 - 3.25)
	Force	(-)	6	-	-	8.66 ± .07	3.16
		(+)	6	-	-	5.51 ± .08	(2.92 - 3.39)
Tropolone + Phentolamine	Rate	(-)	8	229 ± 8[b]	438 ± 5[b]	8.85 ± .05[b]	3.27
		(+)	8	217 ± 9[b]	427 ± 16[b]	5.58 ± .05[b]	(3.11 - 3.43)
	Force	(-)	5	-	-	9.16 ± .04[b]	3.30
		(+)	6	-	-	5.86 ± .18	(2.88 - 3.72)
Tropolone + Phentolamine + Theophylline (10^{-4}M)	Rate	(-)	7	259 ± 17[c]	466 ± 9[c]	9.20 ± .09[c]	3.35
		(+)	7	236 ± 15[c]	439 ± 11[c]	5.86 ± .05[c]	(3.11 - 3.58)
	Force	(-)	6	-	-	9.24 ± .17	3.58
		(+)	6	-	-	5.66 ± .15	(3.08 - 4.09)
Tropolone + Phentolamine + Theophylline (10^{-3}M)	Rate	(-)	7	372 ± 21[cd]	460 ± 17[c]	9.23 ± .12[c]	3.34
		(+)	7	361 ± 17[cd]	456 ± 12[c]	5.89 ± .13[c]	(2.95 - 3.72)
	Force	(-)	4	-	-	9.63 ± .30	3.51
		(+)	4	-	-	6.12 ± .10[d]	(2.74 - 4.28)
Tropolone + Phentolamine	Rate	Desoxy-isoproterenol	6	224 ± 7	471 ± 10	6.00 ± .05	-

TABLE 2 (continued)

---

[a]Isomeric potency difference = [negative log molar ED_{50} of (-)-isomer] - [negative log molar ED_{50} of (+)-isomer].

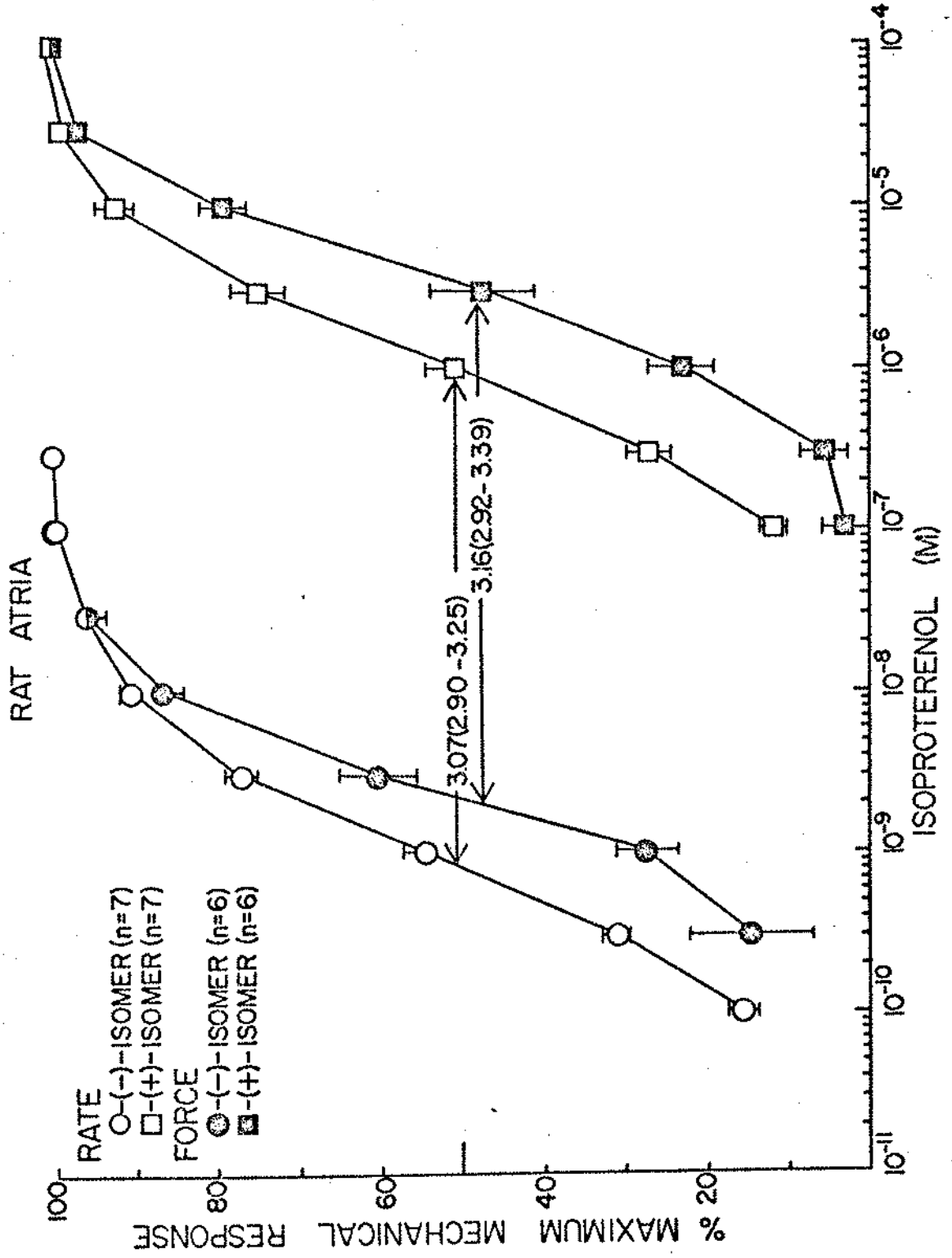
[b]Significantly different ($P < .05$) from corresponding value obtained in the absence of phentolamine.

[c]Significantly different ($P < .05$) from corresponding value obtained in the absence of theophylline.

[d]Significantly different ($P < .05$) from corresponding value obtained in the presence of theophylline, 10^{-4}M.

FIGURE 2

Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat atria in the presence of tropolone. Rate and force were determined from spontaneously beating right and electrically stimulated left atria, respectively. Numbers between the horizontal arrows connecting the curves are isomeric potency differences [(negative log molar ED_{50} of (-)-isomer) - (negative log molar ED_{50} of (+)-isomer)] along with their 95 per cent C. I. in parentheses. Vertical lines indicate S.E.M., and n is the number of observations.


RAT ATRIA
RATE
○-(-)-ISOMER (n=7)
□-(+)-ISOMER (n=7)
FORCE
●-(-)-ISOMER (n=6)
■-(+)-ISOMER (n=6)
3.07(2.90-3.25)
3.16(2.92-3.39)
% MAXIMUM MECHANICAL RESPONSE
ISOPROTERENOL (M)
100
80
60
40
20
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}

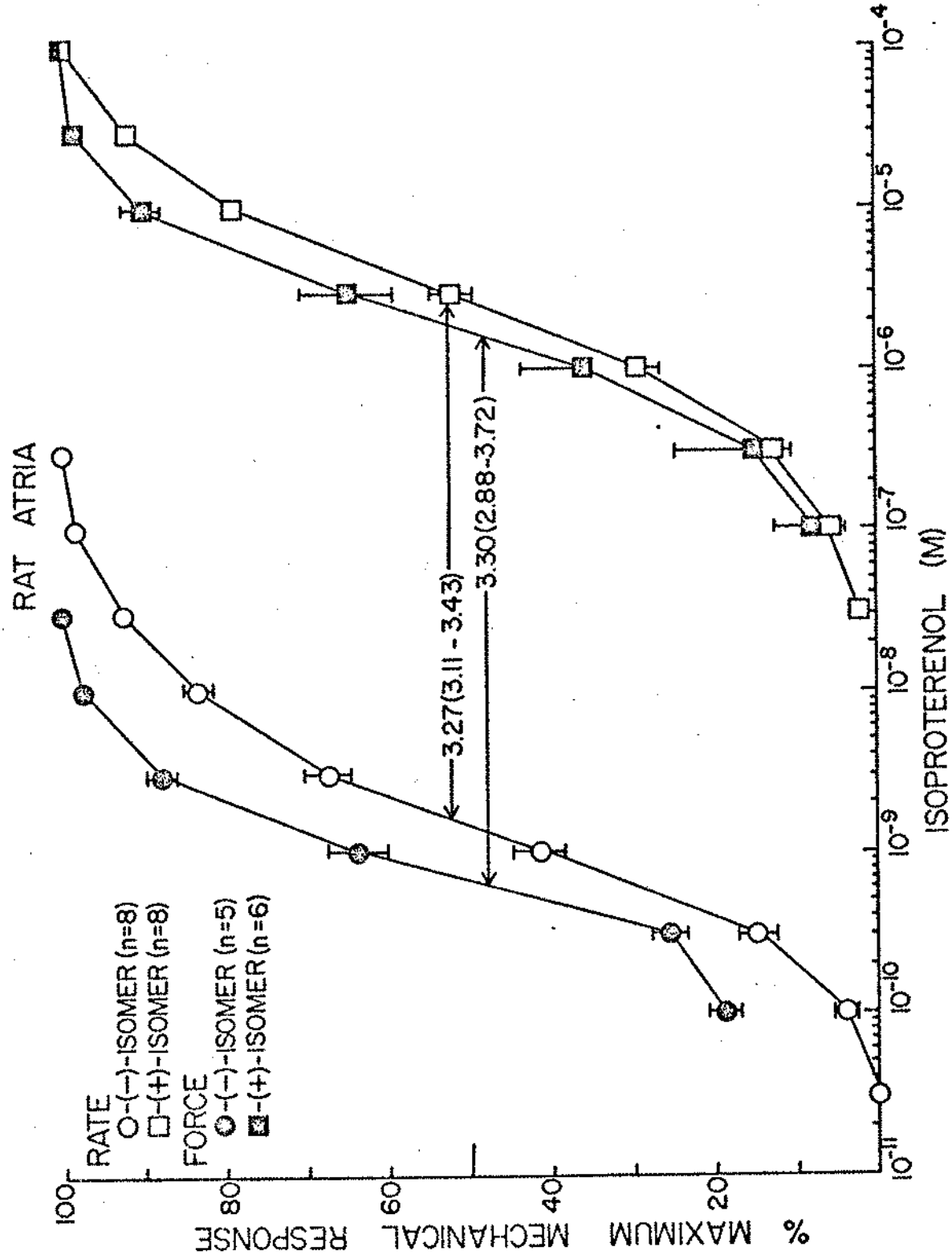
respectively, and the isomeric potency difference is 3.16 log units.

Figure 3 shows the response to isoproterenol in the presence of both tropolone and phentolamine. The latter drug decreased the average basal atrial rate by 29 per cent and the average isoproterenol-induced maximum rate by 9 per cent. Negative log molar ED_{50} values for both isomers are significantly decreased, the (+)-isomer to a greater extent than the (-)-isomer. The resultant increase in potency difference between the isomers to 3.27 is not statistically significant. In contrast to the depressant effects exerted by phentolamine on right atrial rate, on left atria the drug either did not change or slightly increased (average, 4 ± 6 per cent) the force of contraction. Isoproterenol effects on force were potentiated by phentolamine such that the dose-response curves are now situated to the left of those for rate. Only the potentiation of the (-)-isomer is statistically significant; the increased potency for the (+)-isomer and increase in isomeric potency difference to 3.30 log units are not.

Theophylline, 10^{-4}M, significantly increased basal rate by 11 per cent and restored the isoproterenol-induced maximum rate which had been depressed by phentolamine. Potencies of both isomers were significantly increased approximately 0.3 log units, but the isomeric potency difference was unaltered. Small increases in force (average, 5 ± 3 per cent) in response to theophylline were observed in only about half of the left

FIGURE 3

Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat atria in the presence of phentolamine and tropolone. Rate and force were determined from spontaneously beating right and electrically stimulated left atria, respectively. Numbers between the horizontal arrows connecting the curves are isomeric potency differences [(negative log molar ED_{50} of (-)-isomer) - (negative log molar ED_{50} of (+)-isomer)] along with their 95 per cent C.I. in parentheses. Vertical lines indicate S.E.M., and n is the number of observations.


RAT ATRIA
RATE
○-(−)-ISOMER (n=8)
□-(+)-ISOMER (n=8)
FORCE
●-(−)-ISOMER (n=5)
■-(+)-ISOMER (n=6)
3.27(3.11 - 3.43)
3.30(2.88-3.72)
% MAXIMUM MECHANICAL RESPONSE
ISOPROTERENOL (M)
100
80
60
40
20
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}

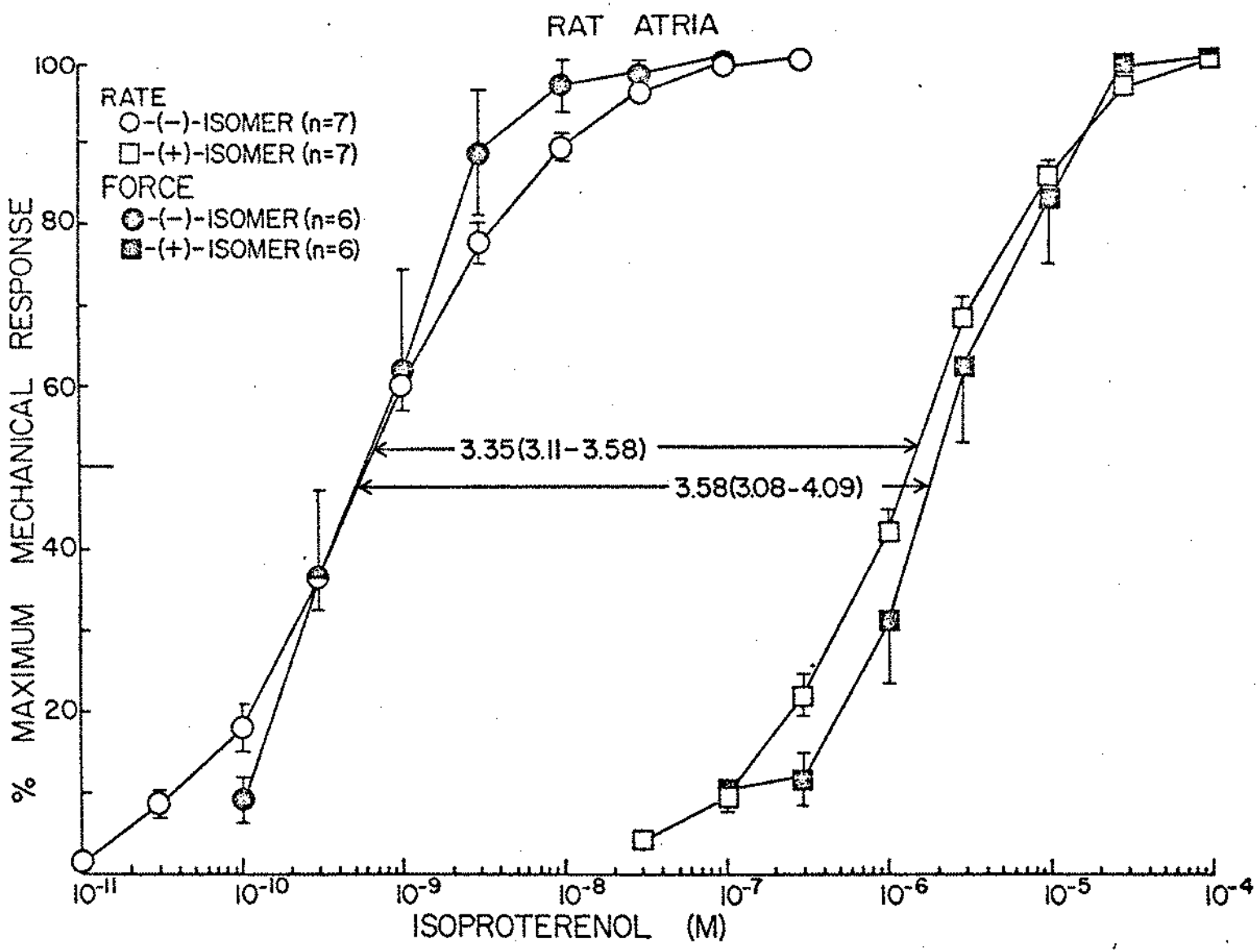
atria tested, and negative log molar ED_{50} values of the isomers and the potency difference between them were not affected. Complete dose-response curves for rate and force in response to isoproterenol, obtained in the presence of theophylline, phentolamine and tropolone are presented in Figure 4.

Increasing the concentration of theophylline to 10^{-3}M resulted in a high incidence of arrhythmias in rate preparations and spontaneous rhythms in force preparations during exposure of atria to isoproterenol. The 10-fold increase in theophylline produced a further significant increase in basal atrial rate (48 per cent) but the maximum isoproterenol-induced rate increase, negative log molar ED_{50} values for the isomers, and potency difference were unaltered. Compared with experiments in which theophylline was absent, 10^{-3}M of this drug increased control atrial rate 64 per cent and the maximum isoproterenol-induced rate 6 per cent and significantly increased the negative log molar ED_{50} values of (-)- and (+)-isomers by 0.38 and 0.31 log units, respectively. There was no change, however, in the isomeric potency difference.

On left atria, theophylline, 10^{-3}M, increased the force of contraction only slightly (4 ± 2 per cent). Compared with experiments in which theophylline, 10^{-4}M, was used, increases in force in response to isoproterenol were potentiated by theophylline, 10^{-3}M. However, only the increase in potency of the (+)-isomer was statistically significant. In addition, the isomeric potency difference was not changed. It is possible

FIGURE 4

Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat atria in the presence of theophylline (10^{-4}M), phentolamine, and tropolone. Rate and force were determined from spontaneously beating right and electrically stimulated left atria, respectively. Numbers between the horizontal arrows connecting the curves are isomeric potency differences [(negative log molar ED_{50} of (-)-isomer) - (negative log molar ED_{50} of (+)-isomer)] along with their 95 per cent C.I. in parentheses. Vertical lines indicate S.E.M., and n is the number of observations.


RAT ATRIA
RATE
○-(−)-ISOMER (n=7)
□-(+)-ISOMER (n=7)
FORCE
●-(−)-ISOMER (n=6)
■-(+)-ISOMER (n=6)
% MAXIMUM MECHANICAL RESPONSE
100
80
60
40
20
3.35(3.11-3.58)
3.58(3.08-4.09)
10-11
10-10
10-9
10-8
10-7
10-6
10-5
10-4
ISOPROTERENOL (M)

that the maximum responses of left atria to isoproterenol were underestimated due to the onset of spontaneous rhythms. If the effects of both isomers were affected similarly, this underestimation would be reflected in an apparent increase in negative log molar ED_{50} values without a concomitant change in the isomeric potency difference. This possibility is consistent with results which were obtained.

In comparing results of experiments in which theophylline was absent to those where it was present at 10^{-3}M, the effects of the (-)-isomer on both rate and force were potentiated to a greater extent than the effects of the (+)-isomer. This tendency is reflected in an increase in the isomeric potency difference from 3.27 to 3.34 in the case of rate, and from 3.30 to 3.51 in the case of force. The lesser potentiating effect of theophylline on (+)-isoproterenol suggested that this isomer might itself be inhibiting phosphodiesterase. As indicated by the studies of Goren and Rosen (1972) on beef heart phosphodiesterase, inhibition of this enzyme by high concentrations of catecholamines is not stereoselective but might appear so in the present experiments since (+)-isoproterenol must be used in higher doses to produce a pharmacological effect. It is possible that high concentrations of isoproterenol, by inhibiting phosphodiesterase, might potentiate responses elicited through beta receptor activation. This was tested by using the beta receptor antagonist practolol to shift the dose-response curve for rate from the (-)-isomer to the right on the log-dose axis. Under these conditions,

effective concentrations of the (-)-isomer are more similar to those of the (+)-isomer. With practolol, 10^{-3}M, the negative log molar ED_{50} of (-)-isoproterenol is approximately 5.0. The results are illustrated in Figure 5. The dose-ratio for practolol (i.e., the difference in potency of (-)-isoproterenol in the absence and presence of practolol) is 1/2 as great without theophylline (5837-fold) as it is with theophylline (11,630-fold). Thus, theophylline potentiates the effects of lower concentrations of isoproterenol more than it does the effects of higher concentrations.

Increases in Atrial Cyclic AMP Levels in Response to Enantiomers of Isoproterenol

A characteristic time course of the isoproterenol-induced accumulation of cyclic AMP is illustrated in Figure 6. The results were obtained in the presence of tropolone and theophylline, 10^{-3}M. Cyclic AMP levels were elevated as early as 5 seconds and reached maximum within 15 seconds after exposure to an effective dose of either isomer. At all doses, the levels of nucleotide at 15, 30, and 60 seconds were statistically the same, and therefore, the values were pooled. In subsequent experiments in which phentolamine was also included, all determinations of cyclic AMP were made after a 60 second exposure to isoproterenol. As was the case for mechanical responses, maximally effective doses of either isomer elicited similar effects on cyclic AMP levels.

Dose-response curves for increases in cyclic AMP levels in response to (-)- and (+)-isoproterenol are shown in Figure 7. Table 3 summarizes the data on which these curves are based.

FIGURE 5

Effects of theophylline, 10^{-3}M, on the increase in atrial rate in response to (-)-isoproterenol in the absence and presence of practolol, 10^{-3}M. Log dose-response curves to (-)-isoproterenol were obtained from spontaneously beating rat atria in the presence of tropolone and phentolamine under one of four conditions: 1) without practolol, without theophylline; 2) without practolol, with theophylline; 3) with practolol, without theophylline; and 4) with practolol, with theophylline. Numbers between the horizontal arrows are the fold difference in potency for (-)-isoproterenol under conditions 1 versus 3 and 2 versus 4. Vertical lines indicate S.E.M. The number of observations under each condition is 8 or 9.

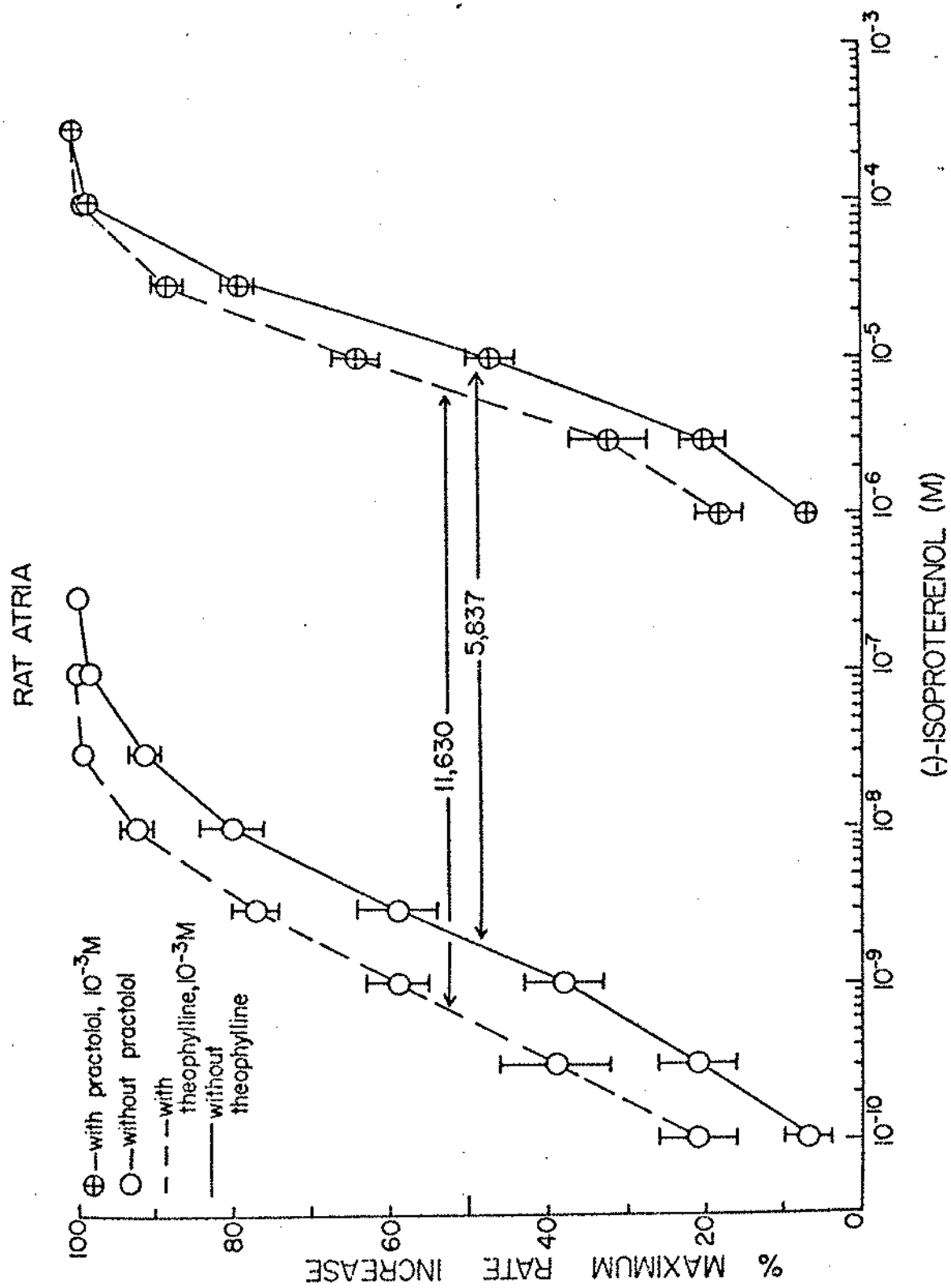

RAT ATRIA
⊕—with practolol, 10⁻³M
○—without practolol
– – –with theophylline, 10⁻³M
——without theophylline
11,630
5,837
% MAXIMUM RATE INCREASE
(-)-ISOPROTERENOL (M)
100
80
60
40
20
0
10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
10⁻³

FIGURE 6

Time-response graph for increases in cyclic AMP levels in response to (-)-isoproterenol, 10^{-5}M. Results were obtained from rat atria in the presence of tropolone and theophylline (10^{-3}M). Spontaneously beating right atria were exposed to isoproterenol for fixed periods of time, and cyclic AMP levels were then determined by the method of Gilman (1970). Vertical lines indicate S.E.M., and n is the number of observations at each time.

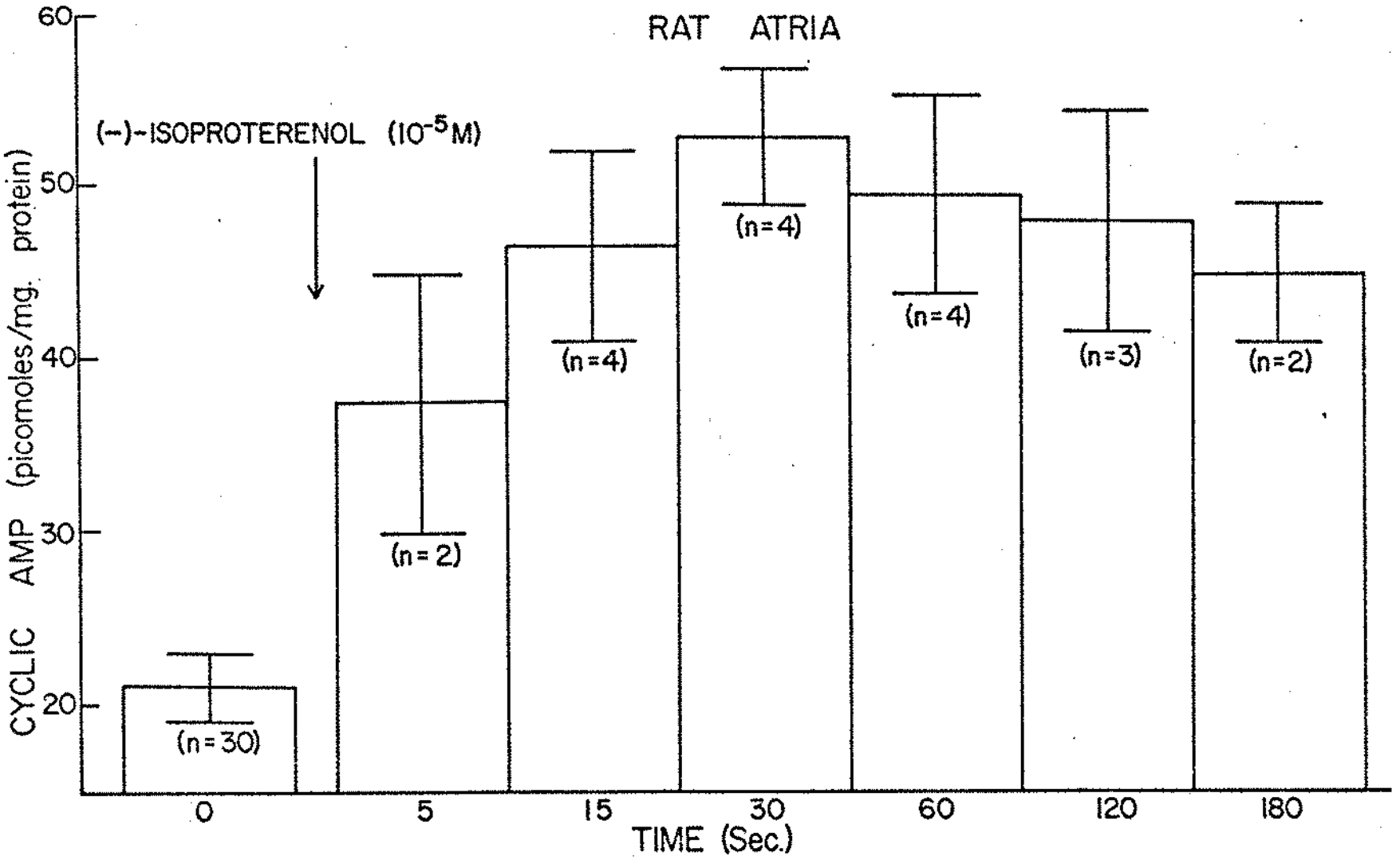

RAT ATRIA
(-)-ISOPROTERENOL (10^{-5}M)
CYCLIC AMP (picomoles/mg. protein)
60
50
40
30
20
(n=30)
(n=2)
(n=4)
(n=4)
(n=4)
(n=3)
(n=2)
0
5
15
30
60
120
180
TIME (Sec.)

FIGURE 7

Log dose-response curves for increases in cyclic AMP levels in response to (-)- and (+)-isoproterenol. Results were obtained from rat atria in the presence of tropolone and theophylline (10^{-3}M) with and without phentolamine. A single dose of one isomer was used on each spontaneously beating righ atrium. Determinations of cyclic AMP levels were made by the method of Gilman (1970). Numbers between the horizontal arrows are isomeric potency differences [(negative log molar ED_{50} of (-)-isomer) - (negative log molar ED_{50} of (+)-isomer)]. Vertical lines indicate S.E.M., and the number of observations at each dose is given within parentheses.

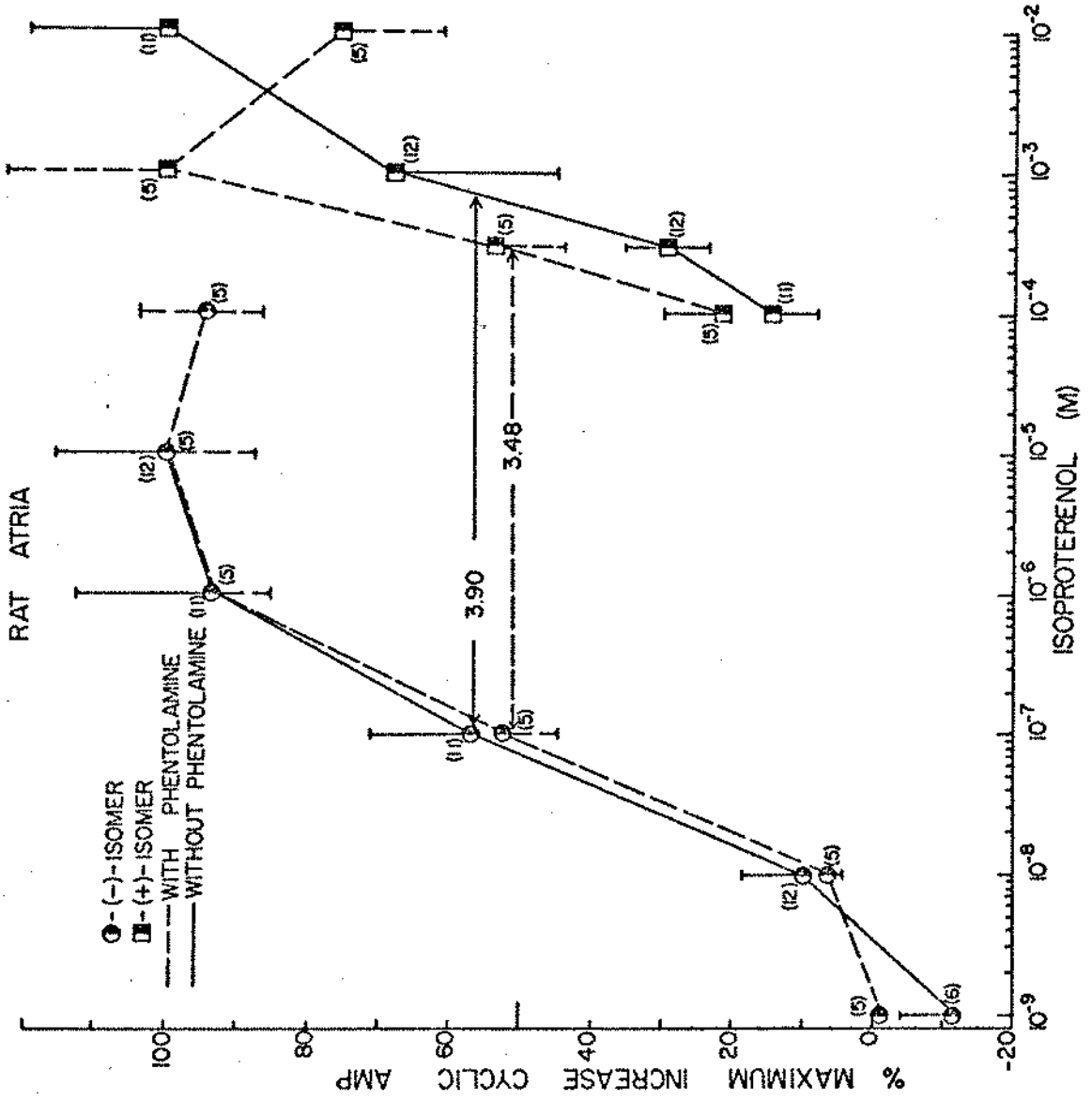

RAT ATRIA
●- (-)-ISOMER
□- (+)-ISOMER
— — WITH PHENTOLAMINE
——— WITHOUT PHENTOLAMINE
3.90
3.48
% MAXIMUM INCREASE CYCLIC AMP
ISOPROTERENOL (M)
100
80
60
40
20
0
-20
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}

TABLE 3

Increases in Cyclic AMP Levels in Response to Isoproterenol in Rat Atria

Treatment	Number of Observations	Concentration and Isomer of Isoproterenol	Picomoles Cyclic AMP per mg Protein with S.E.M.	%Increase[a] with S.E.M.	%Maximum Increase with S.E.M.
Tropolone + Theophylline (10^{-3}M)	30	control[b]	30.9 ± 4.8[g]	-	-
	6	(-) 10^{-9}M	48.5 ± 7.4	-12 ± 13	-12 ± 8[d]
	12	(-) 10^{-8}M	38.8 ± 5.7	10 ± 13	10 ± 9[cd]
	11	(-) 10^{-7}M	51.1 ± 6.4	57 ± 18	57 ± 14
	11	(-) 10^{-6}M	63.3 ± 7.1	95 ± 25	94 ± 19
	12	(-) 10^{-5}M	73.4 ± 10.3[h]	101 ± 16	100 ± 16
	11	(+) 10^{-4}M	48.2 ± 9.5	21 ± 13	15 ± 6
	12	(+) 3×10^{-4}M	78.3 ± 5.0	42 ± 9	29 ± 6[d]
	12	(+) 10^{-3}M	69.3 ± 15.7	98 ± 44	68 ± 23
	11	(+) 10^{-2}M	80.0 ± 12.4[i]	144 ± 28	100 ± 19

TABLE 3 (continued)

Treatment	Number of Observations	Concentration and Isomer of Isoproterenol	Picomoles Cyclic AMP per mg Protein with S.E.M.	%Increase[a] with S.E.M.	%Maximum Increase with S.E.M.
Tropolone + Theophylline + (10^{-3}M) Phentolamine	6	control[b]	10.9 ± 0.5[e]	-	-
	5	(-) 10^{-9}M	10.4 ± 0.4	-4 ± 4	-1 ± 1[cd]
	5	(-) 10^{-8}M	14.2 ± 1.5	31 ± 13	6 ± 2[cd]
	5	(-) 10^{-7}M	38.4 ± 4.6	252 ± 42	53 ± 8[cd]
	5	(-) 10^{-6}M	59.9 ± 1.2	449 ± 11	94 ± 8
	5	(-) 10^{-5}M	63.3 ± 6.5	480 ± 59	100 ± 12
	5	(-) 10^{-4}M	60.4 ± 2.7	454 ± 25	95 ± 9
	5	(+) 10^{-4}M	28.7 ± 4.3	163 ± 40	22 ± 5[cd]
	5	(+) 3×10^{-4}M	55.3 ± 5.9	407 ± 54	54 ± 10
	5	(+) 10^{-3}M	93.2 ± 18.3	755 ± 168	100 ± 22
	5	(+) 10^{-2}M	91.8 ± 11.5	570 ± 84	75 ± 14
Tropolone + Theophylline + (10^{-3}M) Phentolamine + Practolol (10^{-4}M)	4	control[b]	7.5 ± 2.2	-	-[d]
	4	(-) 10^{-7}M	10.5 ± 1.3[f]	35 ± 24[f]	-
	4	(+) 3×10^{-4}M	10.1 ± 1.7[f]	34 ± 17[f]	-

TABLE 3 (continued)

[a]Separate controls were determined for separate experiments.

[b]Controls for all experiments were averaged.

[c]Significantly different ($P < .05$) from value obtained with next highest concentration of isoproterenol.

[d]Significantly different ($P < .05$) from value obtained with second highest concentration of isoproterenol.

[e]Significantly different ($P < .05$) from average control obtained in the absence of phentolamine.

[f]Significantly different ($P < .05$) from values obtained with the same concentration of isoproterenol in the absence of practolol.

[g]Equivalent to 2.71 ± .36 picomoles cyclic AMP per mg wet weight of atrium.

[h]Equivalent to 6.37 ± .74 picomoles cyclic AMP per mg wet weight of atrium.

[i]Equivalent to 7.48 ± 1.10 picomoles cyclic AMP per mg wet weight of atrium.

In the absence of phentolamine, negative log molar ED_{50} values are 7.15 for the (-)-isomer and 3.25 for the (+)-isomer, and the resulting isomeric potency difference is approximately 3.90 log units. The cyclic AMP levels produced by (-)-isoproterenol, 10^{-9}M, were consistently below baseline, but the difference was not statistically significant.

In experiments conducted in the presence of phentolamine, basal levels of cyclic AMP were decreased 65 per cent. However, peak levels attained with maximally effective doses of either isomer were unaltered. Likewise, negative log molar ED_{50} values of 7.05 and 3.57 for (-)- and (+)-isomers and an isomeric potency difference of approximately 3.48 log units do not represent significant alterations from values in the absence of phentolamine. At high concentrations of isoproterenol the levels of cyclic AMP decreased relative to maximum levels, but this was not statistically significant. Results of experiments on isoproterenol-induced increases in cyclic AMP are summarized in Table 4.

The nature of the increase in atrial cyclic AMP in response to isoproterenol was further investigated by testing its susceptibility to blockade by practolol. The selective blockade by this drug of beta receptor-mediated mechanical responses in rat atria has been demonstrated (Refsum and Landmark, 1972). As illustrated in Figure 8, practolol, 10^{-4}M, completely abolished the increases in cyclic AMP induced by intermediately effective doses of either (-)- or (+)-isoproterenol.

TABLE 4

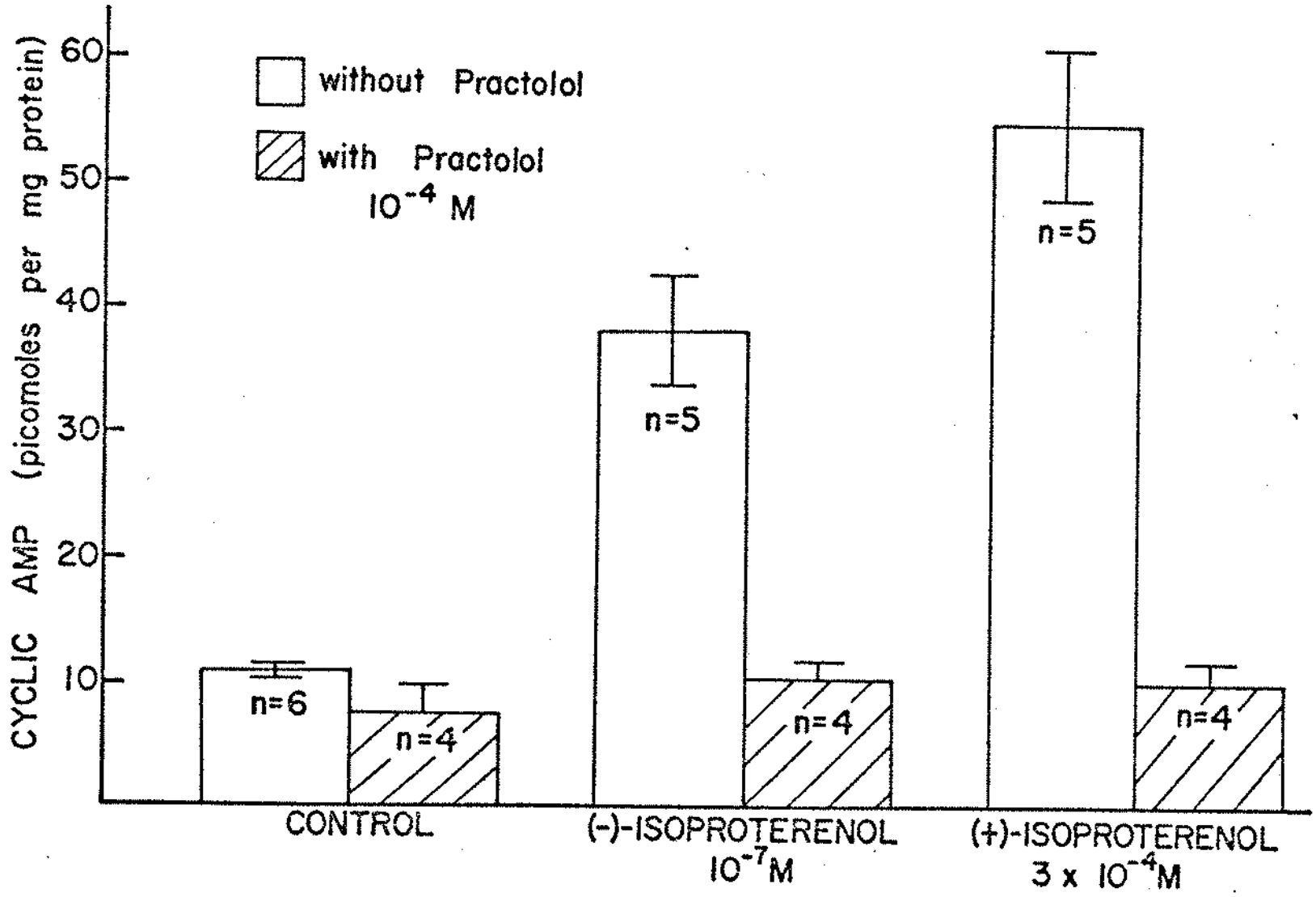
Increases in Cyclic AMP in Response to Isoproterenol in Rat Atria

Treatment	Isomer	Negative log molar ED_{50}	Isomeric potency difference[a]
Tropolone + Theophylline (10^{-3}M)	(-)	7.15	3.90
	(+)	3.25	
Tropolone + Theophylline + (10^{-3}M) Phentolamine	(-)	7.05	3.48
	(+)	3.57	

[a] Isomeric potency difference = [negative log molar ED_{50} of (-)-isomer] - [negative log molar ED_{50} of (+)-isomer].

FIGURE 8

Effect of practolol, 10^{-4}M, on the increase in cyclic AMP levels in response to isoproterenol in rat atria in the presence of tropolone and theophylline (10^{-3}M). Spontaneously beating right atria were exposed for one minute to either (-)-isoproterenol, 10^{-7}M, or (+)-isoproterenol, 3×10^{-4}M, in the absence and presence of practolol. Cyclic AMP levels were determined by the method of Gilman (1970). Vertical lines indicate S.E.M., and n is the number of observations.


RAT ATRIA
without Practolol
with Practolol
10⁻⁴ M
CYCLIC AMP (picomoles per mg protein)
60
50
40
30
20
10
n=6
n=4
n=5
n=4
n=5
n=4
CONTROL
(-)-ISOPROTERENOL
10⁻⁷M
(+)-ISOPROTERENOL
3 x 10⁻⁴M

Uterine Mechanical Responses to Enantiomers of Isoproterenol

Maximum reduction of uterine contractile amplitude usually occurred within 2-4 minutes after addition of each dose of isoproterenol. However, when the rate of spontaneous contraction was particularly high (3 or more per minute), time until maximum was less than 2 minutes. In all preparations, regardless of rate, the maximum effect occurred within 2-3 contractions. Thus, the time course of development of the effect on contractile amplitude may depend on contraction rate.

Results of uterine mechanical responses are summarized in Table 5. Figure 9 shows the average cumulative dose response curves for isoproterenol in the presence of tropolone and phentolamine. Negative log molar ED values are 9.75 and 6.87 for the (-)- and (+)-isomers, respectively, and the resulting potency difference is 2.88 log units. As illustrated in Figure 10, theophylline, 10^{-4}M, potentiates the response to both (-)- and (+)-isomers by equal amounts (0.57 and 0.56 log units), and consequently, the isomeric potency difference (2.90 log units) is unaltered. At a concentration of 10^{-3}M, theophylline completely abolished uterine contractions, and, therefore, it was not possible to study isoproterenol-induced responses under these conditions.

Increases in Uterine Cyclic AMP Levels in Response to Enantiomers of Isoproterenol

Increases in cyclic AMP levels in uterus, obtained in the presence of tropolone and theophylline, 10^{-3}M, were measured at various time intervals after exposure to isoproterenol.

TABLE 5

Mechanical Responses to Isoproterenol in Rat Uteri

Treatment	Isomer	Number of observations	Negative log molar ED_{50} with S.E.M.	Isomeric potency difference[a] (95% C.I.)
Tropolone + Phentolamine	(-)	7	9.75 ± .16	2.88 (2.66 - 3.10)
	(+)	7	6.87 ± .11	
Tropolone + Phentolamine + Theophylline (10^{-4}M)	(-)	7	10.32 ± .13[b]	2.90 (2.72 - 3.07)
	(+)	7	7.43 ± .07[b]	

[a]Isomeric potency difference = [negative log molar ED_{50} of (-)-isomer] - [negative log molar ED_{50} of (+)-isomer].

[b]Significantly different ($P < .05$) from corresponding value obtained in the absence of theophylline.

FIGURE 9

Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat uteri in the presence of phentolamine and tropolone. Decreases in amplitude were determined from spontaneously contracting uterine segments. Numbers between the horizontal arrows connecting the curves are isomeric potency differences [(negative log molar ED_{50} of (-)-isomer) - (negative log molar ED_{50} of (+)-isomer)] along with their 95 per cent C.I. in parentheses. Vertical lines indicate S.E.M., and n is the number of observations.

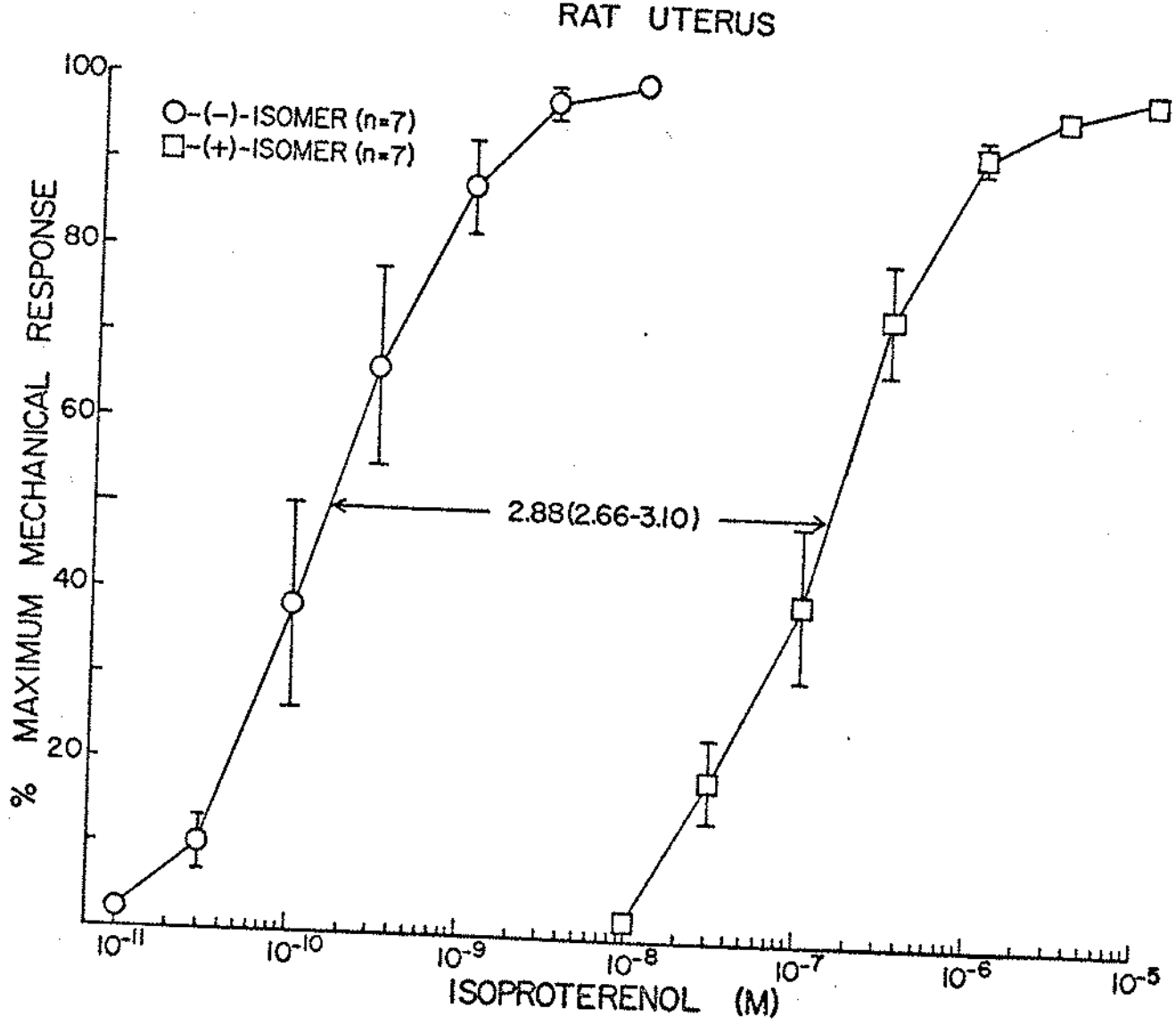

RAT UTERUS
O-(-)-ISOMER (n=7)
□-(+)-ISOMER (n=7)
% MAXIMUM MECHANICAL RESPONSE
100
80
60
40
20
2.88(2.66-3.10)
10⁻¹¹
10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
ISOPROTERENOL (M)

FIGURE 10

Log dose-response curves for mechanical responses to (-)- and (+)-isoproterenol obtained from rat uteri in the presence of theophylline (10^{-4}M), phentolamine, and tropolone. Decreases in amplitude were determined from spontaneously contracting uterine segments. Numbers between the horizontal arrows connecting the curves are isomeric potency differences [(negative log molar ED_{50} of (-)-isomer) - (negative log molar ED_{50} of (+)-isomer)] along with their 95 per cent C.I. in parentheses. Vertical lines indicate S.E.M., and n is the number of observations.

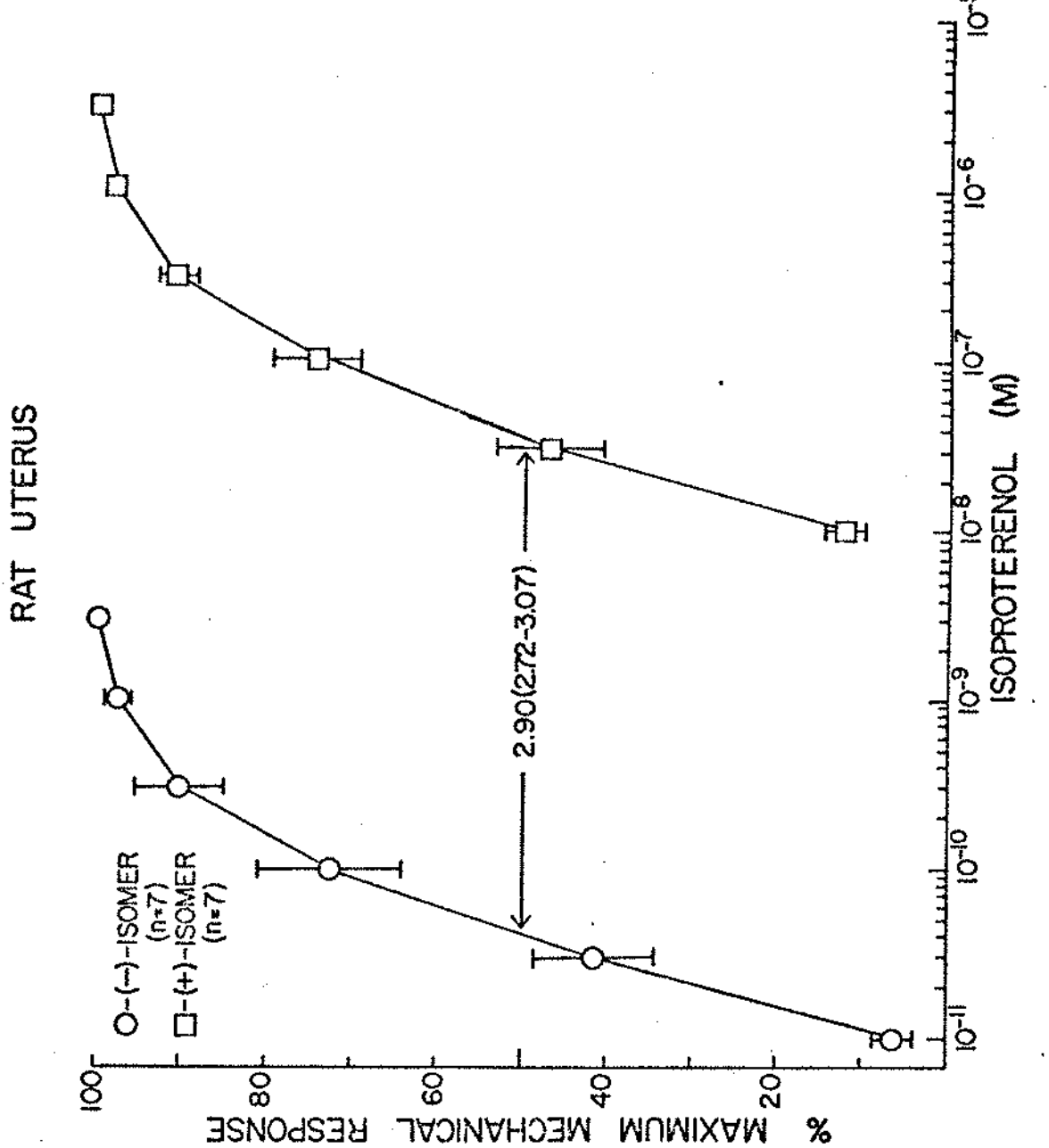

RAT UTERUS
O-(−)-ISOMER (n=7)
□-(+)-ISOMER (n=7)
2.90(2.72-3.07)
% MAXIMUM MECHANICAL RESPONSE
100
80
60
40
20
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
ISOPROTERENOL (M)

Figure 11 shows the time course of this response, plotted from data tabulated in Table 6. Effective doses of isoproterenol produced marked elevations in nucleotide at least as early as 15 seconds after addition. With most concentrations, the maximum response was attained by 120 seconds. Values at 60, 120, and 180 seconds were not significantly different from one another at any concentration and the values were therefore pooled. The cyclic AMP levels attained with maximally effective concentrations of isoproterenol were similar for both isomers.

Dose-response curves, plotted from data summarized in Table 7 are shown in Figure 12. The negative log molar ED_{50}'s are 7.26 and 4.72 for (-)- and (+)-isomers and the resulting potency difference is approximately 2.54 log units. Since there appeared to be a distinct time-response relationship throughout the concentration range of both isomers (Figure 11), a Michaelis "rate constant" or "Km" was determined on the basis of cyclic AMP levels at 15 and 30 seconds. In making the analogy with enzyme kinetics, isoproterenol was considered to be analogous to substrate (S) and cyclic AMP was considered to be the product. Average Km values were calculated from the data in Table 6 using the Michaelis-Menten equation:

$$v = \frac{Vmax \cdot S}{S + Km} \quad ,$$

where v and Vmax are the per cent increase in cyclic AMP per second produced by intermediate and maximally effective concentrations of isoproterenol, respectively. The Km values so

FIGURE 11

Time-response curves for increases in cyclic AMP levels in response to (-)- and (+)-isoproterenol obtained from rat uteri in the presence of tropolone and theophylline (10^{-3}M). Individual uterine segments were exposed to a single dose of one isomer for a fixed period of time, and cyclic AMP levels were then determined by the method of Gilman (1970).

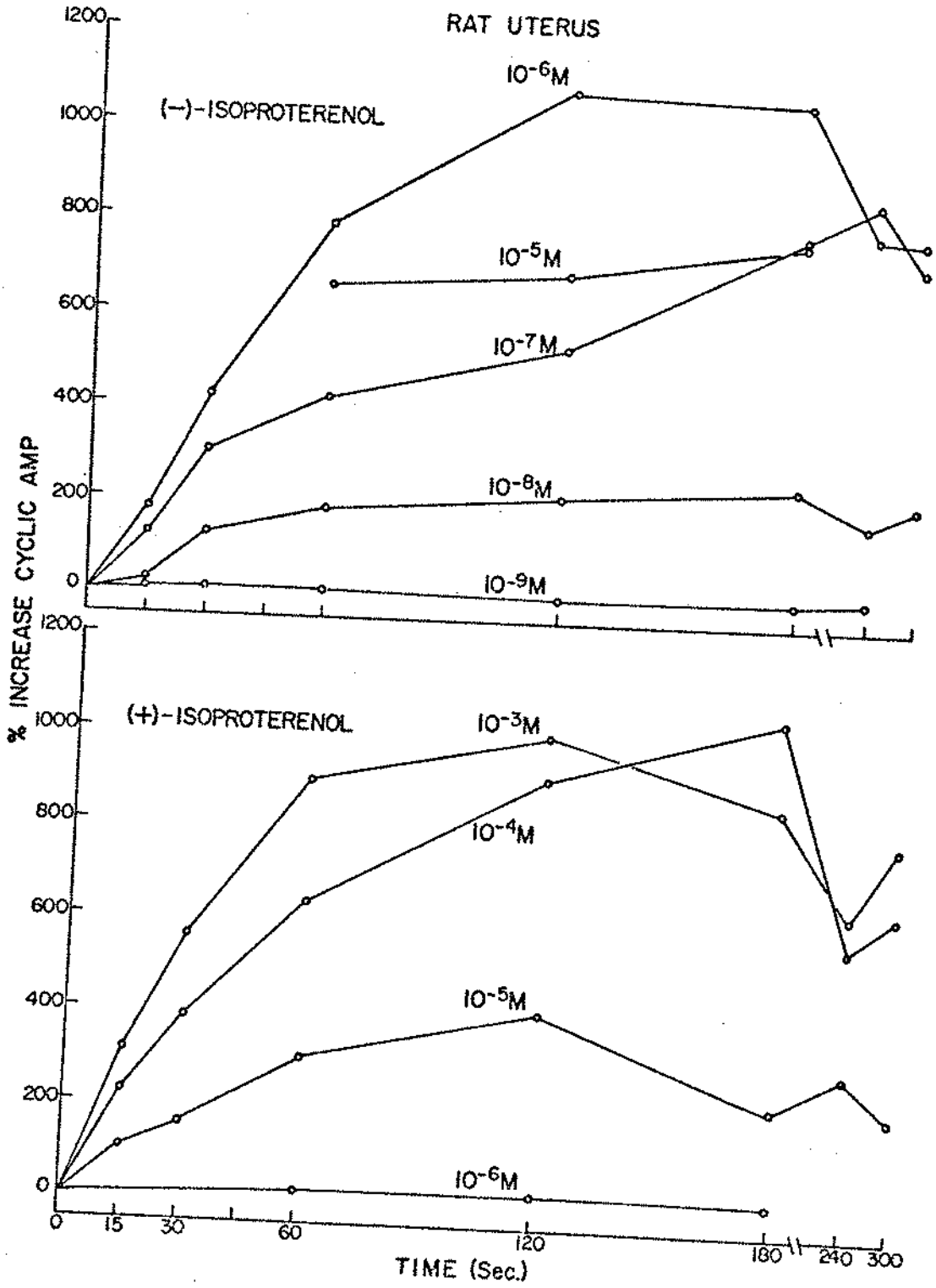

RAT UTERUS
(−)-ISOPROTERENOL
10-6M
10-5M
10-7M
10-8M
10-9M
(+)-ISOPROTERENOL
10-3M
10-4M
10-5M
10-6M
% INCREASE CYCLIC AMP
TIME (Sec.)
0
200
400
600
800
1000
1200
0
15
30
60
120
180
240
300

TABLE 6

Time Response of Isoproterenol-Induced Cyclic AMP Increases in Rat Uteri

Concentration and Isomer	Per Cent Increase in Cyclic AMP Above Control[a] Time (seconds)						
	15	30	60	120	180	240	300
(-) 10^{-10}				22 ± 23	-3 ± 9	-23 ± 7	-5 ± 6
(-) 10^{-9}	7 ± 10	10 ± 30	6 ± 10	0 ± 10	1 ± 9	11 ± 17	45 ± 20
(-) 10^{-8}	22 ± 9	122 ± 35	185 ± 79	216 ± 80	248 ± 38	169 ± 10	217 ± 43
(-) 10^{-7}	123 ± 51	300 ± 33	418 ± 71	632 ± 131	783 ± 178	858 ± 287	719 ± 242
(-) 10^{-6}	175 ± 31	420 ± 62	785 ± 101	1079 ± 136	1060 ± 136	783 ± 137	777 ± 89
(-) 10^{-5}			658 ± 182	693 ± 204	770 ± 201		
(+) 10^{-6}			17 ± 8	21 ± 10	16 ± 6		
(+) 10^{-5}	104 ± 38	160 ± 25	304 ± 72	359 ± 26	218 ± 53	296 ± 95	207 ± 33
(+) 10^{-4}	224 ± 50	388 ± 76	633 ± 145	909 ± 219	1048 ± 174	563 ± 125	640 ± 108
(+) 10^{-3}	310 ± 24	559 ± 84	844 ± 103	998 ± 17	858 ± 167	638 ± 76	787 ± 90

[a]Mean ± S.E.M. of 3 or 4 observations for each time for each concentration.

TABLE 7

Increases in Cyclic AMP Levels in Response to Isoproterenol in Rat Uteri

Treatment	Number of Observations	Concentration and Isomer of Isoproterenol	Picomoles Cyclic AMP per mg Protein with S.E.M.	%Increase[a] with S.E.M.	%Maximum Increase with S.E.M.
Tropolone + Theophylline (10^{-3}M)	81	control[b]	4.5 ± 0.2[g]	-	-
	7	(-) 10^{-10}M	5.2 ± 0.5	8 ± 11	1 ± 1[d]
	11	(-) 10^{-9}M	2.6 ± 0.1	2 ± 5	0 ± 1[cd]
	12	(-) 10^{-8}M	16.8 ± 2.0	216 ± 37	22 ± 4[cd]
	12	(-) 10^{-7}M	36.2 ± 4.5	578 ± 84	60 ± 10[c]
	11	(-) 10^{-6}M	52.3 ± 3.8[h]	966 ± 77	100 ± 11[c]
	12	(-) 10^{-5}M	39.6 ± 4.7	707 ± 96	73 ± 12
	12	(+) 10^{-6}M	5.4 ± 0.2	18 ± 4	2 ± 1[cd]
	12	(+) 10^{-5}M	18.1 ± 1.5	294 ± 33	33 ± 4[cd]
	12	(+) 10^{-4}M	40.5 ± 4.5	863 ± 108	96 ± 14
	12	(+) 10^{-3}M	39.5 ± 2.5[i]	900 ± 63	100 ± 10

TABLE 7 (continued)

Treatment	Number of Observations	Concentration and Isomer of Isoproterenol	Picomoles Cyclic AMP per mg Protein with S.E.M.	%Increase[a] with S.E.M.	%Maximum Increase
Tropolone + Theophylline + (10^{-3}M) Phentolamine	8	control[b]	4.5 ± 0.6	-	-[cd]
	5	(-) 10^{-8}M	7.7 ± 0.9	72 ± 21	21 ± 9[cd]
	5	(-) 10^{-7}M	20.2 ± 5.4	351 ± 121[e]	100 ± 49
	5	(-) 10^{-6}M	14.4 ± 2.3	223 ± 52	64 ± 26
	5	(-) 10^{-5}M	21.8 ± 3.3	387 ± 74	100 ± 43
	5	(+) 10^{-6}M	6.5 ± 0.4	45 ± 10	12 ± 4[cd]
	5	(+) 10^{-5}M	11.9 ± 1.3	166 ± 30	44 ± 15
	5	(+) 10^{-4}M	18.0 ± 4.6	303 ± 102	80 ± 36
	5	(+) 10^{-3}M	21.4 ± 4.9	378 ± 109[e]	100 ± 41
Tropolone + Theophylline + (10^{-3}M) Phentolamine + Practolol (10-4M)	6	control[b]	5.0 ± 0.3	-	-
	6	(-) 10^{-7}M	6.8 ± 0.8[f]	37 ± 16[f]	-
	6	(+) 10^{-5}M	6.3 ± 0.4[f]	26 ± 8[f]	-

TABLE 7 (continued)

[a]Separate controls were determined for separate experiments.

[b]Controls for all experiments were averaged.

[c]Significantly different ($P < .05$) from value obtained with next highest concentration of isoproterenol.

[d]Significantly different ($P < .05$) from value obtained with second next highest concentration of isoproterenol.

[e]Significantly different ($P < .05$) from maximum responses obtained in the absence of phentolamine.

[f]Significantly different ($P < .05$) from values obtained with the same concentration of isoproterenol in the absence of practolol.

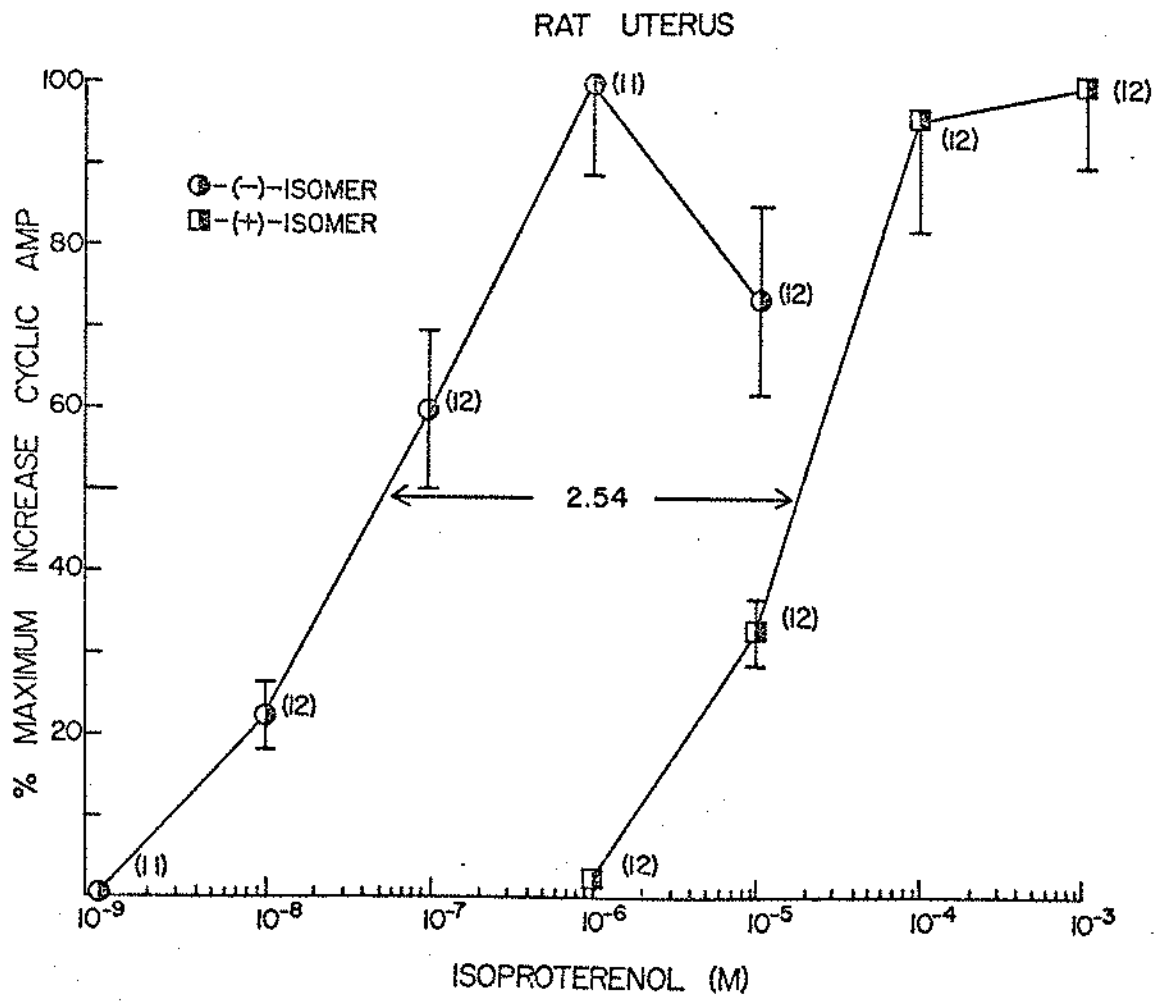
[g]Equivalent to .40 ± .01 picomoles cyclic AMP per mg wet weight of uterus.

[h]Equivalent to 4.75 ± .38 picomoles cyclic AMP per mg wet weight of uterus.

[i]Equivalent to 3.66 ± .24 picomoles cyclic AMP per mg wet weight of uterus.

FIGURE 12

Log dose-response curves for increases in cyclic AMP levels in response to (-)- and (+)-isoproterenol obtained from rat uteri in the presence of tropolone and theophylline (10^{-3}M). A single dose of one isomer was used on each uterine segment. Determination of cyclic AMP levels were made by the method of Gilman (1970). Numbers between the horizontal arrows are isomeric potency differences [(negative log molar ED_{50} of (-)-isomer) - (negative log molar ED_{50} of (+)-isomer)]. Vertical lines indicate S.E.M., and the number of observations at each dose is given within parentheses.


RAT UTERUS
% MAXIMUM INCREASE CYCLIC AMP
100
80
60
40
20
●-(−)-ISOMER
□-(+)-ISOMER
(11)
(12)
(12)
(12)
(12)
(12)
(12)
2.54
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
ISOPROTERENOL (M)

obtained are 4.20 x 10^{-8}M for (-)-isoproterenol and 2.91 x 10^{-5}M for (+)-isoproterenol. If the Km is considered analogous to the ED_{50}, then the potency difference between the isomers is approximately 2.84 log units. These results based on the rate of accumulation of cyclic AMP are quite similar to those obtained on the basis of maximum levels of cyclic AMP.

In subsequent experiments, conducted in the presence of phentolamine, all determinations of cyclic AMP levels were made after a 180 second exposure to isoproterenol. While control levels were not affected by this drug, the maximum isoproterenol-induced increase was depressed by approximately 60 per cent. Since neither the negative log molar ED_{50}'s (7.63 for (-)- and 4.84 for (+)-isomers) nor the isomeric potency difference (2.79) are altered, however, the depressant action is probably unrelated to an effect at the receptor. In experiments with or without phentolamine, doses of (-)-isoproterenol higher than those required to elicit a maximum response significantly decreased the levels of cyclic AMP relative to maximum. Results from preceding experiments are summarized in Table 8.

Practolol, a cardioselective beta receptor antagonist, was evaluated for its ability to block the isoproterenol-induced accumulation of cyclic AMP in uterus. As shown in Figure 13, practolol, 10^{-4}M, almost completely abolished the increase in cyclic AMP induced by either (-)-isoproterenol, 10^{-7}M, or (+)-isoproterenol, 10^{-4}M, without affecting control levels.

TABLE 8

Increases in Cyclic AMP in Response to Isoproterenol in Rat Uteri

Treatment	Isomer	Negative log molar ED_{50}	Isomeric potency difference[a]
Tropolone + Theophylline (10^{-3}M)	(-)[b]	7.26	2.54
	(+)[b]	4.72	
	(-)[c]	7.38	2.84
	(+)[c]	4.54	
Tropolone + Theophylline + (10^{-3}M) Phentolamine	(-)[b]	7.63	2.79
	(+)[b]	4.84	

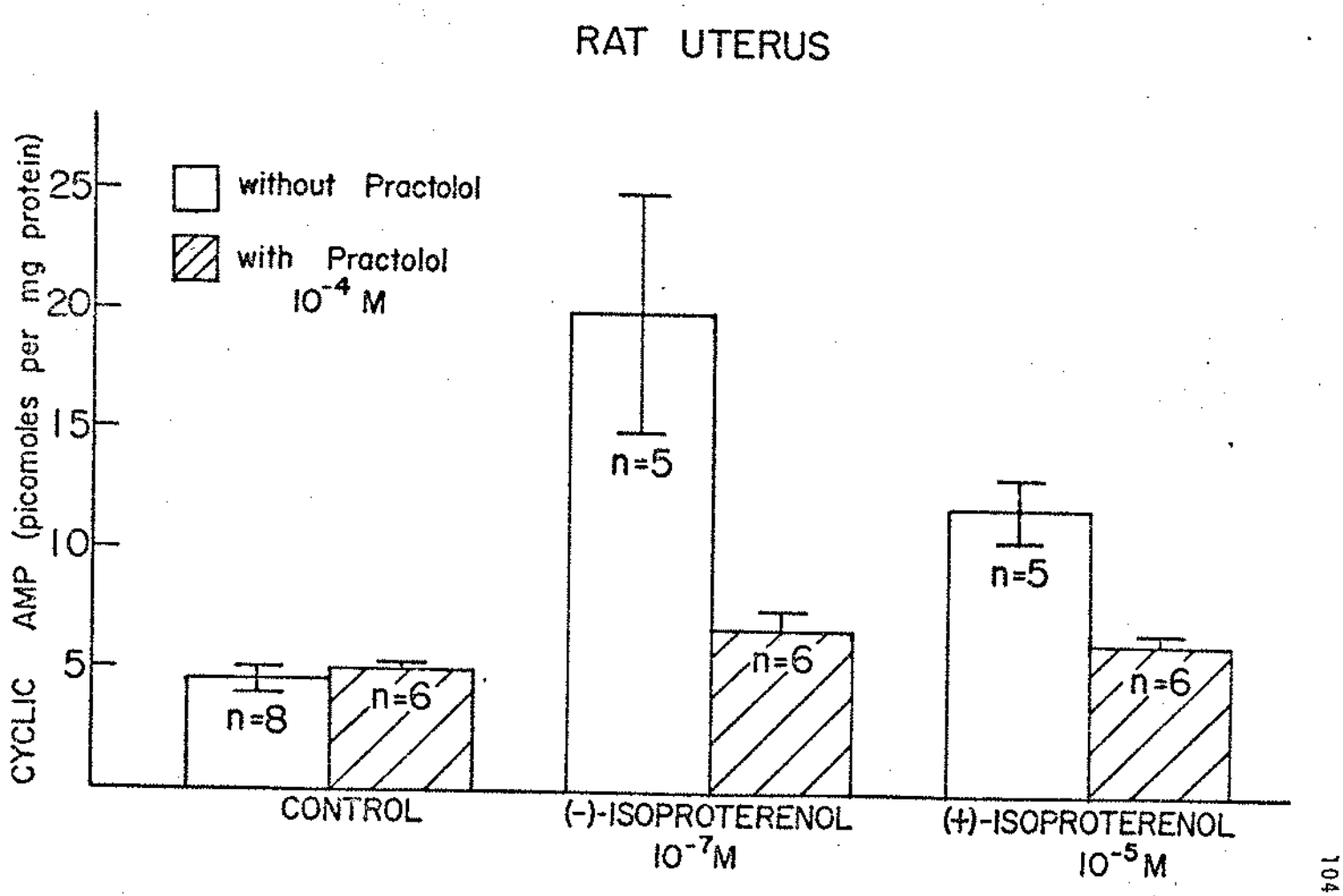
[a]Isomeric potency difference = [negative log molar ED_{50} of (-)-isomer] - [negative log molar ED_{50} of (+)-isomer].

[b]Results based on maximum levels of cyclic AMP.

[c]Results based on rate of accumulation of cyclic AMP.

FIGURE 13

Effect of practolol, 10^{-4}M, on the increase in cyclic AMP levels in response to isoproterenol in rat uteri in the presence of tropolone and theophylline (10^{-3}M). Uterine segments were exposed for three minutes to either (-)-isoproterenol, 10^{-7}M, or (+)-isoproterenol, 10^{-5}M, in the absence and presence of practolol. Cyclic AMP levels were determined by the method of Gilman (1970). Vertical lines indicate S.E.M., and n is the number of observations.


RAT UTERUS
CYCLIC AMP (picomoles per mg protein)
25
20
15
10
5
without Practolol
with Practolol
10^{-4} M
n=8
n=6
n=5
n=6
n=5
n=6
CONTROL
(-)-ISOPROTERENOL
10^{-7}M
(+)-ISOPROTERENOL
10^{-5}M

Effect of DES on the Increases in Atrial Rate in Response to Isoproterenol

Animals used in measuring atrial mechanical responses and cyclic AMP levels were selected without regard to estrogenic status, while those used in parallel experiments measuring uterine relaxation and cyclic AMP levels were either in a state of natural estrus or maintained in estrus with reserpine and DES. It is possible that estrogenic status might influence the activity of isoproterenol in atria, either by some action at the receptor, as has been postulated to occur in the uterus, or at some non-beta receptor site. In cat uterus, for example, there is an apparent transition from alpha receptor predominance in the progesterone-dominated tissue, to beta receptor predominance in the estrogen-dominated tissue (Gruber, 1933; Tsai and Fleming, 1964). Steroids have been reported to inhibit extraneuronal uptake of ^{3}H-norepinephrine in perfused rat heart (Iversen and Salt, 1970), and it has also been suggested that the steroid potentiation of responses to sympathomimetic amines in aorta is a consequence of interference of the o-methylation pathway by which these compounds are metabolized (Kalsner, 1969a; 1969b). Jacobowitz and Brus (1971) were unable to demonstrate by histofluorescence any effect of prolonged estradiol treatment on the extraneuronal uptake of norepinephrine into fibroblasts, blood vessels, connective tissue fibers, or muscle of perfused guinea-pig heart. On the other hand, neuronal uptake is the primary mechanism of norepinephrine inactivation, and in the

absence of a drug to block this mechanism (e.g., cocaine), an effect on extraneuronal uptake may not have been seen. In this light, Kaumann (1972) was able to demonstrate hydrocortisone potentiation in cat heart of both isoproterenol, which is not taken up into adrenergic neurons, as well as of norepinephrine in the presence of cocaine.

To evaluate the possible influence of DES on mechanical responses of atria, rate of contraction was measured on spontaneously beating right atria of animals in estrus which had been pretreated 24 hours prior to use with reserpine and DES. Tissues were exposed to tropolone, phentolamine, and theophylline, 10^{-4}M. While basal rate was found to be decreased approximately 30 per cent in these atria (175 ± 29 beats per minute) compared with parallel experiments with atria from non-DES-pretreated animals, maximum isoproterenol-induced increases (434 ± 33 beats per minute) and the negative log molar ED_{50} values for the (-)-isomer (9.05 ± .01) and (+)-isomer (5.99 ± .02) were not changed. Thus, with respect to atrial rate, DES does not appear to alter the potency of isoproterenol.

Increases in Atrial Rate in Response to Desoxyisoproterenol

The use of the isomeric potency difference to characterize receptors mediating particular responses presupposes that both isomers are active at those receptors and that the responses to the less active (+)-isomer are not merely due to contamination with the (-)-isomer. With respect to

isoproterenol-induced responses in atria and uteri, where the apparent isomeric potency differences are in the order of 3 log units, a 0.1 per cent contamination by the (-)-isomer in a sample of totally inactive (+)-isomer could, in theory, account for such a difference.

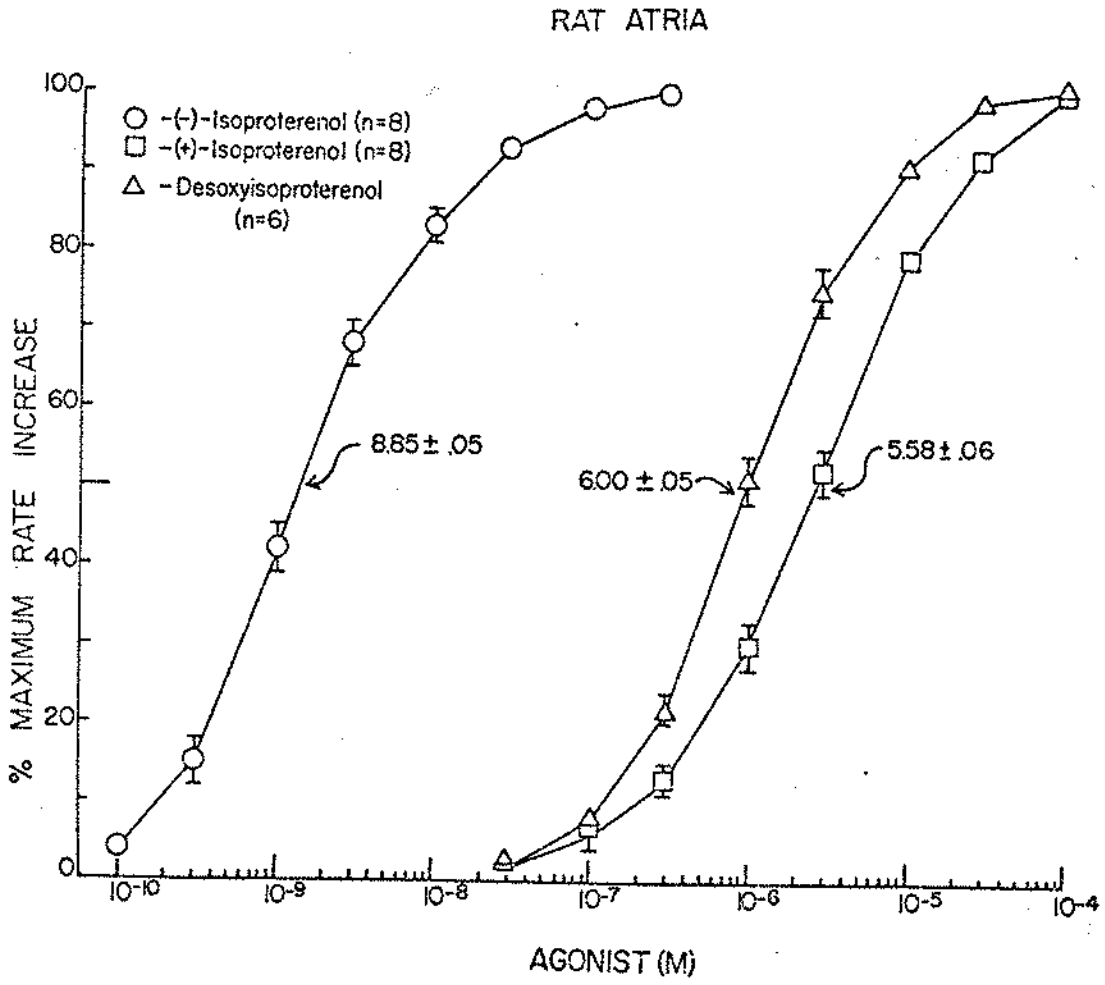
In the absence of a pure standard for comparison, the degree of purity of a given sample of the (+)-isomer cannot be determined directly. In practice the criterion which is employed is repeated recrystallization to constant biological activity, but this does not afford unequivocal assurance of purity. Actually, absolute purity is not a prerequisite for making meaningful determinations of isomeric potency differences. Slight contamination by the (-)-isomer can be tolerated so long as the same sample of (+)-isoproterenol is used in all studies and the activity of the (+)-isomer is not zero. All experiments in the present study were performed with solutions prepared from the same crystalline sample of (+)-isoproterenol. In addition, several experiments were performed which provide indirect evidence that this isomer has activity of its own.

The Easson and Stedman (1933) hypothesis predicts that the (+)-isomers of sympathomimetic compounds like isoproterenol are less potent than their enantiomers because their β-hydroxyl group is in the "wrong position", oriented away from the receptor. This results in a less than perfect interaction with the receptor. Since the β-hydroxyl group is unable to contribute to the drug-receptor interaction, the desoxy-

analog, in which the β-hydroxyl is absent, should be equipotent to the (+)-isomer. Figure 14 illustrates the dose-response curves for atrial rate in response to (-)-, (+)-, and desoxy-isoproterenol obtained in the presence of tropolone and phentolamine. Compared with (-)-isoproterenol, (+)- and desoxy-isoproterenol are quite similar in potency. Negative log molar ED_{50} values are 8.85, 5.58, and 6.00, respectively. Since all functional groups other than the β-hydroxyl moiety are identical on both (+)- and desoxy-compounds, these results suggest that (+)-isoproterenol does indeed have activity of its own. This conclusion is further supported by the fact that different tissues exhibit dissimilar isomeric potency differences (see discussion). If the apparent activity of the (+)-isomer sample were merely a consequence of contamination by the (-)-isomer, then the potency of the (+)-isomer would always be a constant fraction of the (+)-isomer, and the isomeric potency difference would never change. This is not the case.

FIGURE 14

Log dose-response curves for increases in rate in response to (-)-, (+)-, and desoxy-isoproterenol. Responses were obtained from rat atria in the presence of tropolone and phentolamine. Numbers beside the curved arrows are negative log molar ED_{50}'s ± S.E.M. Vertical lines indicate S.E.M. of responses, and n is the number of observations with each agonist.


RAT ATRIA
○ -(-)-Isoproterenol (n=8)
□ -(+)-Isoproterenol (n=8)
△ -Desoxyisoproterenol (n=6)
8.65 ± .05
6.00 ± .05
5.58 ± .06
% MAXIMUM RATE INCREASE
AGONIST (M)
0
20
40
60
80
100
10-10
10-9
10-8
10-7
10-6
10-5
10-4

DISCUSSION

On the basis of isomeric potency differences of isoproterenol, it has been possible to differentiate the beta adrenergic receptors of rat atrium from those of rat uterus. Receptor differences between the two tissues are manifested at the levels of both cyclic AMP formation and mechanical responses. Under proper experimental conditions to control non-receptor processes (Furchgott, 1967; 1970; 1972), the isomeric potency difference is expected to provide a sensitive indication of receptor differences (Patil, 1969). Moreover, the use of the optical isomers of a single agonist in lieu of separate agonists provides a means of controlling accessibility factors which may complicate comparisons of structurally dissimilar compounds in different tissues. Dissimilar isomeric potency differences, obtained for receptors in atria and uteri under various conditions (summarized in Table 9), are consistent with the existence of different beta receptors in the two tissues (Lands et al., 1967a; 1967b). In addition, Patil et al. (1971), previously reported that on the basis of potency differences between (-)- and (+)-norepinephrine, beta receptors of rabbit and guinea-pig atria, guinea-pig trachea, and rabbit aorta were similar to each other but clearly different from those in rat atria. Thus, under the scrutiny of the isomeric potency difference, atrial receptors from the rat appear to retain an identity distinct from other beta receptors. The fact that atria and uteri

TABLE 9

Summary of Isomeric Potency Differences[a] for Isoproterenol in Rat Atria and Uteri

Response	Treatment			
	Tropolone Phentolamine	Tropolone Theophylline (10^{-3}M)	Tropolone Phentolamine Theophylline (10^{-4}M)	Tropolone Phentolamine Theophylline (10^{-3}M)
Atrial Rate	3.27[b]		3.35[b]	3.34
Atrial Force	3.30		3.58[b]	3.51
Atrial Cyclic AMP		3.90		3.48
Uterine Relaxation	2.88[b]		2.90[b]	
Uterine Cyclic AMP		2.54		2.79

[a]Isomeric potency difference = [negative log molar ED_{50} of (-)-isomer] - [negative log molar ED_{50} of (+)-isomer].

[b]Significantly different ($P < .05$) from corresponding isomeric potency difference in uteri or atria.

display dissimilar potency differences may also be regarded as additional indirect evidence that (+)-isoproterenol has activity of its own. Were its apparent activity merely due to contamination by the (-)-isomer, then the potency of the (+)-isomer would always be a constant fraction of the (-)-isomer and the isomeric potency difference would be constant regardless of the tissue.

Beta receptors in atria, mediating increases in rate and force of contraction, have previously been reported to be dissociated by certain agonists and antagonists and by certain experimental conditions. For example soterenol is more of a full agonist (relative to isoproterenol) for production of a positive chronotropic than inotropic response in guinea-pig atria (Farmer et al., 1970; Brittain et al., 1970). In their studies on various derivatives of the primary catecholamines, Lands and Brown (1964) found that α-ethyl-isoproterenol (isoetharine) was 60-times more potent in stimulating force than rate in perfused rabbit heart. Parr and Urquilla (1972) have reported differential blockade by propranolol of epinephrine-induced rate and force increases in rabbit atria, and Broadley (1972) found different orders of potency of the catecholamines for the two responses in perfused guinea-pig heart at 25°C. In many of these experiments, possible influences of rate on the force of contraction (Koch-Weser and Blinks, 1963) and possible actions of agonists and antagonists at non-beta receptor sites were not considered. Most studies using agonists and antagonists

have been unable to differentiate inotropic and chronotropic responses (Blinks, 1967; Kaumann and Blinks, 1967; Bristow and Green, 1970; Refsum and Landmark, 1972). In the present experiments, the same isomeric potency differences for the production of rate and force also indicate that the population of atrial beta receptors which mediate both of these responses have very similar characteristics.

Isomeric potency differences for increases in atrial cyclic AMP levels are nearly identical to those for mechanical responses. Under proper experimental conditions, these values are 3.34, 3.51, and 3.48 log units for rate, force, and cyclic AMP, respectively. Likewise, potency differences between the isomers of isoproterenol in producing uterine relaxation are similar to those observed for increasing uterine cyclic AMP under similar conditions. The potency difference for the mechanical response is 2.90 log units and for increases in cyclic AMP, 2.79. Thus, it may be concluded that in rat uteri, as in rat atria, both isoproterenol-induced mechanical responses and increases in cyclic AMP levels are mediated through similar receptors.

Lefkowitz et al. (1973) reported purification of a microsomal fraction of dog ventricle with a beta receptor profile for both binding of catecholamines and stimulation of adenylate cyclase. However, (±)-norepinephrine and (±)-epinephrine bound equally well as their (-)-isomers, and in addition, (-)- and (+)-epinephrine were equipotent in stimulating adenylate cyclase. The present experiments cast doubt on the authenticity of this "beta receptor" which lacks stereoselectivity. Clearly,

in rat atria and rat uteri beta receptors mediating increases in cyclic AMP and mechanical responses show the same stereoselectivity. It is possible that the conformational state which confers stereoselectivity is lost during homogenization while the order of potency for binding and stimulating adenylate cyclase is still retained.

Evidence from other studies is now accumulating which supports the conclusion that beta receptor subclassification is equally applicable when cyclic AMP formation is measured as an effect as when some mechanical effect is measured. In dog (Murad et al., 1960; 1962; Mayer, 1972) or rat (Burges and Blackburn, 1972) heart homogenates, norepinephrine is either equally or more potent than epinephrine in stimulating adenylate cyclase activity. In similar preparations from rat uterus (Triner et al., 1970a) and rat lung (Burges and Blackburn, 1972) epinephrine is 20 times and 6 times more potent than norepinephrine, respectively.

The selective beta_2 receptor antagonists IMA and TMA are weak or ineffective blockers of catecholamine-induced stimulation of adenylate cyclase in broken cell preparations of dog (Robison et al., 1967; Mayer, 1972) and rat heart (Burges and Blackburn, 1972), and of accumulation of cyclic AMP in perfused rat heart (Robison et al., 1965) or open-chest dog heart (Wastila et al., 1972). In isolated rat uterus, on the other hand, butoxamine is capable of blocking epinephrine-induced cyclic AMP formation in the same dose as propranolol

(Polacek and Daniel, 1971). Conversely, in dog or rat heart homogenates, practolol is either equally or slightly less potent than propranolol or DCI, yet in guinea-pig trachea, practolol only slightly decreases the epinephrine-induced accumulation of cyclic AMP (Murad, 1973). Similar findings were made by Burges and Blackburn (1972) who reported that practolol is approximately 15 times more potent on dog heart than on guinea-pig lung adenylate cyclase, whereas butoxamine is approximately 4 times as potent on the heart than on the lung enzyme. In present experiments, the effectiveness of practolol in blocking isoproterenol-induced cyclic AMP increases in atria and uteri indicates that in both tissues, both isomers are acting on beta receptors. However, in view of the high concentration of drug employed, the results cannot be used to make any decision concerning antagonist selectivity between the two tissues. High concentrations of this cardioselective drug have also been shown to inhibit catecholamine-induced uterine relaxation (Farmer and Levy, 1970).

With respect to agonists, some "selective" action on adenylate cyclase has also been noted. Both salbutamol and soterenol are effective stimulators of lung adenylate cyclase but have no effect on the heart enzyme even in high concentrations (Burges and Blackburn, 1972). Tertbutaline and albuterol, also selective $beta_2$ receptor agonists, have been reported equipotent with isoproterenol in increasing cyclic AMP formation in dog bronchus, rat aorta, and rat uterus, yet 500 times less potent than isoproterenol in rat myocardium

(Vulliemoz et al., 1973). These studies support the subclassification of beta receptors into at least two types and provide additional evidence for a role of cyclic AMP in the mediation of catecholamine-induced mechanical events in these tissues. However, these studies should not be interpreted necessarily as evidence for different adenylate cyclases in different tissues. As postulated by Robison et al. (1967), the membrane-bound adenylate cyclase system consists of two types of subunits. One or more regulatory or discriminator (Bär and Hechter, 1969) subunits, in contact with the extracellular fluid, are responsible for selectively binding catecholamines (or other hormones). A catalytic subunit, facing the interior of the cell, has access to ATP from which cyclic AMP is formed. An intermediate subunit, possibly a phospholipid (Birnbaumer et al., 1970; Levey, 1971; Pohl et al., 1971) or protaglandin (Levey and Klein, 1973), may be involved in the transduction of the stimulus from the regulatory to catalytic subunit. Presumably, it is the regulatory subunit which might be responsible for differences in stereoselectivity between tissues, independent of any differences in the catalytic subunit where enzymatic activity resides.

Consistent with the findings of other investigators (Rall and West, 1963; Kukovetz and Pöch, 1970; Skelton et al., 1971; Dobbs and Robison, 1968; Levey and Wilkenfeld, 1968; Mitznegg et al., 1970b), theophylline potentiated isoproterenol-induced mechanical responses in atria and uteri. This

may be taken as further evidence that cyclic AMP is involved in the mediation of these responses. The assumption is made that the concentration of theophylline employed, 10^{-4} or 10^{-3}M, effectively inhibits phosphodiesterase. This is based on the finding of Butcher and Sutherland (1962) that on partially purified preparations of beef heart phosphodiesterase the K_i for theophylline 1 x 10^{-4}M, yet there is no direct evidence that similar inhibition is achieved in intact muscle. Theophylline is generally regarded as a "dirty" drug, since it exerts a number of actions, particularly at these high concentrations, and it is possible that the potentiation of isoproterenol may be due to a mechanism unrelated to phosphodiesterase inhibition. Several possibilities have been suggested including 1) release of calcium from intracellular storage sites (Nayler, 1967; Massingham and Nasmyth, 1972), 2) inhibition of o-methylation by inhibiting COMT or access to it (Kalsner, 1971), and 3) release of endogenous catecholamines (Westfall and Fleming, 1968; Chiba et al., 1972; Poisner, 1973). Assuming that COMT is already effectively inhibited by tropolone (Kalsner and Nickerson, 1969; Kalsner, 1971) and that endogenous catecholamine stores have been effectively depleted by reserpine pretreatment (Paasonen and Krayer, 1958; Waud et al., 1958; Rudzik and Miller, 1962; Wurtman et al., 1964) in the present studies, then possibilities 2) and 3) seem unlikely to occur here. The possibility that theophylline is exerting part or all of its action through an effect on calcium flux cannot be ruled out by the present

investigation. If theophylline does, in fact, alter calcium flux, it may do so through an effect on cyclic AMP (Entman et al., 1969; Shinebourne and White, 1970; Marshall and Kroeger, 1973). Arguing against a role for calcium is the fact that calcium-induced changes in cardiac contractility were unaltered by theophylline (Skelton et al., 1971).

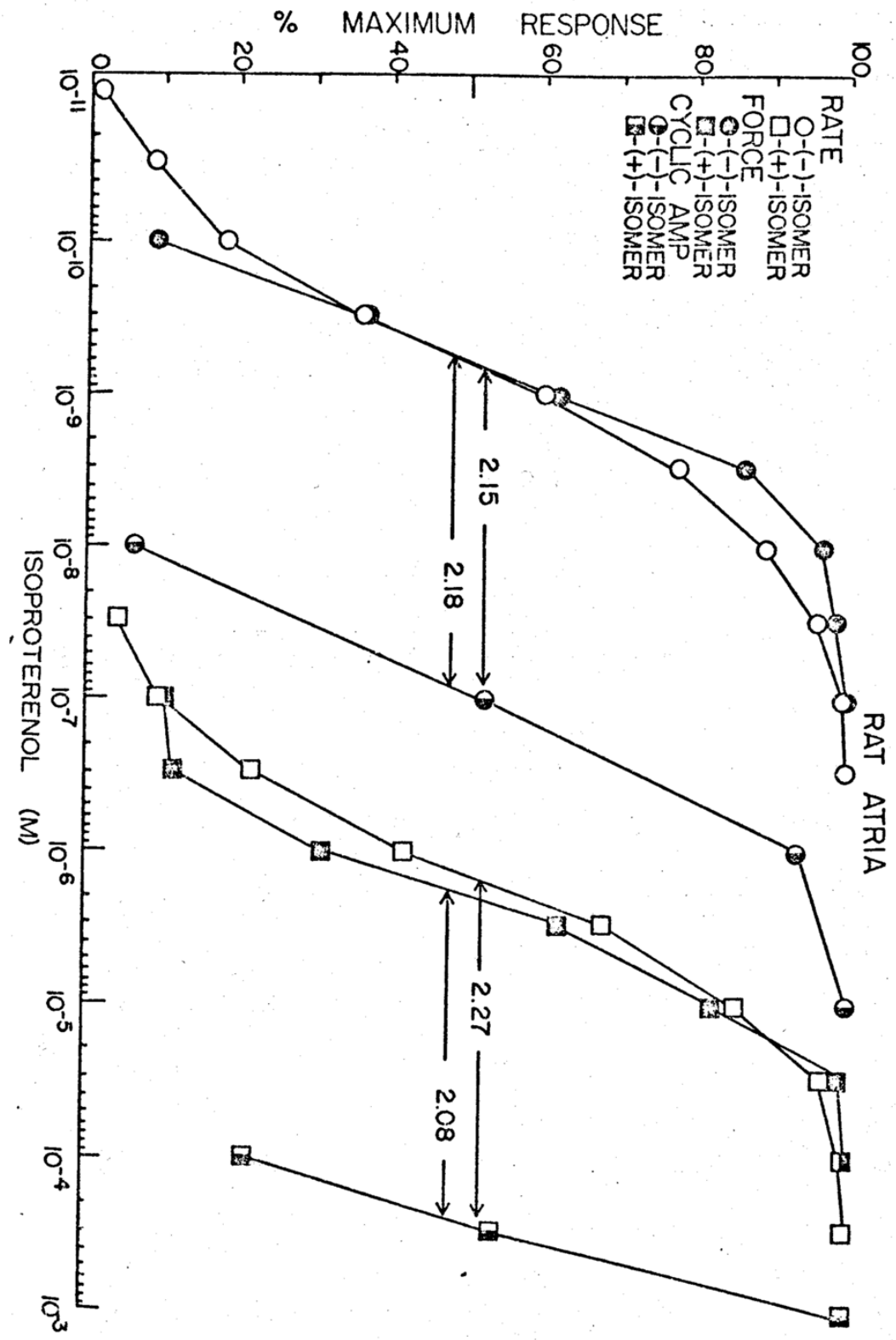
The observation was made that theophylline tended to potentiate the effects of (-)-isoproterenol on atrial rate and force to a greater extent than those of (+)-isoproterenol. This suggested that the (+)-isomer might itself be inhibiting phosphodiesterase. Inhibition of phosphodiesterase by high concentrations of catecholamines is not stereoselective (Goren and Rosen, 1972) but might appear so since the (+)-isomer must be used in higher concentrations to produce a pharmacological effect. Thus, responses elicited through beta receptor activation might be potentiated by a secondary effect of the catecholamine on phosphodiesterase. This is further supported by mechanical experiments utilizing practolol to shift the dose-response curves to (-)-isoproterenol to higher concentration ranges. Under these conditions, theophylline potentiated the effects of (-)-isoproterenol to a lesser extent than it did in the absence of practolol. Results obtained with practolol in experiments measuring isoproterenol-induced increases in cyclic AMP, however, might not support this hypothesis. Increases in cyclic AMP induced by intermediately effective concentrations of both isomers were completely abolished. If it is assumed that there is

ongoing synthesis of cyclic AMP in the absence of beta receptor activation, the (+)-isomer would be expected to elicit some increase in the levels of this nucleotide through its inhibitory effect on phosphodiesterase. The assumption of ongoing synthesis may not be valid, however. Preliminary experiments indicated that after a 15 minute exposure to theophylline, cyclic AMP levels were no higher than without theophylline. On the other hand, the drug potentiated the isoproterenol-induced increase in cyclic AMP. This suggests that basal (in the absence of isoproterenol) synthesis of cyclic AMP is extremely low and/or there is compartmentalization of the cyclic AMP in the tissue. The latter possibility is discussed below.

While similar isomeric potency differences were obtained for mechanical responses and increases in cyclic AMP in atria and uteri, log dose-response curves for cyclic AMP increases are situated more than 2 log units to the right of those for mechanical responses. Figure 15 illustrates the relationship between the curves for the two types of effects in atria. Approximately 100 times the concentration of each isomer is required to elicit 50% of the maximum increase in cyclic AMP than an equal degree of positive chronotropic and inotropic effect. A similar relationship exists in uterus (not illustrated) where the concentrations of the isomers of isoproterenol required to produce increases in cyclic AMP are approximately 400 times those which produce mechanical effects.

FIGURE 15

Log dose-response curves for mechanical responses and increases in cyclic AMP induced by (-)- and (+)-isoproterenol in rat atria. Rate and force were determined from spontaneously beating right and electrically stimulated left atria, respectively. Increases in cyclic AMP were determined from spontaneously beating right atria by the method of Gilman (1970). All experiments were performed in the presence of tropolone, phentolamine, and theophylline, 10^{-4} or 10^{-3}M. Numbers between the horizontal arrows connecting the curves indicate the log unit differences in molar ED_{50} values between mechanical responses and increases in cyclic AMP produced by the same isomer.


% MAXIMUM RESPONSE
0
20
40
60
80
100
RATE
○-(−)-ISOMER
□-(+)-ISOMER
FORCE
●-(−)-ISOMER
■-(+)-ISOMER
CYCLIC AMP
◐-(−)-ISOMER
◨-(+)- ISOMER
2.15
2.18
2.27
2.08
RAT ATRIA
10⁻¹¹
10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
10⁻³
ISOPROTERENOL (M)

On the basis of effective concentration differentials, catecholamine-induced increases in cyclic AMP have appeared to be dissociated from positive inotropism and phosphorylase activation in heart (Shanfeld et al., 1969), from glycogenolysis in liver (Exton et al., 1971) and from lipolysis in adipose tissue (Kuo and DeRenzo, 1969). A consistent observation in broken cell preparations of many tissues is that adenylate cyclase is 2-3 orders of magnitude less sensitive to stimulation by catecholamines than are "physiological" responses in the intact tissues (Robison et al., 1972; Bär and Hechter, 1969; Levey et al., 1969a; Mayer et al., 1972; Lefkowitz et al., 1973). However, an additional consideration here, not applicable to the concentration differentials in intact tissues, is that homogenization may change the adenylate cyclase system, either directly (Øye and Sutherland, 1966) or through release of proteolytic or other enzymes from subcellular organelles. Presumably, any alteration in regulatory, transducer, or catalytic subunits might obscure correlations between homogenate and intact tissue.

Presumably, if production of both mechanical responses and increases in cyclic AMP are consequences of the same receptor event, then both should be elicited by similar concentrations of isoproterenol. A possible explanation for the dissociation of these two types of effects in the present and other experiments is suggested by studies on the activation of protein kinases. In the presence of cyclic AMP, these kinases have the capacity to phosphorylate a number of protein

substrates, both endogenous and foreign to the tissues in which they are found (Walsh et al., 1968; Langan, 1968; Corbin and Krebs, 1969; Miyamoto et al., 1969; Kuo and Greengard, 1969a; 1969b; Brostrom et al., 1970; Kuo et al., 1970; Greengard and Kuo, 1970). It has been proposed (Kuo and Greengard, 1969a; 1969b) that all of the biochemical and mechanical effects of cyclic AMP may be mediated through regulation of the activity of protein kinases in various tissues. If one assumes uniform distribution of cyclic AMP throughout the intracellular water, then the basal concentration of cyclic AMP in heart and uterus is probably between 10^{-7}M and 10^{-6}M (e.g.: Robison et al., 1965; Wastila et al., 1972; Polacek and Daniel, 1971; Marshall and Kroeger, 1973; present experiments). Apparent K_m values reported for activation of protein kinases from bovine heart and uterus range between 4-6 x 10^{-8}M (Kuo et al., 1970; Rubin et al., 1972). Since it is unlikely that the kinases are fully activated under basal conditions, presumably much of the cyclic AMP is metabolically inactive due to compartmentalization or binding. This inactive pool would create a large background against which small increases in metabolically active nucleotide, produced in response to catecholamines, are obscured. Although the catecholamine-induced increases might be small relative to total cyclic AMP, they could represent large increases in a small compartment. Moreover, the small increases in cyclic AMP might induce large responses as a

result of amplification at subsequent steps leading to those responses.

In perfused liver preparations, it has been possible to discriminate active and inactive pools of cyclic AMP. Exton et al. (1971; 1972) have found that glycogenolysis induced by glucagon or epinephrine is more closely related to cyclic AMP released into the perfusing medium than in the liver itself. Approximately 2-fold increases in both glucose output and medium cyclic AMP were observed with a concentration of glucagon which did not increase tissue nucleotide. The medium cyclic AMP represented 1/50 the amount present within the liver. Concentrations of epinephrine which increased the medium cyclic AMP by 500 per cent, produced only 50 per cent increases in tissue cyclic AMP. These workers suggested that cyclic AMP released into the medium is freely diffusable and metabolically active and that it is the most sensitive index of hormonal action. Unfortunately, in most tissues which have been observed in this respect, release of cyclic AMP is not appreciable (Rall, 1972). In the present experiments, cyclic AMP values represent not only that present in the tissues, but also any which may have been released into the PSS medium. Hence, a large background of intracellular cyclic AMP may have obscured smaller extracellular concentrations of this nucleotide.

It is also possible that isoproterenol may continue to produced increases in cyclic AMP over and above those amounts required to elicit a maximum mechanical response. The

ability of cyclic AMP formation to outstrip its apparent usefulness has been reported for catecholamines-induced glycogenolysis in liver (Exton et al., 1971; 1972), calcium accumulation in subcellular preparations of dog heart (Entman et al., 1969), positive inotropism and phosphorylase activation in dog heart in situ and isolated rat heart (Mayer, 1971), lipolysis in rat fat pads (Butcher et al., 1965) and human adipocytes (Robison et al., 1972). The phenomenon reaches something of an extreme for ACTH in adrenal cortex (Grahame-Smith et al., 1967) and for various lipolytic drugs in adipose tissue (Fain, 1971) where the cyclic AMP concentrations attained in response to these agents approach that of ATP. These observations can be interpreted as evidence that the various responses in these tissues do not depend upon activation of adenylate cyclase. Alternatively, it may be postulated that the ability of catecholamines to increase the levels of cyclic AMP exceeds the ability of the responding elements of the tissue to respond to those increases. Sutherland et al. (1968) suggested that this represents an example of the pharmacological concept of spare receptors (Ariens, 1966). Consistent with this is the observation that in hearts from hypothyroid cats, there is a 30 per cent decrease in total amount of adenylate cyclase present, yet no decrease in the contractile response to norepinephrine (Levey et al., 1969b).

In the present experiments, basal levels of cyclic AMP, whether a result of a large inactive pool or the particular experimental design, may have been sufficiently high to

to obscure small increases produced by lower concentrations of isoproterenol. If we assume that the dose-response curve for these large increases in cyclic AMP is merely an extension of the physiologically significant portion of the curve, then regardless of the position of the dose-response curves along the log-dose axis, the isomeric potency difference should reflect receptor events. Moreover, the relatively parallel relationship between the curves for both mechanical effects and cyclic AMP (illustrated for atria in Figure 15) further suggests that both are manifestations of the same receptor event. The less parallel agreement observed in uterus may reflect a more complex relationship between receptor activation and mechanical response in this tissue.

Phentolamine decreased basal atrial rate, the isoproterenol-induced maximum rate, and the potency of isoproterenol. Phentolamine produced a slight positive inotropic effect in left atria and potentiated the inotropic effects of isoproterenol. The question arises as to whether the responses to phentolamine are a consequence of alpha receptor blockade? Whether there are, in fact, alpha receptors in the heart is a matter of controversy. Cotton and coworkers found that in open-chested dogs, dibenamine, phenoxybenzamine, and phentolamine antagonized the inotropic response to catecholamines (Cotton and Walton, 1951; Cotton et al., 1957), but these alpha receptor blocking agents also depressed the responses to calcium, a non-beta-receptor cardiostimulant (Moran and Perkins, 1961; Nickerson and Chan, 1961). On isolated

guinea-pig left atria, the positive inotropic response to low doses of phenylephrine, and a portion of the inotropic response to epinephrine and norepinephrine, can be blocked by phentolamine and phenoxybenzamine, but not by propranolol (Govier, 1967; 1968; Parr and Urquilla, 1972). Chronotropic responses have generally been assumed to involve only beta receptors. Unlike inotropic responses, chronotropic responses to phenylephrine in guinea-pig right atria are not modified by alpha receptor blockade with phentolamine (Krell and Patil, 1969; Parr and Urquilla, 1972). However, the chronotropic responses produced by phenylephrine in hypothyroid rats, or by methoxamine in normothyroid or hypothyroid rats, are antagonized by phentolamine and not by propranolol (Nakashima and Hagino, 1972; Nakashima et al., 1973). Thus, where alpha receptors have been implicated in heart, they generally have been found to mediate responses in the same direction as beta receptors. While it is possible that the depressant effects of phentolamine on atrial rate observed in the present study are due to alpha receptor blockade, this is rather tenuous since the alpha receptor population is not very large but the observed effect is. Furthermore, alpha receptor blockade would not explain the effects on force.

An alternative explanation is that phentolamine is exerting a non-specific effect unrelated to alpha receptors. The drug has been shown to produce a stabilizing effect on the cardiac cell membrane (Ledda and Marchetti, 1971). In addition, an antiarrhythmic activity has been demonstrated

previously both experimentally (Leimdorfer, 1952; Ettinger et al., 1969) and clinically (Morris et al., 1952; Gould et al., 1969) and has been observed in our experiments when atria were exposed to theophylline.

All experiments on mechanical responses in rat uteri were conducted in the presence of phentolamine so that the effect of this agent on contractile amplitude were not examined. The presence in rat uteri of alpha receptors, susceptible to blockade by phentolamine, has been reported by many investigators (Ahlquist, 1962; Rudzik and Miller, 1962a; Brooks et al., 1965; Diamond and Brody, 1966; Tothill, 1967; Miller, 1967; Abdel-Aziz and Bakry, 1972) and with one notable exception (Rudzik and Miller, 1962a), activation of these receptors produces an excitatory response opposite to that mediated through beta receptors.

With respect to depressant effect of phentolamine on basal levels of cyclic AMP in atria and on isoproterenol-induced maximum cyclic AMP levels in uteri, there is no rationale for invoking a mechanism of action involving alpha receptors. Stimulation of peripheral alpha receptors has been associated with either no effect on or a decrease in cyclic AMP (Robison et al., 1971). Therefore, combination of these receptors with phentolamine would be expected to produce either no change or an increase in cyclic AMP levels. The presence of an imidazole nucleus in phentolamine suggests that the drug might stimulate phosphodiesterase (Butcher and Sutherland, 1962; Kukovetz and Pöch, 1967), enhance the

hydrolysis of cyclic AMP, and thereby reduce the levels of this nucleotide produced by theophylline or isoproterenol. However, this has not been found to be the case (Weinryb et al., 1972). Another possible way in which phentolamine might decrease cyclic AMP levels is through inhibition of adenylate cyclase. Rabinowitz et al. (1972) have reported that phentolamine decreases adenylate cyclase activity in myocardial homogenates and the force of contraction in papillary muscle of the cat. Tolazoline, another structurally related alpha blocker, was reported by McNeill et al. (1972) to inhibit adenylate cyclase activity in guinea-pig heart by 18 per cent.

SUMMARY

Beta adrenergic receptors of rat atria and rat uteri have been evaluated by measuring changes in mechanical responses and cyclic AMP levels produced by the enantiomers of isoproterenol. The quantitative difference in potency between the isomers, i.e., isomeric potency difference, was used to characterize the beta receptors of each tissue. Under proper experimental conditions, dissimilar isomeric potency differences are expected to provide a sensitive indication of receptor differences.

Isomeric potency differences for isoproterenol-induced increases in atrial rate, atrial force, and atrial cyclic AMP levels are the same yet different from the isomeric potency differences obtained for uterine relaxation and increases in uterine cyclic AMP levels. Thus, the beta receptors of rat atria and rat uteri can be differentiated on the basis of dissimilar isomeric potency differences manifested at the levels of both cyclic AMP formation and the mechanical response. This is consistent with the existence of different types of beta receptors. The data also indicate that changes in mechanical responses and cyclic AMP levels represent different manifestations of the same receptor event. A role for cyclic AMP in the mediation of mechanical responses is suggested, and this is supported by the potentiation of mechanical responses by theophylline.

APPENDIX

Resolution of (±)-isoproterenol

by Paul D. Olsen
University of Wisconsin
School of Pharmacy

Preparation of (±)-isoproterenol-(+)-bitartrate

Procedure: (±)-isoproterenol sulfate (278.3 g, 0.500 moles) was dissolved in water (1.000 l.) with stirring. (+)-tartaric acid (150.1 g, 1.000 mole) was added, and a clear yellow solution resulted. A slurry was made by adding hot (85°C) water (0.500 l.) to $Ba(OH)_2 \cdot 8\ H_2O$ (161.0 g, 0.510 moles)[1]. The hot slurry was poured into the stirred solution of (±)-isoproterenol sulfate-(+)-tartaric acid, and a heavy white precipitate formed immediately. The reaction mixture was stirred for 5 minutes. Allowing the mixture to stir for longer periods of time did not increase yields. The $BaSO_4$ was removed by filtration. Gravity filtration and vacuum filtration through a cake of celite were both used with about equal success.

The filtrate was lyophilized. The resulting yellow to brown solid (325.8 g, 90%) was a mixture of the two diastereomers, (-)-isoproternol-(+)-bitartrate, and (+)-isoproterenol-(+)-bitartrate.

Separation of (+)-isoproterenol-(+)-bitartrate

Procedure: The solid mixture of diastereomers (325.8 g, 0.900 moles) was dissolved in hot (85°C) water (0.317 l.). The temperature was maintained by a constant temperature bath. The brown solution was hot filtered.[2,3] The filtrate flask was scratched to aid the onset of crystallization and

stored in the refrigerator (1°-5°C) overnight. The crystal cake formed was pulverized, stirred, the walls of the flask scratched and the mixture cooled an additional 6 hours, whereupon more crystals were formed.

The crystal were collected by suction filtration (aspirator). The filtrate was saved for further crystallization. The crystals were washed with several small portions of cold (0°-5°C) 90% methanol. The partially air dried crystals (108.6 g, 30%*) were dried in vacuo (1.0 mm) over $CaCl_2$.

Purification of (+)-isoproterenol-(+)bitartrate

The crude (+)-isoproterenol-(+)-bitartrate was recrystallized seven times in the following manner. Before the seventh recrystallization the hot solution (60°C) was filtered before cooling. A crystalline sample from ninth recrystallization was used for all experiments of the present study. Procedure: (+)-isoproterenol-(+)-bitartrate (108.6 g, 0.300 mole) was dissolved in water, pH = 7.2[4], (0.326 l.) at 60°C in a constant temperature bath. The solution process which took from 10 to 25 minutes was hastened by swirling and crushing any large particles with a spatula. The hot solution was placed in the refrigerator (1°-5°C). Crystallization was hastened by scratching the walls of the flask, and was complete after 3 hours. The crystals (70.6 g, 65%) were filtered, washed with cold 90% methanol, and dried in vacuo (vide supra).

---

*Several runs were made with smaller initial volumes of reagents and the yields at this point were as high as 45%.

Footnotes

1. An excess of 2% $Ba(OH)_2 \cdot 8\ H_2O$ was used in order to insure complete neutralization of the SO_4^-, since isoproterenol and (+)-tartaric acid are susceptible to acid racemization.
2. This was accomplished by wrapping copper tubing in a conical shape around a short stemmed funnel and connecting the tubing to a steam line.
3. A cotton plug placed in the stem of the funnel instead of filter paper, minimizes mechanical loses during hot filtration.
4. Distilled, deionized water is slightly acidic so all water was brought to pH = 7.2 with NH_4OH in usually <1 drop/liter in order to avoid possible acid racemization, though the necessity of this precaution has not been checked.

BIBLIOGRAPHY

Abdel-Aziz, A. and Bakry, N.: The nature of the adrenoceptors in the post-partum rat uterus. Br. J. Pharmacol. 45: 504-509, 1972.

Abel, J.J. and Crawford, A.C.: On the blood-pressure-raising constituent of the suprarenal capsule. Bull. Johns Hopk. Hosp. 8: 151-157, 1897.

Ahlquist, R.P.: A study of the adrenergic receptors. Am. J. Physiol. 153: 586-600, 1948.

Ahlquist, R.P.: The adrenotropic receptor-detector. Arch. Int. Pharmacodyn. Ther. 139: 38-41, 1962.

Ahlquist, R.P. and Levy, B.: Adrenergic receptive mechanism of canine ileum. J. Pharmacol. Exp. Ther. 127: 146-149, 1959.

Ariens, E.J.: Receptor theory and structure-activity relationships. Adv. Drug. Res. 3: 235-285, 1966.

Ariens, E.J.: The structure-activity relationships of beta adrenergic drugs and beta adrenergic blocking drugs. Ann. N.Y. Acad. Sci. 139: 606-631, 1967.

Arnold, A. and McAuliff, J.P.: Guinea-pig adipose tissue responsiveness to catecholamines. Experienta 24: 436, 1968.

Arnold, A. and McAuliff, J.P.: Correlation of calorigenesis with other β-1 receptor mediated responses to catecholamines. Arch. Int. Pharmacodyn. Ther. 179: 381-387, 1969.

Arnold, A. and McAuliff, J.P.: Differentiation of receptors activated by catecholamines. 2. Arch. Int. Pharmacodyn. Ther. 193: 287-293, 1971.

Arnold, A., McAuliff, J.P., Colella, D.F., O'Conner, W.V. and Brown, Jr., Th.G.: The β-2 receptor mediated glycogenolytic responses to catecholamines in the dog. Arch. Int. Pharmacodyn. Ther. 176: 451-457, 1968.

Arnold, A. and Selberis, W.H.: Activities of catecholamines in the dog. Arch. Int. Pharmacodyn. Ther. 176: 451-457, 1968.

Axelrod, J., Inscoe, J.K., Senoh, S. and Witkop, B.: O-methylation, the principal pathway for the metabolism of epinephrine and norepinephrine in the rat. Biochim. Biophys. Acta 27: 210-211, 1958.

Bacq, Z.M.: La pharmacologie du système nerveux autonome, et particulièrement du sympathique d'après la théorie neurohumorale. Ann. Physiol. Physiochim. Biol. 10: 467-528, 1934.

Bär, H.-P. and Hechter, O.: Adenyl cyclase and hormone action: I. Effects of adrenocorticotropic hormone, glucagon, and epinephrine on the plasma membrane of rat fat cells. Proc. Nat. Acad. Sci. 63: 350-356, 1969.

Barger, G. and Dale, H.H.: Chemical structure and sympathomimetic action of amines. J. Physiol. 41: 19-59, 1910.

Barrett, A.M., Growther, A.F., Dunlop, O., Shanks, R.G. and Smith, L.H.: Cardio-selective β-blockade. Arch. Pharmakol. Exp. Path. 259: 152-153, 1968.

Belleau, B. and Burba, J.: Tropolones: a unique class of potent noncompetitive inhibitors of S-adenosylmethionine-catechol methyltransferase. Biochim. Biophys. Acta 54: 195-196, 1961.

Birnbaumer, L., Pohl, S.L., Michiel, H., Krans, J. and Rodbell, M.: The actions of hormones on the adenyl cyclase system. In Advances in Biochemical Psychopharmacology. Vol.3. (P. Greengard and E. Costa, Eds.) 185-208, Raven Press, New York, 1970.

Black, J.W., Crowther, A.F., Shanks, R.G., Smith, L.H. and Dornhorst, A.C.: A new beta-receptor antagonist. Lancet 1: 1080-1081, 1964.

Black, J.W. and Stephenson, J.S.: Pharmacology of a new adrenergic beta-receptor-blocking compound (Nethalide). Lancet 2: 311-314, 1962.

Blaschko, H.: Amine oxidase and amine metabolism. Pharmacol. Rev. 4: 415-458, 1952.

Blinks, J.R.: Evaluation of the cardiac effects of several beta adrenergic blocking agents. Ann. N.Y. Acad. Sci. 139: 673-679, 1967.

Boissier, J.R., Advenier, C., Giudicelli, J.F. and Viars, P.: Studies on the nature of the bronchial β-adrenoreceptors. Eur. J. Pharmacol. 15: 101-109, 1971.

Brick, I., Hutchison, K.J., Roddie, I.C. and Shanks, R.G.: Cardiac adrenergic blockade by 4-(2-hydroxy-3-isopropylaminopropoxy)acetanilide (ICI 50172). Arch. Pharmakol. Exp. Path. 259: 156-157, 1968.

Bristow, M. and Green, R.D.: A quantitative study of beta-adrenergic receptors in rabbit atrium, aorta, stomach and trachea. Eur. J. Pharmacol. 12: 120-123, 1970.

Bristow, M., Sherrod, T.R. and Green, R.D.: Analysis of beta receptor drug interactions in isolated rabbit atrium, aorta, stomach and trachea. J. Pharmacol. Exp. Ther. 171: 52-61, 1970.

Brittain, R.T., Farmer, J.B., Jack, D., Martin, L.E. and Simpson, W.T.: α-[(t-Butylamino)methyl]-4-hydroxy-m-xylene-α^1,α^3-diol (AH 3365): a selective β-adrenergic stimulant. Nature 219: 862-863, 1968.

Brittain, R.T., Jack, D. and Ritchie, A.C.: Recent β-adrenoreceptor stimulants. Adv. Drug. Res. 5: 197-253, 1970.

Broadley, K.J.: The effects of temperature on responses of the isolated perfused heart of the guinea-pig to catecholamines: A separation of the rate and force responses. Eur. J. Pharmacol. 20: 291-299, 1972.

Brooker, G.: Elevation of myocardial cyclic AMP during systole. Fed. Proc. 32: 712, 1973.

Brooks, J.R., Schaeppi, U. and Pincus, G.: Evidence for the presence of alpha adrenergic excitatory receptors in the rat uterus. Life Sci. 4: 1817-1821, 1965.

Brostrom, M.A., Reimann, E.W., Walsh, D.A. and Krebs, E.G.: A cyclic 3',5'-AMP-stimulated protein kinase from cardiac muscle. Adv. Enz. Regul. 8: 191-203, 1970.

Buckner, C.K.: Effects of the optical isomers of soterenol and trimeoquinol in guinea-pig atria and trachea. Abs. of V Int. Pharmacol. Meeting, 32, 1972.

Buckner, C.K. and Patil, P.N.: The rate of onset of beta adrenergic blockade by the optical isomers of alprenolol. Eur. J. Pharmacol. 14: 308-311, 1971a.

Buckner, C.K. and Patil, P.N.: Steric aspects of adrenergic drugs. XVI. Beta-adrenergic receptors of guinea-pig atria and trachea. J. Pharmacol. Exp. Ther. 176: 634-649, 1971b.

Bueding, E., Bulbring, E., Gercken, G., Hawkins, J.T. and Kuriyama, H.: The effect of adrenaline on the adenosine triphosphate and creatine phosphate content of intestinal smooth muscle. J. Physiol. 193: 187-212, 1967.

Burges, R.A. and Blackburn, K.J.: Adenyl cyclase and the differentiation of β-adrenoreceptors. Nature, New Biol. 235: 249-250, 1972.

Burn, J.H. and Rand, M.J.: Sympathetic postganglionic mechanism. Nature 164: 163-165, 1959.

Burns, J.J., Colville, K.I., Lindsay, L.A. and Salvador, R.A.: Blockade of some metabolic effects of catecholamines by N-isoporpylmethoxamine (B.W. 61-43). J. Pharmacol. Exp. Ther. 144: 163-171, 1964.

Burns, J.J. and Lemberger, L.: N-tertiary butylmethoxamine, a specific antagonist of the metabolic actions of epinephrine. Fed. Proc. 24: 298, 1965.

Burns, J.J., Salvador, R.A. and Lemberger, L.: Metabolic blockade by methoxamine and its analogs. Ann. N.Y. Acad. Sci. 139: 833-840, 1967.

Butcher, R.W., Ho, R.J., Meng, H.C. and Sutherland, E.W.: Adenosine 3',5'-monophosphate in biological materials. II. The measurement of adenosine 3',5'-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine. J. Biol. Chem. 240: 4515-4523, 1965.

Butcher, R.W. and Sutherland, E.W.: Adenosine 3',5'-phosphate in biological materials. 1. Purification and properties of cyclic 3',5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3',5'-phosphate in human urine. J. Biol. Chem. 237: 1244-1250, 1962.

Cannon, W.B. and Rosenblueth, A.: Studies on conditions of activity in endocrine organs. XXIX. Sympathin E and Sympathin I. Am. J. Physiol. 104: 557-574, 1933.

Cannon, W.B. and Rosenblueth, A.: Autonomic neuro-effector systems. Macmillan, New York, 1937.

Cheung, W.Y., and Williamson, J.R.: Kinetics of cyclic adenosine monophosphate changes in rat heart following epinephrine administration. Nature 207: 979-981, 1965.

Chiba, S., Hashimoto, K. and Hashimoto, K.: Pharmacological analysis of chronotropic responses of the S-A node to caffeine. Eur. J. Pharmacol. 18: 116-120, 1972.

Colville, K.I., Lindsay, L.A. and Burns, J.J.: Metabolic and other blocking properties of butoxamine: A comparison with β-adrenergic blocking drugs. Pharmacologist I: 187, 1965.

Cook, W.H., Lipkin, D., and Markham, R.: Formation of a cyclic dianhydrodiadenylic acid by the alkaline degradation of adenosine-5'-triphosphoric acid. J. Amer. Chem. Soc. 79: 3607-3612, 1957.

Corbin, J.D. and Krebs, E.G.: A cyclic AMP-stimulated protein kinase in adipose tissue. Biochem. Biophys. Res. Commun. 36: 328-336, 1969.

Cori, C.F.: Regulation of enzyme activity in muscle during work. In: Enzymes, Units of Biological Structure and Function (O.H. Gaebler, Ed.), 573-583, Academic Press, New York, 1956.

Cotton, M. DeV., Moran, N.C. and Stopp, P.E.: A comparison of the effectiveness of adrenergic blocking drugs in inhibiting the cardiac actions of sympathomimetic amines. J. Pharmacol. Exp. Ther. 121: 183-190, 1957.

Cotton, M. DeV. and Walton, R.P.: Dibenamine blockade as a method of distinguishing between inotropic action of epinephrine and digitalis. Proc. Soc. Exp. Biol. Med. 78: 810-815, 1951.

Coutinho, E.M. and Lopes, A.C.V.: Inhibition of uterine motility by aminophylline. Amer. J. Obstet. Gynec. 110: 726-729, 1971.

Csapó, A.: Dependence of isometric tension on temperature and on strength of stimulation. Am. J. Physiol. 177: 348-354, 1954.

Cullum, V.A., Farmer, J.B., Jack, D. and Levy, G.P.: Salbutamol, a new selective β-adrenergic receptor stimulant. Br. J. Pharmacol. 35: 141-151, 1969.

Dale, H.H.: On some physiological actions of ergot. J. Physiol. 34: 163-206, 1906.

Daniel, E.E. and Robinson, K.: Effect of inhibitors of metabolism on ^{22}Na and ^{42}K and net movements in rat uteri at 25°C. Can. J. Physiol. Pharmacol. 49: 205-239, 1971.

Danilo, P., Vulliemoz, Y., Verosky, M. and Rosen, M.R.: Activation of cardiac purkinje fiber adenyl cyclase by β-adrenergic amines. Fed. Proc. 32: 773, 1973.

DePlazas, S.F. and DeRobertis, E.: Isolation of a proteolipid from spleen capsule binding (±)-^{3}H -norepinephrine. Biochim. Biophys. Acta 266: 246-254, 1972.

Diamond, J. and Brody, T.M.: Effect of catecholamines on smooth muscle motility and phosphorylase activity. J. Pharmacol. Exp. Ther. 152: 202-211, 1966.

Dobbs, J.W. and Robison, G.A.: Functional biochemistry of beta receptors in the uterus. Fed. Proc. 27: 352, 1968.

Drummond, G.I., Duncan, L., and Hertzman, E.: Effect of epinephrine on phosphorylase b kinase in perfused rat hearts. J. Biol. Chem. 241: 5899-5903, 1966.

Dungan, K.W., Cho, Y.W., Gomoll, A.E., Aviado, D.M. and Lish, P.M.: Pharmacologic potency and selectivity of a new bronchodilator agent: soterenol (MJ 1992). J. Pharmacol. Exp. Ther. 164: 290-301, 1968.

Dunlop, D. and Shanks, R.G.: Selective blockade of adrenoceptive beta receptors in the heart. Br. J. Pharmacol. Chemother. 32: 201-218, 1968.

Easson, L.H. and Stedman, E.: CLXX. Studies on the relationship between chemical constitution and physiological action. V. Molecular dissymmetry and physiological activity. Biochem. J. 27: 1257-1266, 1933.

Elliott, T.R.: On the action of adrenalin. J. Physiol. 31: XX-XXI, 1904.

Ellis, S., Anderson, H.L.Jr. and Collins, M.D.: Pharmacologic differentiation between epinephrine and HGF-hyperglycemia: application in analysis of cobalt-hyperglycemia. Proc. Soc. Exp. Biol. 84: 383-386, 1953.

Entman, M.L., Levey, G.S. and Epstein, S.E.: Mechanism of action of epinephrine and glucagon on the canine heart. Evidence for increase in sarcotubular calcium stores mediated by cyclic 3',5'-AMP. Circ. Res. 25: 429-438, 1969.

Ettinger, S., Gould, L., Carmichael, A. and Tashjian, R.: Phentolamine: use in digitalis-induced arrhythmias. Am. Heart J. 77: 636-640, 1969.

Euler, U.S.v.: A specific sympathiomimetic ergone in adrenergic nerve fibers (sympathin) and its relation to adrenaline and noradrenaline. Acta Physiol. Scand. 12: 73-97, 1946.

Euler, U.S.v.: Indentification of the sympathomimetic ergone in adrenergic nerves of cattle (Sympathin N) with laevonoradrenaline. Acta Physiol. Scand. 16: 63-74, 1948.

Exton, J.H., Lewis, S.B., Ho, R.J. and Park, C.R.: The role of cyclic AMP in the control of hepatic glucose production by glucagon and insulin. In Advances in Cyclic Nucleotide Research, Vol. 1 (P. Greengard, R. Paoletti, and G. A. Robison, Eds.) 91-101, Raven Press, New York, 1972.

Exton, J.H., Lewis, S.B., Ho, R.J., Robison, G.A. and Park, C.R.: The role of cyclic AMP in the interaction of glucagon and insulin in the control of liver metabolism. Ann. N.Y. Acad. Sci. 185: 85-100, 1971.

Fain, J.N.: Effects of menadione and vitamin K_5 on glucose metabolism, respiration, lipolysis, cyclic 3',5'-adenylic acid accumulation, and adenyl cyclase in white fat cells. Mol. Pharmacol. 7: 465-479, 1971.

Farmer, J.B., Kennedy, I., Levy, G.P. and Marshall, R.J.: A comparison of the β-adrenoreceptor stimulant properties of isoprenaline, with those of orciprenaline, salbutamol, soterenol and trimetoquinol on isolated atria and trachea of the guinea-pig. J. Pharm. Pharmacol. 22: 61-63, 1970.

Farmer, J.B. and Levy, G.P.: New sympathomimetic amines: actions on catecholamine receptors. Br. J. Pharmacol. 34: 698P, 1968.

Farmer, J.B. and Levy, G.P.: Differentiation of β-adrenoreceptors by the use of blocking agents. J. Pharm. Pharmacol. 22: 145-146, 1970.

Furchgott, R.F.: The receptors for epinephrine and norepinephrine (adrenergic receptors). Pharmacol. Revs. 11: 429-441, 1959.

Furchgott, R.F.: Receptor mechanisms. Ann. Rev. Pharmacol. 4: 21-50, 1964.

Furchgott, R.F.: The pharmacological differentiation of adrenergic receptors. Ann. N.Y. Acad. Sci. 139: 553-570 1967.

Furchgott, R.F.: A critical apparaisal of the use of isolated organ systems for the assessment of drug action at the receptor level. In Importance of Fundamental Principles in Drug Evaluation (D.H. Tedeschi and R.E. Tedeschi, Eds.), 277-298, Raven Press, New York, 1970.

Furchgott, R.F.: The classification of adrenoceptors (adrenergic receptor). An evaluation from the standpoint of receptor theory. In Catecholamines (H. Blaschko and E. Muscholl, Eds.), 283-335, Springer-Verlag, New York, 1972.

Gaddum, J.H. and Hameed, K.A.: Drugs which antagonize 5-hydroxy-tryptamine. Br. J. Pharmacol. 9: 240-248, 1954.

Genell, S.: Experimental investigations on the muscular functions of the vagina and uterus of the rat. Acta Obst. Gynecol. 19: 113-175, 1939.

George, W.J., Polson, J.B., O'Toole, A.G. and Goldberg, N.D.: Elevation of guanosine 3',5'-cyclic phosphate in rat heart after perfusion with acetylcholine. Proc. Nat. Acad. Sci. 66: 398-403, 1970.

Gilman, A.G.: A protein binding assay for adenosine 3':5'-cyclic monophosphate. Proc. Nat. Acad. Sci. 67: 305-312, 1970.

Glick, G., Parmley, W.W., Wechsler, A.S. and Sonnenblick, E.H.: Glucagon. Its enhancement of cardiac performance in the cat and dog and persistence of its inotropic action despite beta-receptor blockade with propranolol. Cir. Res. 22: 789-799, 1968.

Goren, E.N. and Rosen, O.M.: Inhibition of a cyclic nucleotide phosphodiesterase from beef heart by catecholamines and related compounds. Mol. Pharmacol. 8: 380-384, 1972.

Gould, L., Zahir, M., Shariff, M. and Giuliani, M.G.: Treatment of cardiac arrhythmias with phentolamine. Am. Heart J. 78: 189-193, 1969.

Govier, W.C.: A positive inotropic effect of phenylephrine mediated by alpha adrenergic receptors. Life Sci. 6: 1361-1365, 1967.

Govier, W.D.: Myocardial alpha adrenergic receptors and their role in the production of a positive inotropic effect by sympathomimetic agents. J. Pharmacol. Exp. Ther. 159: 82-90, 1968.

Grahame-Smith, D.G., Butcher, R.W., Ney, R.L. and Sutherland, E.W.: Adenosine 3',5'-monophosphate as the intracellular mediator of the action of adrenocorticotropic hormone on the adrenal cortex. J. Biol. Chem. 242: 5535-5541, 1967.

Greengard, P. and Kuo, J.F.: On the mechanism of action of cyclic AMP. In Advances in Biochemical Psychopharmacology. Vol.3 (P. Greengard and E. Costa, Eds.) 287-306, Raven Press, New York, 1970.

Greer, C.M., Pinkston, J.O., Baxter, J.H.,Jr. and Brannon, E.S.: Norepinephrine [β-(3,4-dihydroxyphenyl)-β-hydroxyethylamine] as a possible mediator in the sympathetic division of the autonomic nervous system. J. Pharmacol. Exp. Ther. 62: 189-227, 1938.

Gruber, C.M.: The autonomic innervation of the genito-urinary tract. Physiol. Rev. 13: 497-609, 1933.

Hammermeister, K.E., Yunis, A.A., and Krebs, E.G.: Studies on phosphorylase activation in the heart. J. Biol. Chem. 240: 986-991, 1965.

Harbon, S. and Clauser, H.: Cyclic adenosine 3',5' monophosphate levels in rat myometrium under the influence of epinephrine, prostaglandins and oxytocin. Correlations with uterus motility. Biochem. Biophys. Res. Com. 44: 1496-1503, 1971.

Harvey, S.C., Wang, C.Y. and Nickerson, M.: Blockade of epinephrine-induced hyperglycemia. J. Pharmacol. Exp. Ther. 104: 363-376, 1952.

Haugaard, N., Kukovetz, W.R. and Hess, M.E.: The effect of sympathomimetic amines on phosphorylase activity of the isolated rat heart. Pharmacol. Rev. 11: 466-468, 1959.

Hawkins, D.F.: Agents acting on the uterus. In Evaluation of Drug Activities: Pharmacometrics. Vol. 2 (D.R. Laurence and A.L. Bacharach, Eds.), 665-701, Academic Press, New York, 1964.

Hedges, A. and Turner, P.: Beta-receptors in human isolated smooth muscle. Br. J. Pharmacol. 37: 547P-548P, 1969.

Hertting, G.: The fate of H^3-isoproterenol in the rat. Biochem. Pharmacol. 25: 18-33, 1964.

Hess, M.E. and Haugaard, N.: The effect of epinephrine and aminophylline on the phosphorylase activity of perfused contracting heart muscle. J. Pharmacol. Exp. Ther. 122: 169-175, 1958.

Hess, M.E., Hottenstein, D., Shanfeld, J. and Haugaard, N.: Metabolic effects of theophylline in cardiac and skeletal muscle. J. Pharmacol. Exp. Ther. 141: 274-279, 1963.

Ishikawa, E., Ishikawa, S., Davis, J.W. and Sutherland, E.W.: Determination of guanosine 3',5'-monophosphate in tissues of guanyl cyclase in rat intestine. J. Biol. Chem. 244: 6371-6376, 1969.

Iversen, L.L.: The Uptake and Storage of Noradrenaline in Sympathetic Nerves. Cambridge University Press, Cambridge, 1967.

Iversen, L.L. and Salt, P.J.: Inhibition of catecholamine uptake$_2$ by steroids in the isolated rat heart. Br. J. Pharmacol. 40: 528-530, 1970.

Iwasawa, Y. and Kiyomoto, A.: Studies on tetrahydroisoquinolines (THI), (I) Bronchodilator activity and structure-activity relationship. Jap. J. Pharmacol. 17: 143-152, 1967.

Jacobwitz, D. and Brus, R.: A study of extraneuronal uptake of norepinephrine in the perfused heart of the guinea-pig. Eur. J. Pharmacol. 15: 274-284, 1971.

Kalsner, S.: Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. Circ. Res. 24: 383-395, 1969a.

Kalsner, S.: Steroid potentiation of responses to sympathomimetic amines in aortic strips. Br. J. Pharmacol. 36: 582-593, 1969b.

Kalsner, S.: Mechanism of potentiation of contractor responses to catecholamines by methylxanthines in aortic strips. Br. J. Pharmacol. 43: 379-388, 1971.

Kalsner, S. and Nickerson, M.: Disposition of norepinephrine and epinephrine in vascular tissue, determined by the technique of oil immersion. J. Pharmacol. Exp. Ther. 165: 152-165, 1969.

Kaumann, A.J.: Potentiation of the effects of isoprenaline and noradrenaline by hydrocortisone in cat heart muscle. Arch. Pharmakol. Exp. Path. 273: 134-153, 1972.

Kaumann, A.J. and Blinks, J.R.: Comparative potencies of beta-adrenergic blocking agents on isolated heart muscle. Fed. Proc. 26: 401, 1967.

Kjekshus, J.K., Henry, P.D. and Sobel, B.E.: Activation of phosphorylase by cyclic AMP without augmentation of contractility in the perfused guinea-pig heart. Circ. Res. 29: 468-478, 1971.

Koch-Weser, J. and Blinks, J.R.: The influence of the interval between beats on myocardial contractility. Pharmacol. Rev. 15: 601-652, 1963.

Krause, E.-G., Halle, W., Kallabis, E. and Wollenberger, A.: Positive chronotropic response of cultured isolated rat heart cells to N^6,2'-O-dibutyryl-3',5'-adenosine monophosphate. J. Mol. Cell. Cardiol. 1: 1-10, 1970.

Krell, R.D. and Patil, P.N.: Combinations of alpha and beta adrenergic blockers in isolated guinea-pig atria. J. Pharmacol. Exp. Ther. 170: 262-271, 1969.

Kukovetz, W.R., Hess, M.E., Shanfeld, J. and Haugaard, N.: The action of sympathomimetic amines on isomeric contraction and phosphorylase activity of the isolated rat heart. J. Pharmacol. Exp. Ther. 127: 122-127, 1959.

Kukovetz, W.R. and Pöch, G.: Action of imidazole on the effects of methylxanthines and catecholamines on cardiac contraction and phosphorylase activity. J. Pharmacol. Exp. Ther. 156: 514-521, 1967.

Kukovetz, W.R. and Pöch, G.: Cardiostimulatory effects of cyclic 3',5'-adenosine monophosphate and its acylated derivatives. Arch. Pharmakol. Exp. Path. 266: 236-254, 1970.

Kuo, J.F. and DeRenzo, E.C.: A comparison of the effects of lipolytic and antilipolytic agents of adenosine 3',5'-monophosphate levels in adipose cells as determined by prior labeling with adenosine-8-^{14}C. J. Biol. Chem. 244: 2252-2260, 1969.

Kuo, J.F. and Greengard, P.: An adenosine 3',5'-monophosphate-dependent protein kinase from Escherichia coli. J. Biol. Chem. 244: 3417-3419, 1969a.

Kuo, J.F. and Greengard, P.: Cyclic nucleotide-dependent protein kinase in various tissues and phyla of the animal kingdom. Proc. Nat. Acad. Sci. 64: 1349-1355, 1969b.

Kuo, J.F., Krueger, B.K., Sanes, J.R. and Greengard, P.: Cyclic nucleotide-dependent protein kinases. V. Preparation and properties of adenosine 3',5'-monophosphate-dependent protein kinase from various bovine tissues. Biochim. Biophys. Acta 212: 79-91, 1970.

Kuo, J.F., Lee, T.P., Reyes, P.L., Walton, K.G., Donnelly, Jr., T.F. and Greengard, P.: Cyclic nucleotide-dependent protein kinases. X. An assay method for the measurement of quanosine 3',5'-monophosphate in various biological materials and a study of agents regulating its levels in heart and brain. J. Biol. Chem. 247: 16-22, 1972.

Lands, A.M.: The pharmacological activity of epinephrine and related dihydroxyphenylalkylamines. Pharmacol. Rev. 1: 279-309, 1949.

Lands, A.M.: Sympathetic receptor action. Am. J. Physiol. 169: 11-21, 1952.

Lands, A.M., Arnold, A., McAuliff, J.P., Luduena, F.P. and Brown, T.G., Jr.: Differentiation of receptor systems activated by sympathomimetic amines. Nature 214: 597-598, 1967a.

Lands, A.M., Luduena, F.P. and Buzzo, H.J.: Differentiation of receptors responsive to isoproterenol. Life Sci. 6: 2241-2249, 1967b.

Lands, A.M. and Brown, T.G.,Jr.: A comparison of the cardiac stimulating and bronchodilator actions of selected sympathomimetic amines. Proc. Soc. Exp. Biol. Med.116: 331-333, 1964.

Lands, A.M., Groblewski, G.E. and Brown, T.G.,Jr.: Comparison of the action of isoproterenol and several related compounds on blood pressure, heart and bronchioles. Arch. Int. Pharmacodyn. Ther. 161: 68-75, 1966.

Lands, A.M., Nash, V.L., McCarthy, H.M., Granger, H.R. and Dertinger, B.L.: The pharmacology of N-alkyl homologues of epinephrine. J. Pharmacol. Exp. Ther. 90: 110-119, 1947.

Langan, T.A.: Histone phosphorylation: stimulation by adenosine 3',5'-monophosphate. Science 162: 579-580, 1968.

Langley, J.N.: Observations on the physiological action of extracts of the suprarenal bodies. J. Physiol. 27: 237-256, 1901.

Langley, J.N.: On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. J. Physiol. 33: 374-413, 1905.

Langslet, A. and Øye, I.: The role of cyclic 3',5'-AMP in the cardiac response to adrenaline. Eur. J. Pharmacol. 12: 137-144, 1970.

Larsen, A.A., Gould, W.A., Roth, H.R., Cromer, W.T., Vloth, R.H., Dungan, K.W. and Lish, P.M.: Sulfonamides. II. Analogs of catecholamines. J. Med. Chem. 10: 462-472, 1967.

Ledda, F. and Marchetti, P.: Effects of phentolamine on transmembrane action potentials of single purkinje fibers of sheep heart. Pharmacol. Res. Commun. 3: 155-164, 1971.

Lefkowitz, R.J. and Haber, E.: A fraction of the ventricular myocardium that has the specificity of the cardiac beta-adrenergic receptor. Proc. Nat. Acad. Sci. 68: 1773-1777, 1971.

Lefkowitz, R.J., Sharp, G.W.G. and Haber, E.: Specific binding of β-adrenergic catecholamines to a subcellular fraction from cardiac muscle. J. Biol. Chem. 248: 342-349, 1973.

Leimdorfer, A.: Prevention and abolition of cardiac arrhythmias by Regitine. Am. J. Physiol. 171: 742-743, 1952.

Levey, G.S.: Solubilization of myocardial adenyl cyclase: Loss of hormone responsiveness and activation by phospholipids. Ann. N.Y. Acad. Sci. 185: 449-457, 1971.

Levey, G.S. and Epstein, S.E.: Activation of adenyl cyclase by glucagon in cat and human heart. Circ. Res. 24: 151-156, 1969.

Levey, G.S. and Klein, I.: Solubilized myocardial adenyl cyclase: Activation by prostaglandins. Life Sci. 13: 41-46, 1973.

Levey, G.S., Skelton, C.L. and Epstein, S.E.: Influence of hyperthyroidism on the effects of norepinephrine on myocardial adenyl cyclase activity and contractile state. Endrocrinology 85: 1004-1009, 1969a.

Levey, G.S., Skelton, C.L. and Epstein, S.E.: Decreased myocardial adenyl cyclase activity in hypothyroidsm. J. Clin. Invest. 48: 2244-2250, 1969b.

Levine, R.A., Dixon, L.M. and Franklin, R.B.: Effects of exogenous 3',5'-monophosphate in man: I. Cardiovascular responses. Clin. Pharmacol. Ther. 9: 168-179, 1968.

Levine, R.A. and Vogel, L.A.: Cardiovascular and metabolic effects of adenosine 3',5'-monophosphate in vivo. Nature 207: 987-988, 1965.

Levy, B.: Alterations of adrenergic responses by N-isopropyl-methoxamine. J. Pharmacol. Exp. Ther. 146: 129-138, 1964.

Levy, B.: The adrenergic blocking activity of N-tert.-butylmethoxamine (Butoxamine). J. Pharmacol. Exp. Ther. 151: 413-422, 1966.

Levy, B.: Selective beta receptor blockade in rat smooth muscle. Arch. Int. Pharmacodyn. Ther. 170: 418-427, 1967.

Levy, B. and Wilkenfeld, B.E.: The potentiation of rat uterine inhibitory responses to noradrenaline by theophylline and nitroglycerine. Br. J. Pharmacol. Chemother. 34: 604-612, 1968.

Levy, B. and Wilkenfeld, B.E.: An Analysis of selective beta receptor blockade. Europ. J. Pharmacol. 5: 227-234, 1969.

Lish, P.M., Weilel, J.H. and Dungan, K.W.: Pharmacological and toxicological properties of two new β-adrenergic receptor antagonists. J. Pharmacol. Exp. Ther. 149: 161-173, 1965.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275, 1951.

Lucchesi, B.R.: Cardiac actions of glucagon. Circ. Res. 22: 777-787, 1968.

Luduena, F.P., Ananenko, E., Siegmund, O.H. and Miller, L.C.: Comparative pharmacology of the optical isomers of arterenol. J. Pharmacol. Exp. Ther. 95: 155-170, 1949.

McNeill, J.H., Davis, R.S. and Muschek, L.D.: Phenylephrine effects on cardiac contractility, adenyl cyclase, and phosphorylase. Arch. Int. Pharmacodyn. Ther. 197: 317-327, 1972.

Marshall, J.M. and Kroeger, E.A.: Adrenergic influences on uterine smooth muscle. Phil. Trans. R. Soc. Lond. B 265: 138-148, 1973.

Massingham, R. and Nasmyth, P.A.: The nature of the positive inotropic response of the isolated frog heart to theophylline and to imidazole. Br. J. Pharmacol. 45: 229-239, 1972.

Mayer, S.E.: Discussion. Ann. N.Y. Acad. Sci. 185: 489, 1971.

Mayer, S.E.: Effects of adrenergic agonists and antagonists on adenylate cyclase activity of dog heart and liver. J. Pharmacol. Exp. Ther. 181: 116-125, 1972.

Mayer, S.E. and Moran, N.D.: Relation between pharmacologic augmentation of cardiac contractile force and the action of myocardial glycogen phosphorylase. J. Pharmacol. Exp. Ther. 129: 271-281, 1960.

Mayer, S.E., Moran, N.C. and Fain, J.: The effect of adrenergic blocking agents on some metabolic actions of catecholamines. J. Pharmacol. Exp. Ther. 134: 18-27, 1961.

Mayer, S.E., Namm, D.H. and Rice, L.: Effect of glucagon on cyclic 3',5'-AMP, phosphorylase activity and contractility of heart muscle of the rat. Circ. Res. 26: 225-233, 1970.

Miller, J.M.: Adrenergic receptors in the myometrium. Ann. N.Y. Acad. Sci. 139: 788-798, 1967.

Mitznegg, P., Heim, F. and Meythaler, B.: Influence of endogenous and exogenous cyclic 3',5'-AMP on contractile responses induced by oxytocin and calcium in isolated rat uterus. Life Sci. 9: 121-128, 1970a.

Mitznegg, P., Hach, B. and Heim, F.: The influence of caffiene on endogenous cyclic 3',5'-AMP on uterine smooth muscle contraction induced by oxytocin. Life Sci. 9: 975-981, 1970b.

Miyamoto, E., Kuo, J.F. and Greengard, P.: Cyclic nucleotide-dependent protein kinases. III. Purification and properties of adenosine 3',5'-monophosphate-dependent protein kinase from bovine brain. J. Biol. Chem. 244: 6395-6402, 1969.

Moran, N.C.: Pharmacological characterization of adrenergic receptors. Pharmacol. Revs. 18: 503-512, 1966.

Moran, N.C.: The development of beta adrenergic blocking drugs: A retrospective and prospective evaluation. Ann. N.Y. Acad. Sci. 139: 649-660, 1967.

Moran, N.C. and Perkins, M.E.: Adrenergic blockade of the mammalian heart by a dichloro analog of isoproterenol. J. Pharmacol. Exp. Ther. 124: 233-237, 1958.

Moran, N.C. and Perkins, M.E.: An evaluation of adrenergic blockade of the mammalian heart. J. Pharmacol. Exp. Ther. 133: 192-201, 1961.

Moran, J.F. and Triggle, D.J.: Quantification and isolation of pharmacological receptors. In Fundamental Concepts in Drug-Receptor Interactions (J.F. Danielli, J.F. Moran and D.J. Triggle, Eds.), 133-176, Academic Press, New York, 1970.

Morris, L.E., Yein, C.S., Haid, B. and White, Jr., J.M.: Laboratory and clinical observations of the effect of Regitine (C-7337) on cardiac irregularities during cyclopropane anesthesia. J. Pharmacol. Exp. Ther. 106: 49-54, 1952.

Murad, F.: Beta-blockade of epinephrine-induced cyclic AMP formation in heart, liver, fat and trachea. Biochim. Biophys. Acta 304: 181-187, 1973.

Murad, F., Chi, Y.M., Rall, T.W., and Sutherland, E.W.: Adenyl cyclase: III. Effect of catecholamines and choline esters on the formation of adenosine 3',5'-phosphate by preparations from cardiac muscle and liver. J. Biol. Chem. 237: 1233-1238, 1962.

Murad, F., Manganiello, V. and Vaughan, M.: A simple, sensitive protein binding assay for quanosine 3',5'-monophosphate. Proc. Nat. Acad. Sci. 68: 736-739, 1971.

Murad, F., Rall, T.W. and Sutherland, E.W.: Formation of adenosine-3',5'-phosphate (3,5-AMP) by particulate preparations of ventricular muscle. Fed. Proc. 19: 296, 1960.

Murad, F. and Vaughan, M.: Effect of glucagon on rat heart adenyl cyclase. Biochem. Pharmacol. 18: 1053-1059, 1969.

Nakashima, M. and Hagino, Y.: Evidence for the existence of alpha adrenergic receptor in isolated rat atria. Jap. J. Pharmacol. 22: 227-233, 1972.

Nakashima, M., Tsuru, H. and Shigei, T.: Stimulant action of methoxamine in the isolated atria of normal and 6-propyl-2-thiouracil-fed rats. Jap. J. Pharmacol. 23: 307-312, 1973.

Namm, D.H., and Mayer, S.E.: Effects of epinephrine on cardiac cyclic 3',5'-AMP, phosphorylase kinase and phosphorylase. Molec. Pharmacol. 4: 61-69, 1968.

Nayler, W.G.: Calcium exchange in cardiac muscle: A basic mechanism of drug action. Am. Heart J. 73: 379-384, 1967.

Nickerson, M.: The pharmacology of adrenergic blockade. Pharmacol. Revs. 1: 27-101, 1949.

Nickerson, M. and Chan, G.C.M.: Blockade of responses of isolated myocardium to epinephrine. J. Pharmacol. Exp. Ther. 133: 186-191, 1961.

Nickerson, M. and Goodman, L.S.: Pharmacological properties of a new adrenergic blocking agent N,N-dibenzyl-β-chloroethylamine (Dibenamine). J. Pharmacol. Exp. Ther. 89: 167-185, 1947.

Ochoa, E., DePlazas, S.F. and DeRobertis, E.: The effect of (-)-noradrenaline on artifical lipidic membranes containing a proteolipid with adrenoceptor properties. J. Pharm. Pharmacol. 24: 75-77, 1972.

Øye, I. and Sutherland, E.W.: The effect of epinephrine and other agents on adenyl cyclase in cell membrane of avian erythrocytes. Biochim. Biophys. Acta 127: 347-354, 1966.

Paasonen, M.K. and Krayer, O.: The release of norepinephrine from mammalian heart by reserpine. J. Pharmacol. Exp. Ther. 123: 153-160, 1958.

Parr, J.J. and Urquilla, P.R.: Analysis of the adrenergic receptors of pacemaker and myocardial cells. Eur. J. Pharmacol. 17: 1-7, 1972.

Patil, P.N.: Use of isomeric-ratio as a criterion to differentiate adrenergic receptors. J. Pharm. Pharmacol. 21: 628-629, 1969.

Patil, P.N., LaPidus, J.B. and Tye, A.: Steric aspects of adrenergic drugs. J. Pharm. Sci. 59: 1205-1234, 1970.

Patil, P.N., Patel, D.G. and Krell, R.: Steric aspects of adrenergic drugs. XV. Use of isomeric-activity-ratio as a criterion to differentiate adrenergic receptors. J. Pharmacol. Exp. Ther. 176: 622-633, 1971.

Paton, W.D.M.: A theory of drug action based on the rate of drug-receptor combination. Proc. Roy. Soc. Ser. B Biol. Sci. 154: 21-69, 1961.

Peart, W.S.: The nature of splenic sympathin. J. Physiol. 108: 491-501, 1949.

Pfeiffer, C.C.: Optical isomerism and pharmacological action, a generalization. Sci. 124: 29-31, 1956.

Pohl, S.L., Krans, H.M.J., Kozyreff, V., Birnbaumer, L. and Rodbell, M.: The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. J. Biol. Chem. 246: 4447-4454, 1971.

Poisner, A.M.: Caffeine-induced catecholamine secretion: similarity to caffeine-induced muscle contraction. Proc. Soc. Exp. Biol. Med. 142: 103-105, 1973.

Polacek, I., Bolan, J. and Daniel, E.E.: Accumulation of adenosine 3',5'-monophosphate and relaxation in the rat uterus in vitro. Can. J. Physiol. Pharmacol. 49: 999-1004, 1971.

Polacek, I. and Daniel, E.E.: Effect of α-and β-adrenergic stimulation on the uterine motility and adenosine 3',5'-monophosphate level. Can. J. Physiol. Pharmacol. 49: 988-998, 1971.

Posternak, Th., Sutherland, E.W., and Henion, W.F.: Derivatives of cyclic 3',5'-adenosine monophosphate. Biochim. Biophys. Acta 65: 558-560, 1962.

Powell, C.E. and Slater, I.H.: Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. J. Pharmacol. Exp. Ther. 122: 480-488, 1958.

Rabinowitz, B., Parmley, W.W. and Katz, J.P.: Effects of phentolamine on myocardial contractility and adenyl cyclase activity. Fed. Proc. 31: 556, 1972.

Rall, T.W.: Role of adenosine 3',5'-monophosphate (cyclic AMP) in actions of catecholamines. Pharmacol. Rev. 24: 399-409, 1972.

Rall, T.W., Sutherland, E.W., and Berthet, J.: Relationship of epinephrine and glucagon to liver phosphorylase: IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J. Biol. Chem. 224: 463-475, 1957.

Rall, T.W. and Sutherland, E.W.: Formation of a cyclic adenine ribonucleotide by tissue particles. J. Biol. Chem. 232: 1065-1076, 1958.

Rall, T.W., and Sutherland, E.W.: Adenyl cyclase: II. Enzymatically catalyzed formation of adenosine 3',5'-phosphate and inorganic pyrophosphate from adenosine triphosphate. J. Biol. Chem. 237: 1228-1232, 1962.

Rall, T.W. and West, T.C.: The potentiation of cardiac inotropic responses to norepinephrine by theophylline. J. Pharmacol. Exp. Ther. 139: 269-274, 1963.

Rasmussen, H. and Tenenhouse, A.: Cyclic adenosine monophosphate. Ca^{++}, and membranes. Proc. Nat. Acad. Sci. 59: 1364-1370, 1968.

Refsum, H. and Landmark, K.: The action of practolol on the isolated rat atrium. Acta Pharmacol. et Toxicol. 31: 97-106, 1972.

Robison, G.A., Butcher, R.W., Øye, I., Morgan, H.E. and Sutherland, E.W.: The effect of epinephrine on adenosine 3',5'-phosphate levels in the isolated perfused rat heart. Molec. Pharmacol. 1: 168-177, 1965.

Robison, G.A., Butcher, R.W., and Sutherland, E.W.: Adenyl cyclase as an adrenergic receptor. Ann. N.Y. Acad. Sci. 139: 703-723, 1967.

Robison, G.A., Butcher, R.W. and Sutherland, E.W.: On the relation of hormone receptors to adenyl cyclase. In Fundamental Concepts in Drug-Receptor Interactions (J.F. Danielli, J.F. Moran and D. J. Triggle, Eds.), 59-91, Academic Press, New York, 1970.

Robison, G.A., Butcher, R.W. and Sutherland, E.W.: Cyclic AMP. Academic Press, New York, 1971.

Robison, G.A., Langley, P.E. and Burns, T.W.: Adrenergic receptors in human adipocytes-- Divergent effects on adenosine 3',5'-monophosphate and lipolysis. Biochem. Pharmacol. 21: 589-592, 1972.

Robison, G.A. and Sutherland, E.W.: Cyclic AMP and the function of eukaryotic cells: An introduction. Ann. N.Y. Acad. Sci. 185: 5-9, 1971.

Rothlin, E., Konzett, H. and Cerletti, A.: Antagonism of ergot alkaloids toward the inhibitory response of the isolated rabbit intestine to epinephrine and norepinephrine. J. Pharmacol. Exp. Ther. 112: 185-190, 1954.

Rubin, C.S., Erlichman, J. and Rosen, O.M.: Molecular forms and subunit composition of a cyclic adenosine 3',5'-monophosphate-dependent protein kinase purified from bovine heart muscle. J. Biol. Chem. 247: 36-44, 1972.

Rudzik, A.D. and Miller, J.W.: The effect of altering the catecholamine content of the uterus on the rate of contractions and the sensitivity of the myometrium to relaxin. J. Pharmacol. Exp. Ther. 138: 88-95, 1962.

Rudzik, A.D. and Miller, J.W.: The mechanism of uterine inhibitory action of relaxin-containing ovarian extracts. J. Pharmacol. Exp. Ther. 138: 82-87, 1962a.

Salvador, R.A., Colville, K.I., April, S.A. and Burns, J.J.: Inhibition of lipid mobilization by N-isopropylmethoxamine (B.W. 61-43). J. Pharmacol. Exp. Ther. 144: 172-180, 1964.

Sato, M., Yamaguchi, I. and Kiyomoto, A.:. Studies on tetrahydroisoquinolines (THI). (II) Pharmacological action on cardiovascular system. Jap. J. Pharmacol. 17: 153-158, 1967.

Shanfeld, J., Frazer, A. and Hess, M.E.: Dissociation of the increased formation of cardiac adenosine 3',5'-monophosphate from the positive inotropic effect of norepinephrine. J. Pharmacol. Exp. Ther. 169: 315-320, 1969.

Shinebourne, E. and White, R.: Cyclic AMP and calcium uptake of the sarcoplasmic reticulum in relation to increased rate of relaxation under the influence of catecholamines. Cardiovasc. Res. 4: 194-200, 1970.

Sim, M.K. and Chantharaksri, U.: Rat uterine contractility and the activities of uterine adenyl cyclase and phosphodiesterase during the estrus cycle. Biochem. Pharmacol. 22: 1417-1422, 1973.

Skelton, C.L., Karch, F.E., Hougen, T.J., Marcus, M.L. and Epstein, S.E.: Potentiation of the inotropic effects of norepinephrine and dibutyryl cyclic AMP by theophylline. J. Mol. Cell. Cardiol. 3: 243-253, 1971.

Skelton, C.L., Levey, G.S. and Epstein, S.E.: Positive inotropic effects of dibutyryl cyclic adenosine 3',5'-monophosphate. Circ. Res. 26: 35-43, 1970.

Sutherland, E.W.: Effect of the hypterglycemic factor of the pancreas and of epinephrine on glycogenolysis. Recent Progr. Hormone Res. 5: 441-459, 1950.

Sutherland, E.W. and Cori, C.F.: Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase. J. Biol. Chem. 188: 531-543, 1951.

Sutherland, E.W. and Rall, T.W.: Fractionation and characterization of a cyclic adenosine ribonucleotide formed by tissue particles. J. Biol. Chem. 232: 1077-1091, 1958.

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

Sutherland, E.W., Rall, T.W., and Menon, T.: Adenyl cyclase: I. Distribution, preparation, and properties. J. Biol. Chem. 237: 1220-1227, 1962.

Sutherland, E.W., Robison, G.A. and Butcher, R.W.: Some aspects of the biological role of adenosine 3',5'-monophosphate (cyclic AMP). Circulation 37: 279-306, 1968.

Takamine, J.: The isolation of the active principle of the supra-renal gland. J. Physiol. 27: 29P-30P, 1901.

Thienes, C.H.: Action of ergot alkaloids on intestine and uterus. Proc. Soc. Exp. Biol. Med. 26: 501-502, 1929.

Tothill, A.: Investigation of adrenaline reversal in the rat uterus by the induction of resistence to isoprenaline. Br. J. Pharmacol. 29: 291-301, 1967.

Trendelenberg, U., DeLaSierra, B.G.A. and Muskus, A.: Modification by reserpine of the atrial pacemaker to sympathomimetic amines. J. Pharmacol. Exp. Ther. 141: 301-309, 1963.

Triner, L., Vulliemoz, Y., Verosky, M. and Nahas, G.G.: The effect of catecholamines on adenyl cyclase activity in rat uterus. Life Sci. 9: 707-712, 1970a.

Triner, L., Overweg, N.I.A. and Nahas, G.G.: Cyclic 3',5'-AMP and uterine contractility. Nature 225: 282-283, 1970b.

Triner, L., Nahas, G.G., Vulliemoz, Y., Overweg, N.I.A., Verosky, M., Habif, D.V. and Ngai, S.H.: Cyclic AMP and smooth muscle function. Ann. N.Y. Acad. Sci. 185: 458-476, 1971.

Tsai, T.H. and Fleming, W.W.: The adrenotropic receptors of the cat uterus. J. Pharmacol. Exp. Ther. 143: 268-272, 1964.

VanDeripe, D.R., Ablad, B. and Moran, N.C.: β-adrenergic blockade by four methyl substituted N-isopropylphenyl-ethanolamines. Fed. Proc. 23: 124, 1964.

VanDeripe, D.R. and Moran, N.C.: Comparison of cardiac and vasodilator adrenergic blocking activity of DCI and four analogs. Fed. Proc. 24: 712, 1965.

Van Rossum, J.M.: Cumulative dose-response curves. II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. Ther. 143: 299-330, 1963.

Vulliemoz, Y., Verosky, M., Katz, R. and Triner, L.: Effect of some synthetic beta adrenergic agonists on the cyclic AMP system in smooth muscle. Fed. Proc. 32: 712, 1973.

Walsh, D.A., Perkins, J.P. and Krebs, E.G.: An adenosine 3',5'-monophosphate-dependent protein kinase from rabbit skeletal muscle. J. Biol. Chem. 243: 3763-3765, 1968.

Wasserman, M. A. and Levy, B.: Selective Beta adrenergic receptor blockade in the rat. J. Pharmacol. Exp. Ther. 182: 256-263, 1972.

Wastila, W.B., Su, J.Y., Friedman, W.F. and Mayer, S.E.: Blockade of biochemical and physiological responses of cardiac muscle to norepinephrine by N-tert-butyl-methoxamine (butoxamine). J. Pharmacol. Exp. Ther. 181: 126-138, 1972.

Weinryb, I., Chasin, M., Free, C.A., Harris, D.N., Goldenberg, H., Michel, I.M., Paik, V.S., Phillips, M., Samaniego, S. and Hess, S.M. Effects of therapeutic agents on cyclic AMP metabolism in vitro. J. Pharm. Sci. 61: 1556-1567, 1972.

Westfall, D.P. and Fleming, W.W.: Sensitivity changes in the dog heart to norepinephrine, calcium and aminophylline resulting from pretreatment with reserpine. J. Pharmacol. Exp. Ther. 159: 98-106, 1968.

Waud, D.R., Kottegoda, S.R. and Krayer, O.: Threshold dose and time course of norepinephrine depletion of the mammalian heart by reserpine. J. Pharmacol. Exp. Ther. 124: 340-346, 1958.

Weston, A.H.: Changes in the amounts of high energy phosphate compounds associated with the actions on smooth and cardiac muscle. Br. J. Pharmacol. 40: 153P, 1970.

Wurtman, R.J., Axelrod, J. and Potter, L.T.: The disposition of catecholamines in the rat uterus and the effect of drugs and hormones. J. Pharmacol. Exp. Ther. 144: 150-155, 1964.

Yamato, E., Hirakura, M. and Sugasawa, S.: Synthesis of 6,7-dihydroxy-1,2,3,4,-tetrahydroisoquinoline derivatives. Tetrahedron, Suppl. 8: 129-134, 1966.

Youmans, W.B., Aumann, K.W. and Haney, H.F.: Relation of the various groups of the adrenalin molecule to its intestine inhibiting function in unanesthetized dogs. Am. J. Physiol. 126: 237-246, 1939.

Youmans, W.B., Haney, H.F. and Aumann, K.W.: Relation of the groups of the adrenaline molecule to its cardioaccelerator action. Am. J. Physiol. 130: 190-196, 1940.