BETA-ADRENERGIC RECEPTORS MEDIATING INHIBITION OF ANTIGEN-INDUCED HISTAMINE RELEASE FROM GUINEA-PIG HEART AND LUNG

BY STANLEY K. WONG

Under the Supervision of Associate Professor Carl Kenneth Buckner

The beta adrenergic receptors mediating inhibition of antigen-induced histamine release from guinea-pig heart and lung were evaluated by the relative potencies of the beta agonists. Potency differences between the optical isomers of isoproterenol were used as an additional criterion for the classification of the beta receptors in each tissue. Under proper experimental conditions, similar relative potencies of the beta agonists and similar isomeric-potency-differences in two tissues suggest that these tissues have the same type of beta receptors.

Guinea-pigs were actively sensitized by i.p. injections of ovalbumin on days 1, 3 and 5. Fragments of heart and lung tissue were obtained from these animals beginning 21 days later. In vitro challenge with ovalbumin resulted in concentration-dependent histamine release. Pretreatment of the tissues with phenoxybenzamine, an agent that impedes processes which could influence observed effects of catecholamines, did not alter the dose-response curve to ovalbumin. Indomethacin, a prostaglandin synthetase

inhibitor, enhanced ovalbumin-induced histamine release in the lung, but not in the heart.

Prior incubation of (-)-isoproterenol, in the presence of phenoxybenzamine and indomethacin, resulted in a shift to the right of the ovalbumin dose-response curve and reduction of the maximum histamine release obtained with ovalbumin in both tissues. (-)-Soterenol and (±)-sulfonterol were equipotent in antagonizing the dose-response effects of ovalbumin. Both produced a smaller maximum effect than (-)-isoproterenol in the lung, but were as effective as (-)-isoproterenol in the heart. Dose-response curves to ovalbumin were not altered by (-)-H 80/62, a cardio-selective beta agonist, except at $1x10^{-3}$M. Relative potencies obtained for (-)-isoproterenol, (-)-soterenol and (±)-sulfonterol in descending order in shifting the ovalbumin dose-response curve to the right and reduction of the maximum histamine release were 1, 0.23 and 0.07, respectively, and 1, 0.027 and 0.023, respectively, in the heart. In the lung, the values for producing these responses were 1, 0.29 and 0.11, respectively, and 1, 0.040 and 0.015, respectively. Therefore, the order of potency obtained for these beta agonists for shifting the ovalbumin dose-response curves to the right in guinea-pig heart and lung was (-)-isoproterenol > (-)-soterenol = (±)-sulfonterol >>> (-)-H 80/62. Similar order of potency

was also obtained for reducing the maximum histamine release in these tissues. However, within guinea-pig heart or lung, dissimilar relative potencies were obtained for the beta agonists in eliciting these two responses.

Dose-response curves to ovalbumin were inhibited by (+)-isoproterenol in a manner similar to its enantiomer. Both isomers produced the same maximum effect on all measured responses. Isomeric-potency-differences (in log units) for shifting the ovalbumin dose-response curve to the right and reduction of the maximum histamine release were 1.92 and 2.46, respectively, in the heart. In the lung, the values for producing these responses were 2.02 and 2.83, respectively. Therefore, isomeric-potency-differences obtained for shifting the ovalbumin dose-response curve to the right in guinea-pig heart and lung were similar. Similar isomeric-potency-differences were also obtained for reducing the maximum histamine release in these tissues. However, within guinea-pig heart or lung, dissimilar isomeric-potency-differences were obtained for eliciting these two responses.

The present data suggest the following: 1) the beta receptors mediating the shift to the right of the antigen dose-response curve in guinea-pig heart and lung are similar. Also, the beta receptors mediating the

reduction of the maximum histamine release in these tissues are similar. 2) The beta receptors mediating the shift to the right of the antigen dose-response curve and reduction of the maximum histamine release are different in each tissue.

APPROVED: Carl K. Buckner

Carl K. Buckner, Ph.D.
Associate Professor

DATE: August 3, 1979

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BY

STANLEY K. WONG

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

DOCTOR OF PHILOSOPHY

(Pharmacy)

at the

UNIVERSITY OF WISCONSIN-MADISON

1979

Dedicated to

my wife,

Nancy Nan-Fun Wong

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to:

Dr. Carl K. Buckner for his instruction, guidance and support in class and research activities.

My wife, Nancy for her encouragement, patience and understanding.

My son, Andrew for his love.

My parents for their love and invaluable support.

The graduate students of the Pharmacology group for their friendship and cooperation.

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INTRODUCTION

Chemical Mediators of Immediate Hypersensitivity

It has been demonstrated that challenging sensitized tissues with specific antigen results in the release of potent pharmacologically active mediators (Lewis and Austin, 1977; Piper, 1977). The primary mediators of immediate type hypersensitivity include histamine, slow-reacting substance of anaphylaxis (SRS-A), Eosinophil chemotactic factor of anaphylaxis (ECF-A) and others. In contrast, mediators such as bradykinin and prostaglandins, which are formed due to the alteration of membrane components, are considered secondary mediators (Austin and Orange, 1975). Because prostaglandins (Tauber et al., 1973) and bradykinin (Stoner et al., 1973) can alter tissue cyclic nucleotides levels in the lung, they may also serve as modulators for the release or action of primary mediators. The primary mediators differ not only in structure and function, but also in the time course of their release. In a study with human nasal polyps (Kaliner et al., 1973), histamine was rapidly released followed by the slow appearance of SRS-A. Maximal SRS-A release was reached at 15 minutes, with only 50 per cent being formed at 5 minutes, when the histamine release was

already maximal. This may relate to the fact that histamine and ECF-A are stored preformed, whereas SRS-A is generated immediately before release.

The association of histamine to allergy and bronchial asthma begins as early as 1932 when Bartosch et al. discovered the presence of histamine in the perfusate of an isolated guinea-pig lung during anaphylactic reaction. However, anti-histamines do not prevent all the manifestations of an anaphylactic reaction and are only marginally effective in asthma (Feinberg, 1947), indicating that other mediators may also be involved. Histamine is bound to an acidic heparin-like molecule in the granules of mast cells (Kobayashi, 1962; Bergquist et al., 1971), which are located predominantly in the perivascular connective tissue of the lung. It is also found in large amount in circulating basophils and in the parietal region of the stomach (Haverback et al., 1965). Histamine is not absorbed from food, but is biosynthesized from histidine; free histamine is rapidly degraded by either oxidative deamination or by methylation and oxidative deamination (Schayer, 1965). The in vivo pathophysiological effects of histamine include increase in vascular permeability in blood vessels resulting in edema (Majno, 1964), and increase in pulmonary resistance with concommitant reduction

in compliance in guinea-pigs (Drazen and Austin, 1974), cats (Colebatch et al., 1966) and man (Laitinen et al., 1976). Although histamine is capable of constricting the airway directly, at least part of the airway constriction of this mediator in vivo is due to vagal reflexes initiated by airway irritant receptors (Colebatch et al., 1966; Mills et al., 1969). The effects of histamine on pulmonary mechanics of the guinea-pig has been shown to be abolished by pretreatment with atropine (Drazen and Austin, 1975).

SRS-A is generally considered to be the substance responsible for greater antihistamine-resistance bronchoconstriction in bronchial asthma since it has been demonstrated that SRS-A is released during anaphylactic reaction in perfused sensitized guinea-pig lungs (Kellaway and Trethewie, 1940). However, little information is available about its chemistry and structure, or how and from where it is formed. SRS-A, an acidic sulphate of approximately 500 molecular weight, is inactivated by human eosinophil arylsulfatase (Wasserman et al., 1975). It is not preformed in tissues, but is formed, as well as released, immediately after immunological activation (Lewis et al., 1974). Immunological in vitro release of SRS-A has been produced by IgE-dependent reactions in

human lung fragments (Orange et al., 1971a) and human leukocytes (Grant and Lichtenstein, 1974), and by IgG-mediated reactions in guinea-pig lung fragments (Stechschulte et al., 1973). SRS-A causes a prolonged contraction of the isolated guinea-pig ileum and contracts isolated bronchial smooth muscles from human and guinea pig in vitro (Brocklehurst, 1960; Berry and Collier, 1964). Upon intracutaneous injection, SRS-A enhances vascular permeability (Orange et al., 1969). It also decreases pulmonary compliance independent of vagal reflexes when injected intravenously into guinea pigs (Drazen and Austin, 1974). Furthermore, SRS-A has been observed to cause bronchoconstriction in asthmatic patients although not in control subjects (Herxheimer and Streseman, 1963).

Recently, it has been proposed that SRS-A may be a metabolite of arachidonic acid, possibly produced by the lipoxygenase pathway of metabolism (Jakschik and Parker, 1976). This view is based on the capacity of the calcium ionophore to generate increased amount of SRS-A-like material from rat leukemic basophils in the presence of arachidonic acid and by the capacity of eicosatetraynoic acid (TYA), an inhibitor of both cyclo-oxygenase and lipoxygenase pathways, to block this effect. In addition,

indomethacin, a selective cyclo-oxygenase inhibitor, appeared to enhance SRS-A formation rather than inhibit it (Jakschik et al., 1977). The fact that immunological release of SRS-A from guinea-pig lung has been augmented by indomethacin and inhibited by TYA lends support for the hypothesis that SRS-A is derived from arachidonic acid.

ECF-A, another primary mediator, is an acidic polypeptide of molecular weight less than 1000 and is stored in mast cells (Wasserman et al., 1974). It is released along with histamine during anaphylaxis and causes the accumulation of eosinophils at the site of reaction (Wasserman et al., 1974; Lewis et al., 1975).

Other primary mediators such as platelet-activating factor, kallikrein and neutrophil chemotactic factor of anaphylaxis are also released in anaphylaxis (Austin and Orange, 1975) but will not be discussed because these mediators have no actions on the bronchial smooth muscle.

Along with the primary mediators, secondary mediators such as bradykinin and prostaglandins, are also released during anaphylaxis. Bradykinin is formed in the blood after the release of kallikrein from guinea-pig lung in anaphylaxis (Jonasson and Becker, 1966). It is a potent agent for increasing vascular permeability, contracting

isolated smooth muscles of intestine and uterus, and dilating small blood vessels (Erdos, 1966). Bradykinin is also a potent bronchoconstrictor in the guinia-pig *in vivo* and *in vitro* (Collier *et al.*, 1960) but is not very effective in causing acute bronchoconstriction in man. Asthmatic patients, however, are very sensitive to bradykinin aerosol (Herxheimer and Stresemann, 1961).

Formation of prostaglandins may result from alteration of the membrane during anaphylactic reaction or after the effects of histamine and SRS-A in the smooth muscle of the lung. Both PGE_1 and PGE_2 appear to cause bronchodilatation *in vivo* and *in vitro*, whereas $PGF_{2\alpha}$ causes bronchoconstriction (Main, 1964; Sweatman and Collier, 1968). The prostaglandins are effective potentiators of increased vascular permeability induced by histamine and bradykinin, and in endogenous inflammatory reactions (Williams and Morley, 1973). It has been shown that concentrations of PGE_1 and $PGF_{2\alpha}$ which increase tissue levels of adenosine-3',5'-monophosphate (cyclic AMP) in human lung fragments, inhibit antigen-induced release of histamine; whereas low concentrations of PGE_1 and $PGF_{2\alpha}$ decrease tissue levels of cyclic AMP and enhance histamine release. This suggests that formation of prostaglandins during anaphylaxis can facilitate or even suppress mediator release (Tauber *et al.*, 1973).

Mechanisms of Mediator Release

Previous studies have demonstrated that a single immunological class, IgG_1 in guinea pigs (Baker et al., 1964) and IgE in the human (Orange et al., 1971a), is responsible for release of chemical mediators. The sequence of biochemical events in antigen-induced release of chemical mediators from human lung tissue has been described (Austin and Orange, 1975). The process starts with the activation of the cell by antigen bridging between molecules of IgE attached to the cell membrane and ends with the release of mediators. It can be divided into several discrete steps. The triggering event results in alteration of the membrane permeability to calcium ion and leads to calcium ion influx into the cell. Removal of calcium ion by chelating agent can prevent the release process in a reversible way (Douglas and Ueda, 1973), and the use of an ionophore to carry calcium ions across the membrane into the cell results in release of mediators (Foreman et al., 1973). The presence of calcium ions leads to the activation of a serine esterase from its precursor. This active enzyme is susceptible to inactivation by diisopropyl flurophosphate (DFP) (Orange et al., 1971b). The proenzyme, however, is not susceptible to inactivation by DFP. The subsequent energy-dependent step

is inhibitable by 2-deoxyglucose, followed by a release phase which is calcium-dependent. This step is also modulated by intracellular cyclic nucleotides levels prior to the release of diverse chemical mediators such as histamine, SRS-A and ECF-A.

Several in vitro studies reveal that the release of mediators after an immunological reaction is by exocytosis (Lichtenstein and Osler, 1964, 1966). The storage granule is brought into contact with the plasma membrane probably by interaction with the microtubules. Energy and intracellular calcium ions are required in this process to convert the microtubules from the A to B form. This process is also inhibited by elevated cyclic AMP levels which may act via the associated protein kinase in returning the microtubules to the A form (Gillespie, 1971). Alternatively, the cyclic nucleotides may act at a final stage of the reaction, that is, the fusion of the granule and plasma membranes leading to mediator release. Histamine is loosely held to heparin-like molecules in the granule and can be released by displacement with sodium ions (Uvnäs, 1974). As soon as the granule membrane is reestablished, histamine is resynthesized by the mast cell and restored in the granules after removal of sodium ions by the membrane pump. This

process applies to other chemical mediators as well, but not to SRS-A. SRS-A, being an acidic protein, is formed only after immunological reaction immediately before release (Lewis et al., 1974). Nevertheless, the requirement for release of all the mediators appears to include sources of energy, calcium ions, plasma membrane, normal levels of cyclic nucleotides and microtubules.

A variety of substances are also capable of releasing histamine from the mast cells. These agents include compound 48/80, basic drugs such as morphine and tubocurare, basic peptides, polymer amino acids, dextrans and lectins (Goth, 1978) and they release histamine by causing degranulation of the mast cells (Bloom and Haegermark, 1965).

Pharmacological Modulation of Mediator Release

Almost forty years ago, Schild (1936) demonstrated that epinephrine prevented the release of histamine from sensitized guinea-pig lungs. Lichtenstein and Margolis observed (1968) that beta adrenergic agonists and methylxanthines act individually and synergistically to inhibit antigen-induced release of histamine from IgE-sensitized human peripheral leukocytes. Subsequently, it was established in human lung that agents capable of activating adenylate cyclase, such as beta adrenergic agonists,

phosphodiesterase inhibitors, and prostaglandins increase tissue concentration of cyclic AMP and inhibit the immunological release of histamine (Orange et al., 1971a; Tauber et al., 1973). Stimulation of phosphodiesterase with imidazole depletes tissue cyclic AMP levels and enhances the release of histamine and SRS-A (Kaliner and Austin, 1974). However, it has been recently shown that imidazole inhibits the metabolism of arachidonic acid to thromboxane and diverts it to prostaglandins, mainly $PGF_{2\alpha}$ (Nijkamp et al., 1977). Hence, an alternative explanation for the enhancing effect of imidazole on mediator release is through the formation of prostaglandins. In this regard, alpha adrenergic agonists and low concentrations of prostaglandins, especially of the $PGF_{2\alpha}$ class, decrease tissue levels of cyclic AMP and enhance the release of chemical mediators (Kaliner et al., 1972; Tauber et al., 1973). In addition, histamine itself has been shown to inhibit antigen-induced histamine release from human leukocytes and increase tissue cyclic AMP levels via stimulation of the histamine H_2 receptors (Lichtenstein and Gillespie, 1975). Thus, there appears to be an inverse relationship between tissue concentration of cyclic AMP and the degree of mediator release. These observations also suggest the presence of alpha, beta,

prostaglandin and histamine H_2 receptors on the target cells in the human lung.

Cholinergic stimulation of sensitized human lung fragments with acetycholine or carbachol results in enhanced release of both histamine and SRS-A. This effect is blocked by pretreatment with atropine, a muscarinic receptor blocking agent. The enhancement is not associated with a fall in tissue concentration of cyclic AMP (Kaliner et al., 1972), but addition of 8-bromo-derivative of guanosine-3', 5'-monophosphate (cyclic GMP) enhances immunological release of mediators. This suggests that cyclic GMP may be the intracellular mediator of cholinergic responses. In various tissues, cholinergic stimulation results in an increase of tissue levels of cyclic GMP, and cyclic AMP and cyclic GMP appear to mediate opposing responses in these tissues (George et al., 1970; Hadden et al., 1973; Schultz et al., 1973) as well as to modulate immunological release of mediators (Kaliner et al., 1972). However, agents which affect cyclic GMP levels have been shown to produce little or no effects on histamine release from human basophils (Lichtenstein et al., 1972) and these same workers were unable to demonstrate the presence of alpha adrenergic receptors on these cells (Lichtenstein, 1977). Furthermore, the physiological role of cyclic GMP as an intracellular

mediator has been questioned and remains obscure in most tissues (Schultz, 1977).

Although the evidence supporting the concept of cyclic AMP modulation of mediator release appears quite convincing, it is questionable that the data obtained from mixed cell sources such as human lung fragments and leukocytes could reflect the actual changes occurring within the mast cells. Thus, unless definite experiments are carried out with isolated lung mast cells, the data will remain circumstantial.

Development of Adrenergic Receptor Concept

The concept of the adrenergic receptors is due to the fundamental contributions of Langley and Dale. Langley (1905) introduced the term 'receptive substance' to describe the mechanism with which the cell responds to nerve stimulation. He suggested that two types of 'receptive substances' must be present, motor and inhibitory. The observed tissue responses should also indicate which of these receptive substances is dominant in that tissue.

The existence of two distinct types of receptive substances was soon substantiated by the studies of Dale (1906) on the physiological actions of ergot alkaloids. He demonstrated that excitatory or motor responses of various organs to epinephrine and nerve stimulation were

'paralyzed' by the ergot alkaloids, whereas the inhibitory responses were not.

However, the marked parallelism of the effects of epinephrine and nerve stimulation led Elliott (1904) to believe erroneously that epinephrine was the chemical mediator released by the sympathetic nerve. Barger and Dale (1910) subsequently refuted this concept and they concluded, after studying the 'sympathomimetic' actions of several series of synthetic analogs of epinephrine, that primary amines (including norepinephrine) more closely 'mimicked' the effects of sympathetic nerve stimulation than did secondary amine analogs of the same series. However, the probable mediator role of norepinephrine was unnoticed for several years.

The adrenergic receptor concept suffered a major setback in 1933 when Cannon and Rosenbleuth proposed the 'Sympathin' theory of sympathetic neurotransmission. According to the theory, epinephrine or a related substance liberated from the nerve unites in the cell with another substance to form a combination product, sympathin E (excitatory) or sympathin I (inhibitory), depending on the particular cell. The sympathin is defined as the actual chemical mediator of sympathetic nerve impulses which induce contraction or relaxation in the cell. The

substances E and I were assumed to correspond to Langley's 'receptive substances.'

In an attempt to investigate the structural requirements of the epinephrine molecule, Youmans et al. (1939) observed that no consistent relationship existed between the smooth muscle-relaxing (intestine) and the smooth muscle-contracting properties (nictitating membrane) of several analogs of epinephrine. They concluded that at least two different types of 'receptive mechanisms' must exist in these smooth muscles.

The dispute over the transmitter of the adrenergic nerve was finally put to an end by the extensive studies of U.S.v Euler (1946; 1948) in which extracts were prepared from several sympathetic nerves, and the activity was determined and analyzed both pharmacologically and chemically. The physiological transmitter of the adrenergic system was accurately identified as norepinephrine.

Classification of Adrenergic Receptors

Ahlquist (1948) studied the order of potency of six different catecholamines--1) (-)-epinephrine, 2) (±)-epinephrine, 3) (±)-norepinephrine, 4) (±)-alpha-methyl-norepinephrine, 5) (±)-alpha-methyl-epinephrine, 6) (±)-isoproterenol--on a variety of tissue responses both in

isolated organs and intact animals. He found the relative potencies in descending order to be 1,2,3,4,5,6 for producing excitation of the smooth muscle of the peripheral blood vessels (vasoconstriction), nictitating membrane, uterus, ureter and pupillary dilator, and inhibition of the smooth muscle of the intestine; but 6, 2,5, 1, 4, 3 for producing inhibition of the smooth muscle of blood vessels (vasodilation) and the uterus and excitation (increase in rate and force) of the heart. The two distinct orders of potencies led him to conclude that there were two distinct types of adrenergic receptors mediating the responses, which he designated as alpha and beta, respectively. One support for this classification scheme was that all of the alpha receptor mediated responses, except inhibition of intestinal smooth muscle, were blocked by the adrenergic blocking agents, e.g., dibenamine, available at that time.

For some obvious reasons, this dual receptor classification was not readily accepted. Firstly, Ahlquist originally assigned the alpha receptor to the smooth muscle of the intestine. This was an obvious anomaly since alpha receptors elsewhere are related to smooth muscle contraction rather than relaxation. Secondly, the differentiation of drug receptors depends, to a large

extent, on the use of blocking agents; but none of the adrenergic blocking agents available at that time were able to block beta receptor mediated responses. It was not until 1958 that dichloroisoproterenol (DCI) was discovered and observed to block both adrenergic inhibitory responses in a variety of smooth muscles (Powell and Slater, 1958) and adrenergic stimulatory responses in the heart (Moran and Perkins, 1958) at concentrations which did not block adrenergic stimulatory responses in smooth muscle. The fact that the blockade by DCI was selective for adrenergic responses which Alhquist had classified as being mediated by beta adrenergic receptors greatly strengthened the suggestion that there were two major types of receptors. The concept of alpha and beta receptors was soon generally accepted, and it provided a simple way to predict and classify adrenergic drug responses. Two potent beta receptor blocking agents, pronethalol (Black and Stephenson, 1962) and propranolol (Black et al., 1964) were subsequently available. Compounds that block alpha receptor responses were termed alpha adrenergic antagonists, and those that block beta receptor responses were classified as beta adrenergic antagonists (Moran and Perkins, 1958). In addition, two pharmacological procedures were available to differentiate

adrenergic receptors: 1) relative potencies of the agonists and 2) specific blockade of the antagonist.

With the aid of DCI, Furchgott (1959) reexamined the intestinal receptor and observed that the inhibitory effect of the isolated rabbit intestine to epinehprine was not completely blocked by either DCI or by dibenamine. He proposed a third type of adrenergic receptor for the smooth muscle of the intestine called delta.

The 'anomalous' intestinal receptor was classified later that year by Ahlquist and Levy (1959) using selective alpha and beta receptor agonists and antagonists. They observed in dogs that the intestinal inhibitory effect of phenylephrine, a selective alpha receptor agonist, was blocked by small doses of dibenamine, and the intestinal inhibitory effect of isoproterenol, a selective beta receptor agonist, was blocked by small doses of DCI. The effect of epinephrine on the canine ileum was blocked by neither kind of antagonists but was blocked by the combination of the two. Thus, it became apparent that both alpha and beta receptors are present in the intestine, and that both receptors mediate relaxation or inhibition.

Subclassification of Beta Receptors

Lands and Brown (1964) investigated the structure-activity relationship of several selected catecholamines and observed that both α-methyl and α-ethyl derivatives of

isoproterenol were much less potent in producing cardiac stimulating than bronchodilating actions than isoproterenol. Two distinct orders of potencies of these amines were demonstrated, suggesting that the beta receptors mediating these responses may have different characteristics.

In a study in which careful considerations were given to experimental conditions, Furchgott (1967) examined the relative potencies of selected adrenergic agonists in eliciting responses mediated by alpha and beta receptors in a number of isolated tissues from rabbit and guinea pig. The dissociation constants (K_B values) for phentolamine with alpha receptors and those for pronethalol with beta receptors were also estimated. On the basis of the orders of relative potencies of selected agonists and the K_B values for phentolamine and for pronethalol, he concluded that there was only one type of alpha receptor, but at least three types of beta receptors.

About the same time, Lands and co-workers (1967a,b) extended their previous investigations of the adrenergic receptors with a large number of catecholamines in producing responses in both isolated tissues and intact animals. On the basis of two distinct orders of relative potencies obtained for different responses, they suggested that the beta receptors should be subclassified as beta$_1$

and $\underline{\text{beta}}_2$. Beta receptors responsible for positive inotropic and chronotropic responses of the rabbit heart, inhibition of the rabbit small intestine, and lipolysis in rat adipose tissue were classified as $\underline{\text{beta}}_1$. Beta receptors that mediate relaxation of the rat uterus, guinea pig trachea and vasodilation of the anesthetized dog were classified as $\underline{\text{beta}}_2$.

Support for this subclassification scheme was obtained from testing new beta receptor antagonists. Two derivatives of methoxamine, N-isopropylmethoxamine (IMA) and N-tertbutylmethoxamine (TMA) or butoxamine, displayed selective blocking actions of responses mediated by beta receptors. Like other beta receptor antagonists, IMA and TMA effectively blocked catecholamine-induced increases in plasma free-fatty-acid, blood glucose and blood lactic acid in anesthetized dogs (Burns et al., 1964) and catecholamine-induced relaxation of the rat uterus (Levy, 1964; 1966). In addition, TMA could effectively block isoproterenol-induced vasodilation (Levy, 1966). Unlike other beta antagonists, neither IMA nor TMA were able to block cardiac stimulating and intestinal inhibiting effects, at concentrations that effectively blocked vasodilating and uterine inhibitory effects of catecholamines (Levy, 1964; 1966).

VanDeripe and Moran (1965) studied the α-methyl derivatives of DCI in anesthetized dogs. They observed that α-methyl DCI, in contrast to DCI, selectively blocked the vasodilator effect, but not the cardiac stimulating effect, of isoproterenol. DCI was at least 15 times more potent than α-methyl DCI in blocking the positive inotropic effect of isoproterenol. Thus α-methyl DCI, IMA and TMA all displayed selective blockade of the adrenergic responses mediated by the $\underline{\text{beta}}_2$ receptors. So far, evidence from studies of both adrenergic agonists and antagonists suggests that alkyl substitution on the α-carbon atom of the catecholamine molecule decreases the affinity of the compound for beta receptors in the heart, but may increase the affinity for those in the uterus and blood vessels.

The discovery of selective beta adrenergic agonists and subsequent application for treatment of bronchial asthma lends additional support for the $\underline{\text{beta}}_1/\underline{\text{beta}}_2$ receptor classification. Among these compounds are soterenol (Larsen et al., 1967; Dungan et al., 1968), salbutamol (Brittain et al., 1968), trimetoquinol (Yamato et al., 1966), terbutaline (Bergman et al., 1969) and sulfonterol (Kaiser et al., 1975). These agents selectively relax tracheal smooth muscle at concentrations that do not stimulate the heart and are often referred to as $\underline{\text{beta}}_2$ receptor agonists.

Recently, another class of beta receptor agonists have become available which includes dobutamine (Tuttle and Mills, 1975) and H 80/62 (Carlsson et al., 1977). These compounds selectively stimulate the heart at concentrations that produce only little effect on peripheral vascular resistance and may be referred to as $beta_1$ receptor agonists.

Practolol, another selective beta receptor antagonist, has been subsequently discovered (Dunlop and Shanks, 1968). Practolol, in contrast to butoxamine (TMA), is capable of selectively blocking 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 and butoxamine are often referred to as $beta_1$ and $beta_2$ selective antagonists, respectively.

When more beta receptor antagonists became available, Moran (1967) foresaw the tendency for receptors being overclassified and urged that in future studies, rigorous analysis and a skeptical attitude must be maintained. Furchgott (1967; 1970; 1972) further emphasized the need for proper experimental precautions and for quantitative measurements with agonists and antagonists, and he outlined the optimal experimental conditions for the pharmacological characterization of receptors as follows:

(1) The use of isolated tissue preparations has certain advantages over *in vivo* preparations. In *in vitro* studies, complicating factors such as absorption, distribution and metabolism encountered *in vivo* are greatly eliminated.

(2) The response to an agonist should be due solely to its direct action on one type of receptor. Any action dependent on the release of an endogenous transmitter by the agonist should be eliminated. The response should not be the result of actions on more than one type of receptor (e.g., both *alpha* and *beta*). Therefore, in order to study one receptor, exclusive of the other, an appropriate antagonist of the other receptor must be applied.

(3) Factors which tend to decrease or increase the concentration of a free drug (agonist or antagonist) in the region of the receptor should be controlled. The removal processes include both uptake of the agonist into cells and enzymatic inactivation. For agonists like norepinephrine and epinephrine, the dominant

removal process is the active transport into the adrenergic nerve terminal (Iversen, 1967) that results in lowering the concentration of the drug at the receptor, such that the observed response is reduced. Therefore, in order to accurately evaluate the potency of an agonist at the receptor, the uptake process must be eliminated by sympathetic denervation or by applying an inhibitor such as cocaine. Removal of catecholamines by degradative enzymes, for example catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO), can be prevented by applying appropriate inhibitors.

(4) The antagonism produced by an antagonist should be due solely to the competition of the antagonist with the agonist for the receptor. At the time a response is measured, the concentration of a free drug (agonist or antagonist) in the bathing solution should be in equilibrium (steady-state) and known. According to the currently accepted receptor theory, these criteria must be applied for accurate determination of the K_B of the receptor-antagonist complex. Under these conditions, equal responses are produced by equal receptor occupancies before and after the

antagonist and the K_B value of the antagonist should be independent of its concentration (Arunlakshana and Schild, 1959). However, if the antagonist interferes with the uptake process for the agonist, this will result in an erroneously high K_B value for the antagonist. To avoid this kind of action, the removal process should be eliminated by some means. In addition, the tissue should be left in contact with the antagonist for a time sufficient to achieve equilibrium.

(5) The response following the addition of a given dose of an agonist should be measured at the maximum level reached. In the most suitable tissues, this maximal level is maintained for a reasonable length of time and does not 'fade' rapidly.

Thus, the results of several studies suggesting different or extensive classification of beta receptors (Bristow et al., 1970; Farmer and Levy, 1970; Farmer et al., 1970; Wasserman and Levy, 1972) can be criticized on the basis that proper experimental conditions were not provided. Therefore, the validity of these observations in differentiating the receptors involved is in doubt.

Though the $\underline{\text{beta}}_1/\underline{\text{beta}}_2$ receptor concept has received considerable support, it is probably an oversimplification and may only represent extremes of a variable spectrum of beta 'isoreceptors' which have different affinities for beta receptor agonists and antagonists (Brittain et al., 1970). On the other hand, it has been suggested that $\underline{\text{beta}}_1$ and $\underline{\text{beta}}_2$ adrenergic receptors may be present in varying proportions in the same tissue (Furchgott et al., 1976; Carlsson et al., 1977). On the basis of a large range of difference in the molar potency ratio of isoproterenol to norepinephrine in inducing relaxation of guinea-pig tracheal strips from different animals, and the different pA_2 (negative log K_B) values for selective beta receptor antagonists, practolol and isopropylmethoxamine, against three adrenergic agonists, Furchgott and co-workers (1976) concluded that both $\underline{\text{beta}}_1$ and $\underline{\text{beta}}_2$ receptor types were present in the tracheal muscle and that the ratio of these two receptor types varied from one guinea-pig to another.

In addition to characterizing adrenergic receptors with agonists and antagonists, another method utilizes optical isomers of the same compound to evaluate stereochemical selectivity for receptor interaction. Optical isomers of adrenergic drugs bind selectively to adrenergic receptors (Arien, 1967), and the levorotatory or (-)-isomer is considerably more potent than the corresponding

dextrorotatory or (+)- isomers (Patil et al., 1970). Patil (1969) suggested that the isomeric potency ratio should provide a useful and sensitive criterion to differentiate the adrenergic receptors. If the receptors in various tissues are of a same type, then under proper experimental conditions, the potency difference between optical isomers should be the same in each tissue. Conversely, if the receptors are of different types, the isomeric potency ratio should be different.

Owing to their identical physico-chemical properties, optical isomers have equal access to the same receptor site and their concentrations in the receptor region of a single tissue are expected to be the same when equal concentrations are added. Thus, differences of the potency ratio in various tissues should reflect differences in the configuration of the ligand binding site of the receptors involved. Hence, results from optical isomers are expected to provide better indications of drug-receptor interaction and receptor configuration than relative potencies of agonists and antagonists.

In a study in which optical isomers of norephinephrine were used to study adrenergic receptors in a variety of isolated tissues, Patil and co-workers (1971) observed, under proper experimental conditions, that the isomeric-potency-ratio from six different tissues were

approximately the same suggesting alpha adrenergic receptors in these tissues are of a single type. In contrast, the isomeric-potency-ratios from the tissues examined for beta adrenergic receptors were markedly different indicating the presence of at least three different types of beta receptors. It is interesting, however, the similar beta adrenergic receptors were suggested for guinea-pig trachea and atria. This observed similarity was studied in greater detail and confirmed by Buckner and Patil (1971) using isomeric-potency-differences of both agonists and antagonists.

Recently, Birnbaum et al. (1975) studied the adrenergic receptors mediating mechanical and cyclic AMP responses in rat atria and uteri using optical isomers of isoproterenol. They concluded that different beta receptors existed in the two tissues and that both cAMP formation and mechanical responses induced by isoproterenol might be manifestations of the same receptor event. Using the same optical isomers, Buckner and Wong (1978) subsequently concluded that the beta receptors in rat atria and guinea-pig trachea were also different.

Adrenergic Receptors Modulating Mediator Release in the Lung

The mechanism of action of beta receptor agonists in inhibiting mediator release has been extensively studied recently. Assem and Schild (1969) studied the order of potency of several catecholamines to inhibit immunological release of histamine in passively sensitized human lung in vitro. Isoproterenol was the most potent and its effect could be antagonized by propranolol suggesting that this action is mediated by the beta adrenergic receptor. Because the adenylate cyclase system is also affected by beta adrenergic agonists and antagonists (Sutherland et al., 1968), it is proposed that the inhibitory effect of catecholamines on the anaphylactic mechanism may be related to their ability to increase formation of cyclic AMP (Assem and Schild, 1969). It was subsequently established in human lung and leukocytes that agents such as beta adrenergic agonists and prostaglandins, which increase intracellular level of cyclic AMP, inhibit anaphylactic release of chemical mediators (Orange et al., 1971a; Bourne et al., 1972).

In an attempt to further characterize the type of beta adrenergic receptors that mediate inhibition of histamine release, Assem and Schild (1971) studied the antagonism of the inhibitory effect of isoproterenol on

histamine release by several beta receptor antagonists in sensitized human and guinea-pig lungs. They observed that the inhibiting effect of isoproterenol could be blocked by beta receptor antagonists and that both practolol, a cardioselective beta antagonist, and butoxamine, a broncho-selective beta antagonist, were equally potent in their action. These results led them to suggest that the beta receptors involved in inhibition of histamine release in lung mast cells are different from those responsible for chronotropic effects in the heart ($beta_1$) and relaxation in the vascular smooth muscle ($beta_2$). On the basis of different orders of potency of several beta receptor agonists and different K_B values for a cardioselective beta receptor antagonist, H 93/26, against isoproterenol in isolated guinea-pig lung, atria and trachea, Malta and Raper (1975) suggested that the beta receptors involved in inhibition of antigen-induced histamine release in the guinea-pig lung are different from those found in guinea-pig atria and trachea. In this study, however, a broncho-selective beta antagonist, H35/25, failed to differentiate between beta receptors in the three preparations. In contrast, Sorenby (1975) suggested, on the basis of relative potencies of beta receptor agonists, that the beta receptors mediating inhibition of antigen-induced

release of histamine are more related to those mediating tracheal relaxation than those mediating cardiac stimulation.

Thus the results of several studies suggest different classifications for the beta adrenergic receptors modulating antigen-induced histamine release in the lung. Such an inconsistency of the data may result from failure to provide proper experimental conditions and to recognize changes in regression coefficients when calculating pA_2 or K_B values. Thus drug disposition factors can unequally influence the observed potencies of the agonists unless these factors are controlled.

Adrenergic Receptors Modulating Mediator Release in the Heart

Even though the lung is often regarded as the target tissue in anaphylactic shock, the heart also responds to antigen challenge with the release of the same pharmacologically active substances as released in the lung, leading to the development of cardiac abnormalities (Feigen and Prager, 1969; Liebig et al., 1975). The cardiac symptoms include sinus tachycardia, atrioventricular block, decrease in coronary flow and brief stimulation of ventricular contraction followed by failure. Most of the functional changes which occur during cardiac anaphylaxis

can be attributed to histamine (Capurro and Levy, 1973). However, the marked decrease in coronary flow cannot be accounted for by histamine release alone, and thus, may involve the actions of other mediators. Since $PGF_{2\alpha}$ is released from the anaphylactic heart and is known to produce a decrease in coronary flow in the guinea-pig heart (Förster and Mentz, 1973), it is suggested that $PGF_{2\alpha}$ may be the major causative factor of the anaphylactic decrease in coronary flow (Levi et al., 1976).

There is very little information regarding the role of catecholamines in modulating antigen-induced release of mediators in the heart. It has been demonstrated that the beta receptor antagonist, DCI, reduced the anaphylactic release of histamine in isolated guinea-pig hearts (Giotti et al., 1966), suggesting that beta adrenergic agonists may enhance histamine release during cardiac anaphylaxis. However, it must be recalled that DCI, in addition to its adrenergic blocking properties, also behaves as a partial agonist and will produce similar effects as other beta agonists. Since beta adrenergic agonists have been shown to inhibit antigen-induced histamine release from the guinea-pig lung (Malta and Raper, 1975; Sorenby, 1975), it seems likely that they may also inhibit histamine release from the heart. This is in

agreement with the observation that agents which increase intracellular cyclic AMP levels, inhibit the immulological release of histamine during cardiac anaphylaxis (Capurro and Levy, 1973).

Statement of the Problem

The beta adrenergic receptors mediating inhibition of antigen-induced histamine release in the heart and lung of the guinea-pig have not been classified using proper experimental conditions. In the heart, no attempts have ever been made to characterize these beta receptors; whereas several previous studies in the lung attempting to classify these receptors have yielded ambiguous results due to variable data obtained with both agonists and antagonists. Most of the data can be criticized on the basis of failure to provide proper experimental conditions as outlined by Furchgott (1967; 1970; 1972) and therefore, drug disposition factors can unequally influence the observed potencies of the agonists. These variable factors must be eliminated in characterization of receptors using pharmacological agents.

The present studies have been undertaken to critically evaluate the beta receptors mediating inhibition of antigen-induced histamine release in the heart and lung of the guinea-pig. Experiments have been designed to provide optimal experimental conditions under which the

antianaphylactic potencies of both 'full' and 'partial' beta adrenergic agonists will be examined in each tissue. It is expected that data obtained from partial agonists will provide information regarding their affinities for each beta receptor type and the presence of 'spare receptors' for their effects in each tissue. The quantitative difference in potency between optical isomers of isoproterenol will be used as additional criterion for the classification of the beta receptors in each tissue, as suggested by the study with the agonists. Under proper experimental conditions, dissimilar isomeric potency differences in each tissue for a particular response should be a sensitive indicator of different receptors (Patil, 1969). In addition, the use of optical isomers should control pharmacokinetic factors which influence access of the drugs to the receptor. The specific experimental goals may be outlined as follows:

1) To establish the optimal experimental conditions for the classification of the beta adrenergic receptors.
2) To determine relative potencies of (-)-isoproterenol, (-)-soterenol, (±)-sulfonterol and (-)-H 80/62.

3) To determine isomeric-potency-difference of (-) and (+) isomers of isoproterenol.

Under proper experimental conditions, if beta receptors mediating antianaphylactic responses in the heart and lung are similar, the relative potencies of the agonists and the isomeric-potency-difference of isoproterenol are expected to be similar.

METHODS

Preparation of Isolated Tissues

Female albino guinea pigs (Bio-Labs, St. Paul, Minn.; Charles River, Wilmington, Mass.; CAMM, Wayne, New Jersey; O'Brien Farms, Madison, WI) weighing 300-400 grams were actively sensitized with ovalbumin (10 mg/Kg, i.p.) on days 1,3, and 5. Beginning 21 days after the last injection, animals were killed by a blow to the head and the lungs and hearts were removed. The lungs were trimmed of large blood vessels and bronchi, and the hearts were cleaned of excess connective tissue. The tissues were then minced finely with scissors. Minced lung tissues from three animals and heart tissues from four animals were pooled and divided into 20 samples of 400 and 250 mg wet weight each, respectively. The tissues were incubated in test-tubes containing 5 ml. of a physiological salt solution of the following composition: NaCl, 118mM; KCl, 4.7mM; $CaCl_2 \cdot 2H_2O$, 2.5mM; $MgCl_2 \cdot 6H_2O$, 0.5mM; $NaH_2PO_4 \cdot H_2O$, 1mM; $NaHCO_3$, 25mM; and glucose, 11mM. The solution bathing the tissue and the stock salt solution were gassed with a mixture of oxygen (95%) and carbon dioxide (5%) and maintained at 37° to 38° C in a Dubnoff metabolic incubation without shaking. The samples were equilibiated for 90 minutes and washings were made at 30 minute intervals by

aspiration and replacement with fresh physiological salt solution.

Histamine Release from Minced Lung and Heart

Dose-response curves for antigen-induced histamine release were obtained in a non-cumulative fashion by incubating each sample with a single concentration of ovalbumin. Of the 20 samples used in each experiment, two were used to determine the degree of spontaneous histamine release (without antigen) and the remaining samples were divided equally into 3 sets of 6 samples so that three ovalbumin dose-response curves could be obtained. One set served as control, while the other two sets each received a single concentration of a beta agonist. The beta agonist was allowed to incubate with the tissue samples for a fixed period of time prior to adding ovalbumin. This time of incubation represented the time at which maximum inhibition of ovalbumin-induced histamine release was obtained with the beta agonist. It was determined by challenging the tissues with a single submaximal concentration of ovalbumin in the presence of a high concentration of the beta agonist. The histamine release obtained in the samples exposed to ovalbumin were corrected by the average spontaneous histamine release determined in each experiment. None of the drug treatments employed in the study altered the spontaneous

histamine release. The maximum spontaneous histamine release in the heart and lung were 3% and 2%, respectively.

When optical isomers of isoproterenol were studied, four ovalbumin dose response curves consisting of 5 samples, were obtained in each experiment. Of these curves, one served as control, one received a single concentration of the (-)-isoproterenol, and the remaining two each received a single concentration of the (+)-isoproterenol. In initial experiments, the spontaneous histamine release in the heart and lung were not altered by the presence of the isomers of isoproterenol. Therefore, the spontaneous histamine release were not determined in subsequent experiments. Instead, the histamine release obtained in all samples exposed to ovalbumin were corrected by the average spontaneous histamine release determined from previous experiments with (-)-isoproterenol in each tissue. The average spontaneous histamine from the heart and lung were 2.0± 0.1% (n=17) and 1.1± 0.1% (n=20), respectively. All drugs were added to the samples in 50 µl volumes.

After a fixed exposure time to ovalbumin, the reaction was terminated by placing the test-tubes in an ice bath for 5 minutes. They were then centrifuged for 5 minutes at 4,000 g in a cold room (at 0 to 4 C) to remove the tissue particles. Histamine contents in

the supernatent and pellet were then determined fluorometrically.

Experimental Protocol

Since various factors are known to influence observed effects of the beta adrenergic receptor agonists (Furchgott, 1967; 1970; 1972), it is desirable to impede these processes with appropriate inhibitors. In all experiments, phenoxybenzamine, 10^{-5}M, was present for 45 minutes prior to addition of the beta agonist. Since release of endogenous norepinehprine, as produced by phenoxybenzamine, may influence observed effects of direct-acting agonists (Trendelenburg, 1968), tissues were taken from guinea-pigs which had been pretreated with reserpine (5 mg/Kg, i.p.) 16-24 hours previously. Phenoxybenzamine is chosen in this study for several reasons. In addition to irreversible alpha adrenergic receptor blockade (Triggle, 1965), phenoxybenzamine also blocks the adrenergic neuronal membrane uptake mechanism (Furchgott, 1966) as well as the extraneuronal uptake process, and hence, the influence of catechol-0-methyl-transferase on externally applied adrenergic agonists (Eisenfeld et al., 1967).

Several factors which are known to influence immunological release of histamine were examined. Hence, dose-response effects to ovalbumin were obtained in the presence of indomethacin, 10^{-5}M, to inhibit prostaglandin

synthesis, and metiamide, 10^{-4}M, to inhibit histamine H_2 receptors. Indomethacin was added to the stock salt solution at the beginning of the experiment and metiamide was incubated with the samples for 15 minutes before ovalbumin challenge.

In some experiments, the effects of several inhibitors of histamine metabolism on the release of histamine were examined. Dose-response effects to ovalbumin were obtained in the presence of: 1) aminoguanidine, 10^{-4}M, a histaminase (diamine oxidase) inhibitor; 2) iproniazid, 10^{-4}M, a monoamine oxidase inhibitor; 3) quinacrine, 10^{-4}M, a N-methyl-transferase inhibitor; 4) a combination of the three inhibitors. The time of incubation with these inhibitors was one hour prior to ovalbumin challenge. None of the inhibitors altered the ovalbumin effects and therefore were not employed in subsequent experiments (see Table 21).

Fluorometric Determination of Histamine

Measurement of histamine content in the supernatent and tissue pellets were determined by the fluorometric procedure described by Shore et al. (1959). The method involved the extraction of histamine into n-butanol from alkalinized perchloric acid tissue extracts and return of the histamine to an aqueous solution. Subsequent

condensation of histamine with o-phthalaldehyde (OPT) at a highly alkaline PH yields a product which upon acidification produces a stable and strong fluorescence that is measured in a spectrofluometer. Histamine concentration as low as 10 ng/ml could be assayed.

Purification of Histamine from Tissue Samples

The tissues were transferred to a glass homogenizer containing 2 ml of 0.4N perchloric acid before being homogenized. They were then centrifuged for 10 minutes at 6,500 g. After centrifugation, 0.5 ml of the lung supernatant and 1 ml of the heart supernatant was used and diluted to 4 ml with 0.4N perchloric acid in a 40-ml glass-stoppered shaking tube. The tube contained 1.5 grams of solid sodium chloride, 0.5 ml of 5N sodium hydroxide and 10 ml of butyl alcohol. The tube was then shaken for 15 minutes and centrifuged for 5 minutes at 1,350 g. After centrifugation, the aqueous phase was removed by aspiration and replaced with 5 ml of salt-saturated 0.1N NaOH. The tube was shaken again for 5 minutes and centrifuged. An 8 ml-aliquot of the butanol was transferred to another 40-ml glass-stoppered shaking tube containing 4 ml of 0.1N HCL and 15 ml of water-saturated heptane. After being shaken for 10 minutes, the tube was centrifuged and 1.8 ml of the aqueous phase was transferred to a test tube for histamine assay.

Fluorometric Assay of Histamine

To estimate histamine in the acid extract, a 1.8 ml-aliquot was transferred to a test tube and 0.4 ml of 1N NaOH was added followed by 0.1 ml of OPT reagent. The reaction mixture was allowed to stand at room temperature for 4 minutes; 0.2 ml of 3N HCl was then added. The tube was shaken after each addition. The solution was then transferred to a cuvette, and the fluorescence at 450 mμ resulting from activation at 360 mμ was measured in a spectrofluorometer (Perkin-Elmer, MPF-4). The fluorescence intensity is proportional to histamine concentration over a wide range.

The fluorescence of each sample was corrected for by the reagent blank, which was prepared by reversing the order of addition of OPT and 3N HCl. Under these conditions, no reaction of histamine and OPT is possible since the condensation reaction does not proceed in acid. Each fluorometric assay was prepared in duplicate. Standard curves for histamine were determined from solutions treated in the same manner as tissue samples in order to control possible interference by the drug treatment with the fluorometric procedure. In initial experiments, recovery of histamine from the extraction procedure was 68 ± 2% (n=10). This value did not change throughout the entire study, and therefore, the

histamine contents of the samples were not corrected for recovery.

According to Shore et al. (1959), the condensation reaction with OPT is not entirely specific for histamine. Histidine, histamine ester and histidyhistidine will react with OPT to produce fluorophores spectrally similar to that of histamine, but do not interfere since they are not extracted by butanol from an alkaline solution. Ammonia will interfere if present in high concentration, such as in urine and gastric juice, and the use of special extraction procedure is required. Amines such as serotonin, catecholamines do not interfere under assay condition. However, high concentration of spermidine and other contaminants in brain tissues causes interference with the assay.

In order to confirm that interfering substances such as spermidine are not present in the fluorometric assay, the following purification (Shore, 1971) was made on several samples. The final 0.1N-HCl extract was neutralized to PH 6.0 with 0.1N NaOH, diluted to 10 ml with 0.03M phosphate buffer, PH 6.0, and applied to a 0.6 x 4 cm column of Cellex-P cation exchange resin (Bio-Rad Laboratories). The column was washed with 5 ml of water and the histamine eluted with 5 ml of 0.2M NaCl. Histamine

was then condensed with OPT and assayed fluorometrically. In this manner, the fluorescence of the samples was completely recovered in 0.2M NaCl and, thus the purification step was not employed in subsequent studies.

Analyses

In both heart and lung tissues, the effect of each concentration of ovalbumin was calculated as a percentage of histamine released from the tissues. The amount of histamine present in the supernatant was expressed as a percentage of the total histamine content in each sample (supernatant plus pellet). These values were then converted to a percentage of the maximum percent released and the ED_{50} for ovalbumin was determined visually by plotting percent maximum response vs. log concentration. All ED_{50} values were converted to negative log values ($-\log ED_{50}$) and the standard error of the mean (S.E.M.) calculated from values obtained in each series of experiment. Differences between two means were determined by Student's paired t tests.

Dose-response curves for beta agonists in inhibiting antigen-induced histamine release in each tissue were calculated by the three following methods:

I) Dose ratio, where the dose ratio is the antilog of [(-log ED_{50} ovalbumin without beta agonist) - (-log ED_{50} ovalbumin with beta agonist)].

II) % inhibition, where % inhibition is calculated as (1 - maximum histamine release with beta agonist/maximum histamine release without beta agonist) x 100%.

III) Dose ratio/% of control maximum response.

The response to each concentration of the agonist was calculated as a percentage of the maximum response produced by that agonist. The ED_{50} value and standard error of the mean (S.E.M.) were calculated by the probit analysis described by Miller and Tainter (1944). All ED_{50} values were converted to negative log units (-log ED_{50}). The relative potency value was calculated as the reciprocal of the potency ratio of the beta agonist to isoproterenol.

Potency differences between the (-)- and (+)- isomers of isoproterenol were determined as the difference between their negative log molar ED_{50} values. The S.E.M. for isomeric-potency-differences were calculated as $[(\text{S.E.M. of (+)- isomer})^2 + (\text{S.E.M. of (-)- isomer})^2]^{1/2}$ for unpaired data.

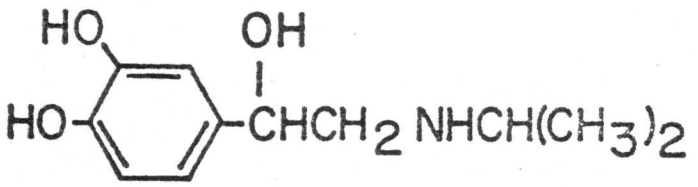
Drugs and Solutions

Chemical structures of the beta receptor agonists used in this study are shown in figure 1. All drug solutions were prepared on the day of each experiment and kept refrigerated until shortly before use. Dilutions of the enantiomers of isoproterenol were made from 10^{-2}M or higher refrigerated stock solutions prepared in 0.9% saline with 0.05% sodium metabisulphite to minimize spontaneous oxidation. Dilutions of ovalbumin were made from a 10 mg/ml solution prepared daily with 0.9% saline. Other drug solutions were prepared with 0.9% saline and final bath concentration are expressed in molar strengths. Indomethacin was dissolved in 2 ml of 95% ethanol and 0.2 ml was removed and added into one liter of physiological salt solution at the beginning of the experiment. Reserpine was prepared by dissolving 250 mg reserpine and 250 mg anhydrous citric acid in 2 ml benzyl alcohol in a 100-ml volumetric flask. This was followed by adding 10 ml of polysorbate '80' and deionized water to make up to 100 ml. Reserpine was stored in brown bottles and kept refrigerated before use (Martindale: The Extra Pharmacopoeia, 1977).

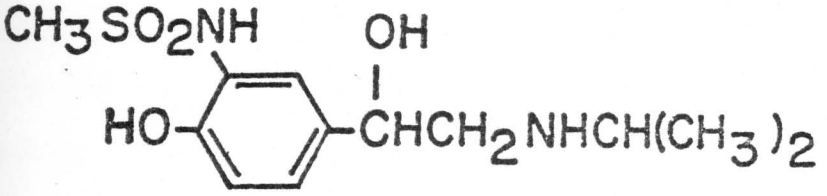
The following drugs were used: (-)-isoproterenol -(+)-bitartrate (Sigma Chemical Company, St. Louis, MO); (-)-soterenol hydrochloride (Mead Johnson & Company,

FIGURE 1

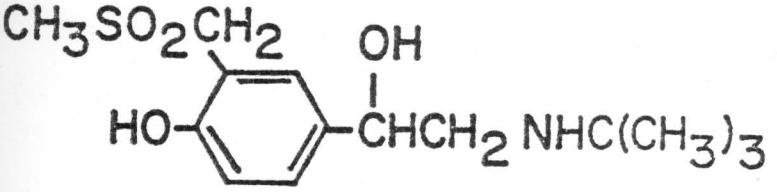
Chemical structures of the beta receptor agonists used in the present experiments.



ISOPROTERENOL



SOTERENOL



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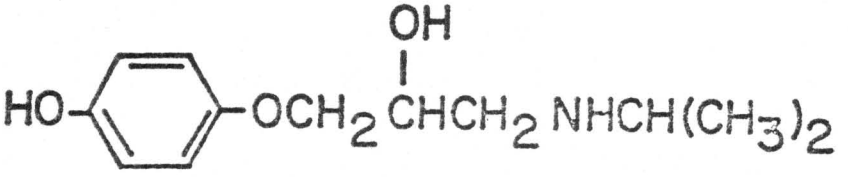


H 80/62

Evansville, IN.); (±)-sulfonterenol hydrochloride (Smith, Kline and French Laboratories, Philadelphia, Pa.); (-)-H 80/62 (AB Hassle, Goteborg, Sweden); (+)-isoproterenol - (+)-bitartrate; phenoxybenzamine HCl, metiamide (Smith, Kline and French Laboratories, Philadelphia, Pa.); aminophylline (Merck & Company, Inc., Rahway, N.J.); egg albumin - Grade V (ovalbumin), reserpine, indomethacin, aminoguamide hemi-sulphate, quinacrine dihydrochloride and iproniazid phosphate (Sigma Chemical Company, St. Louis, Mo.).

The signs (-) and (+) refer to the direction of rotation of polarized light, levo and dextro, respectively. The sign (±) refers to the racemic mixture.

RESULTS

Determination of Optimal Experimental Conditions

Histamine release from guinea-pig minced lung after ovalbumin challenge was apparent within 5 minutes. It reached the maximum in 15 minutes and remained elevated for at least 30 minutes. The time course of histamine release induced by a single concentration of ovalbumin is illustrated in figure 2. The time to peak response remained the same regardless of ovalbumin concentrations and drug treatments (Table 1). Hence, 15 minutes was selected to obtain the ovalbumin dose-response effects.

Figure 3 illustrates the time course of histamine release induced by a single concentration of ovalbumin in guinea-pig minced heart. Maximal release of histamine occurred at the same time regardless of the experimental conditions (Table 2) and a 5 minute incubation time was chosen to obtain the dose-response curves to ovalbumin in the guinea-pig heart.

The dose-response curves of ovalbumin in guinea-pig minced lung and heart were not altered by pretreating the animals with reserpine, or in the presence of phenoxybenzamine in reserpine-pretreated animals (Tables 3 and 4). Furthermore, the ovalbumin dose-response curve in the lung was not altered in the presence of metiamide, 5×10^{-4}M. Subsequently, in all experiments, dose-response effects of

FIGURE 2

Time-response curves for increases in histamine release from guinea-pig minced lung induced by ovalbumin, 1 mg/ml. Curves represent tissues obtained from ovalbumin-sensitized animals (●); reserpine-pretreated, ovalbumin-sensitized animals (■); reserpine-pretreated, ovalbumin-sensitized animals, in the presence of phenoxybenzamine and (▲) reserpine-pretreated, ovalbumin-sensitized animals, in the presence of indomethacin and phenoxybenzamine (◆). Vertical lines indicate S.E.M. Each point represents the mean of two to four experiments.

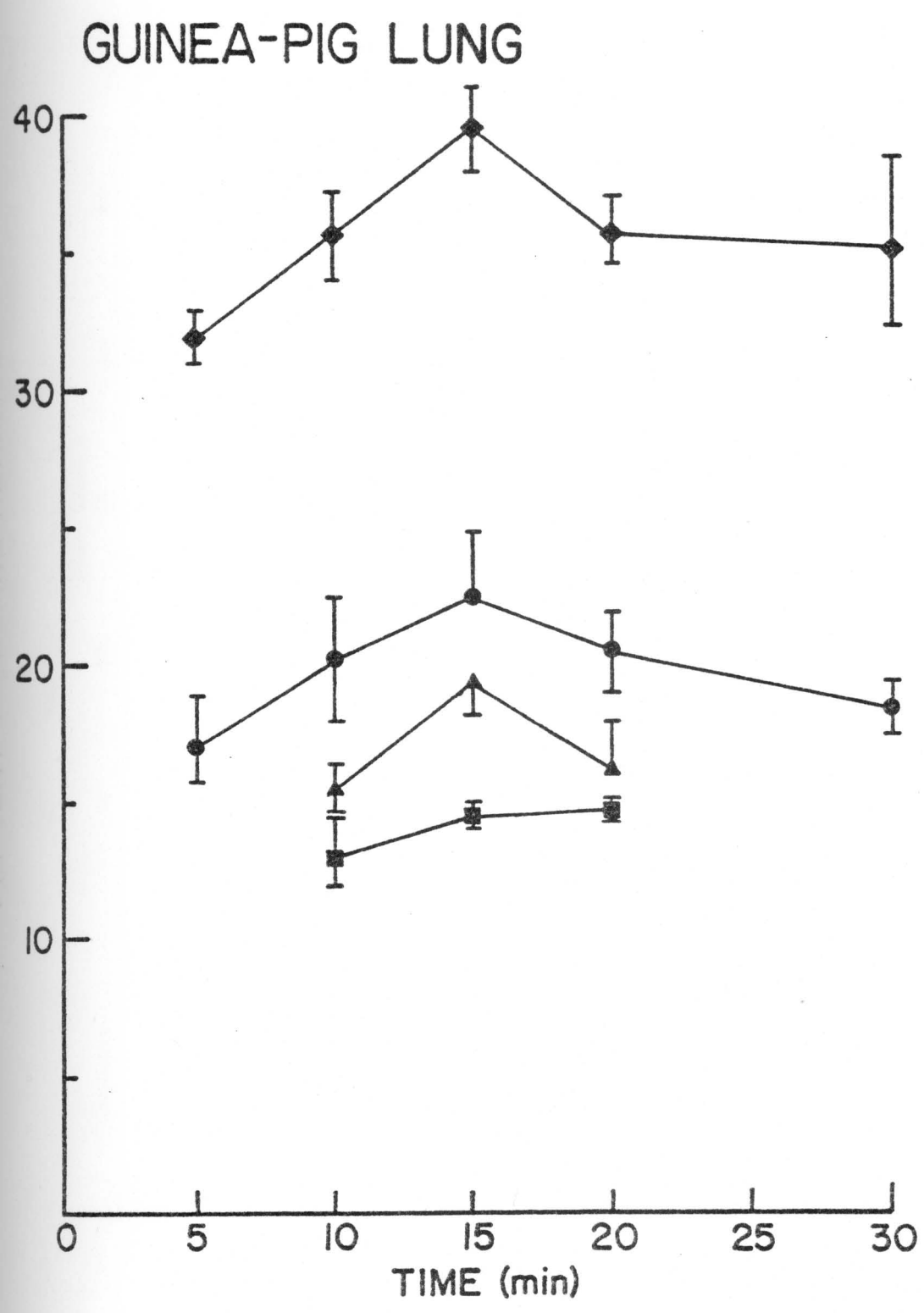

GUINEA-PIG LUNG
40
30
20
10
0
5
10
15
20
25
30
TIME (min)

Table 1 - Time Response of Ovalbumin-Induced Histamine Release from Guinea-Pig Minced Lung.

Treatment	Per Cent Histamine Release[a] Time (Min.) 5	10	15	20	30
Control[b]	17 ± 2	20 ± 2	23 ± 2	21 ± 2	19 ± 1
Control[c]	-	4 ± 4	5 ± 1	4 ± 1	4 ± 2
Reserpine[b,d]	-	13 ± 1	15 ± .3	15 ± .2	-
Phenoxybenzamine[b,d] 10^{-5} M	-	16 ± 1	19 ± 1	16 ± 2	-
Indomethacin[b,e] 10^{-5} M	32 ± 1	36 ± 1	40 ± 2	36 ± 2	35 ± 3
Indomethacin[b,e] 10^{-5} + Metiamide $5x10^{-5}$ M	25 ± 1	32 ± 2	37 ± 3	34 ± 2	34 ± 4

[a] Mean ± S.E.M. of 2-4 observations for each time of incubation.

[b] Tissues were taken from ovalbumin-sensitized animals and challenged with ovalbumin, 1 mg/ml.

[c] Tissues were taken from ovalbumin-sensitized animals and challenged with ovalbumin, 10^{-4} mg/ml.

[d] Tissues were taken from ovalbumin-sensitized animals pretreated with reserpine (5 mg/Kg i.p.) 16-24 hours previously.

[e] Tissues were taken from reserpine-pretreated, ovalbumin sensitized animals and exposed to phenoxybenzamine.

FIGURE 3

Time-response curves for increases in histamine release from guinea-pig minced heart induced by ovalbumin, 10^{-1} mg/ml. Curves represent tissues obtained from ovalbumin-sensitized animals (●); reserpine-pretreated, ovalbumin-sensitized animals (■) and reserpine-pretreated, ovalbumin-sensitized animals, in the presence of indomethacin and phenoxybenzamine (◆). Vertical lines indicate S.E.M. Each point represents the mean of two to three experiments.

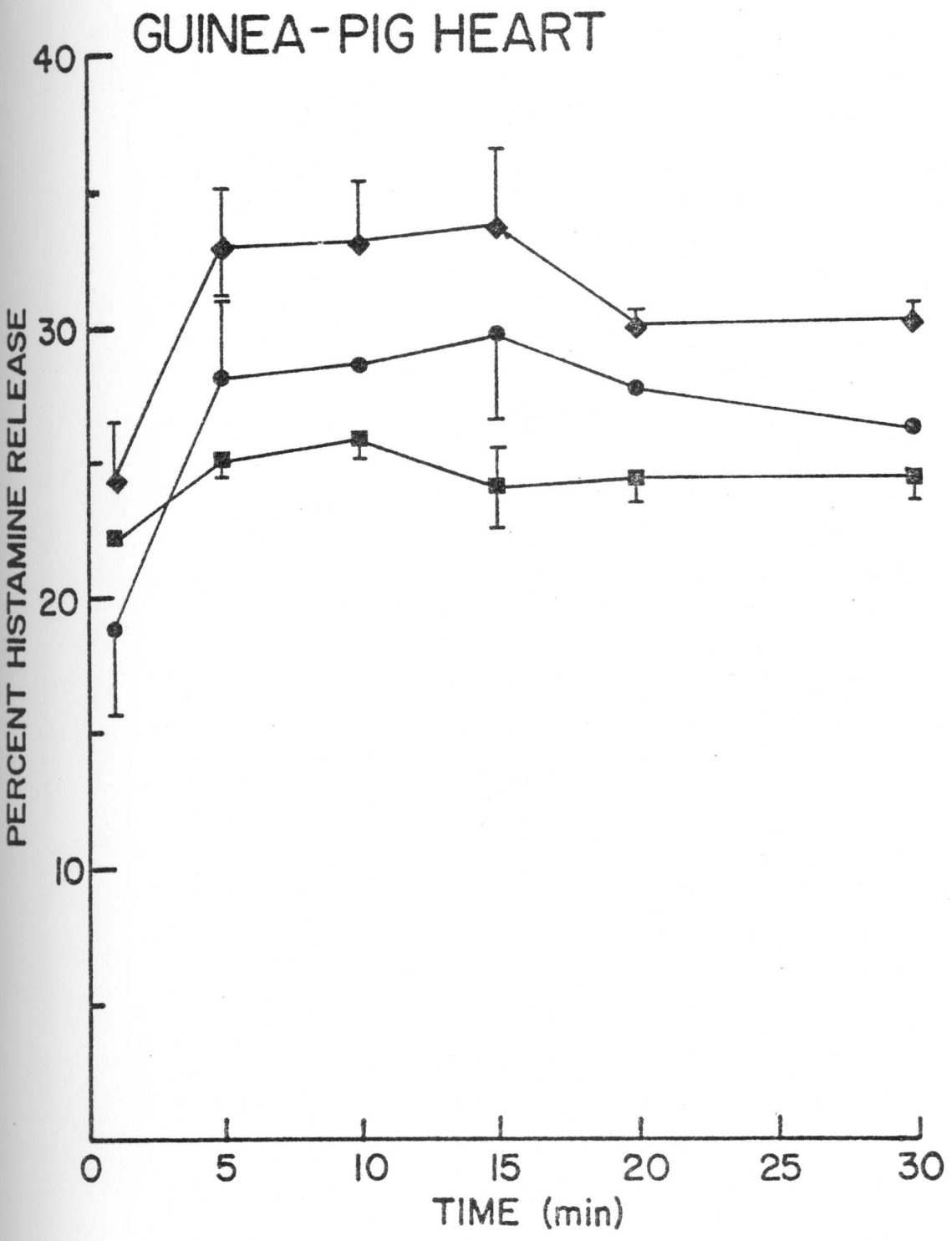

GUINEA-PIG HEART
PERCENT HISTAMINE RELEASE
40
30
20
10
0
5
10
15
20
25
30
TIME (min)

Table 2 - Time Response of Ovalbumin-Induced Histamine Release from Guinea-Pig Minced Heart.

Treatment	Per Cent Histamine Release[a] Time (Min.) 1	5	10	15	20	30
Control[b]	19 ± 3	28 ± 3	29 ± 5	30 ± 3	28 ± 2	26 ± 4
Reserpine[b,c]	22 ± 1	25 ± 1	26 ± 1	24 ± 1	24 ± 1	24 ± 1
Phenoxybenzamine[b,c] 10^{-5} M	26 ± 6	28 ± 6	27 ± 2	31 ± 7	32 ± 7	30 ± 5
Indomethacin[b,d] 10^{-5} M	24 ± 2	33 ± 2	33 ± 2	34 ± 3	30 ±.2	30 ±.3

[a] Mean ± S.E.M. of 2-3 observations for each time of incubation.

[b] Tissues were taken from ovalbumin-sensitized animals and challenged with ovalbumin, 10^{-1} mg/ml.

[c] Tissues were taken from ovalbumin-sensitized animals pretreated with reserpine (5 mg/Kg i.p.) 16-24 hours previously.

[d] Tissues were taken from reserpine-pretreated, ovalbumin sensitized animals and exposed to phenoxybenzamine.

ovalbumin were examined in the presence of phenoxybenzamine in reserpine-pretreated animals (see "Methods").

Incubation of the lung tissues with indomethacin, 10^{-5}M, resulted in a shift to the left of the ovalbumin dose-response curve and a substantial enhancement of the maximum response to ovalbumin (figure 4, Table 3). The potentiation of the ovalbumin effects by indomethacin may be due to its ability to inhibit prostaglandin synthesis and, hence, the modulation of prostaglandins on antigen-induced histamine release (Tauber et al., 1973). Therefore, we examined dose-response effects of ovalbumin in all subsequent experiments in the presence of indomethacin.

In contrast to the guinea-pig lung, however, indomethacin, 10^{-5}M, produced no significant effect on the ovalbumin dose-response curve in the heart (figure 5, Table 4). The results suggest that the modulatory role of prostaglandin on histamine release in the heart is minor in comparison to that in the lung. Nevertheless, we included indomethacin in all subsequent experiments in the heart so that identical experimental conditions were provided in both tissues for comparing the effects of the beta receptor agonists on histamine release.

In the presence of indomethacin, the -log ED50 values for ovalbumin in guinea-pig lung and heart were 3.12 ± .05 (n=41) and 3.73 ± .05 (n=21), respectively; whereas in the

FIGURE 4

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced lung in the absence and presence of indomethacin. All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to phenoxybenzamine. The data taken from these curves are summarized in Table 3. Each curve represents the mean of 2 experiments. Vertical lines indicate S.E.M.

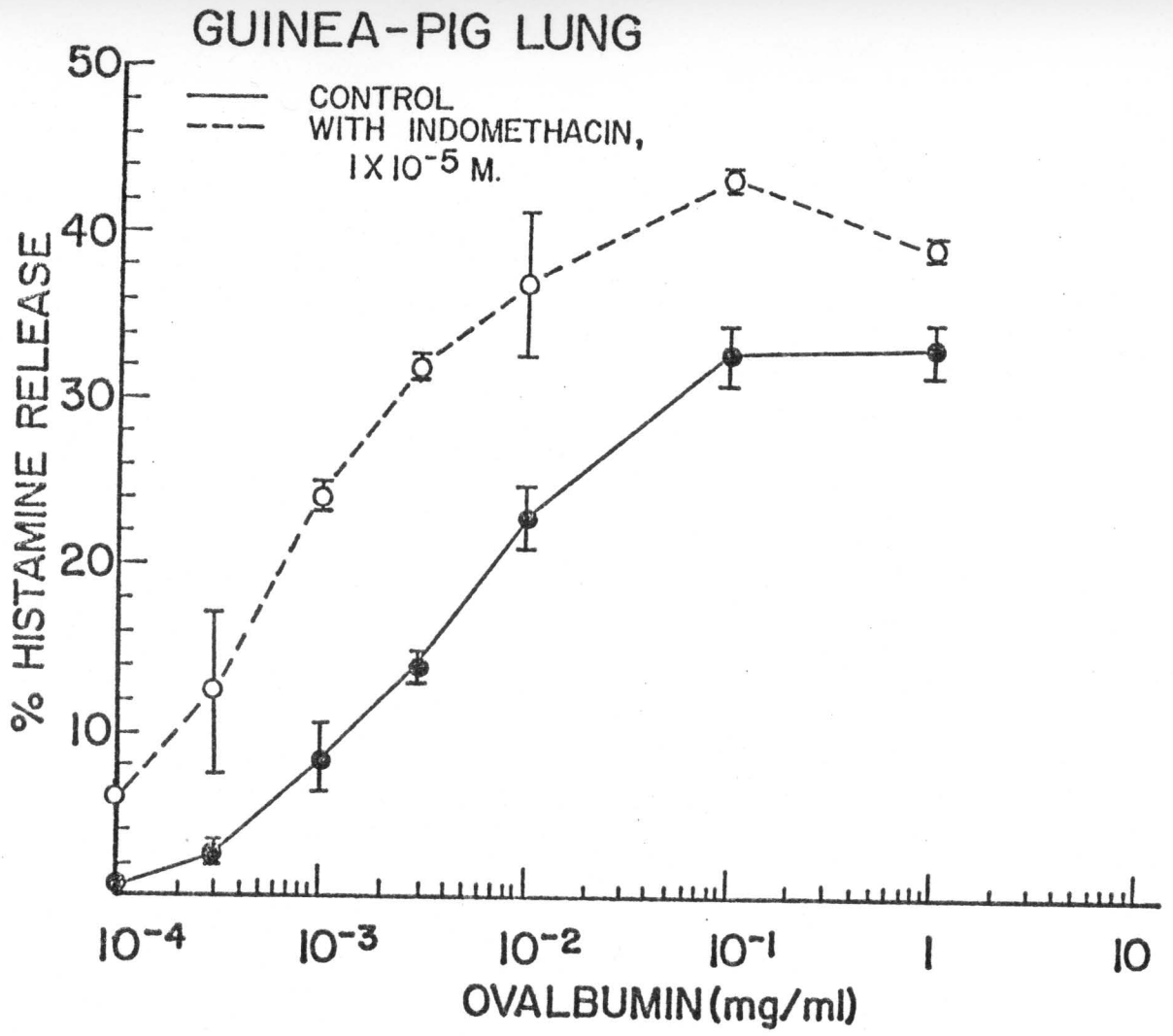

GUINEA-PIG LUNG
CONTROL
WITH INDOMETHACIN,
1X10-5 M.
% HISTAMINE RELEASE
50
40
30
20
10
10-4
10-3
10-2
10-1
1
10
OVALBUMIN (mg/ml)

Table 3 - Effects of Drug Treatments on Ovalbumin-Induced Histamine Release from Guinia Pig Minced Lung.

Treatment	Ovalbumin[a] -Log ED50 With S.E.M. Control	Ovalbumin[a] -Log ED50 With S.E.M. Treated	p[c]	Maximum % Histamine Release[b] With S.E.M. Control	Maximum % Histamine Release[b] With S.E.M. Treated	p[c]	n[d]
Reserpine[e]	2.57 ± .05	2.80 ± .27	>0.05	25 ± 2	24 ± 1	>0.05	2
Phenoxybenzamine[e] 10^{-5} M	2.52	2.55	>0.05	24	28	>0.05	1
Indomethacin[f] 10^{-5} M	2.41 ± .02	3.14 ± .08	<0.05	35 ± 3	43 ± 2	<0.01	2
Indomethacin, 10^{-5} M[f] + Metiamide, $5x10^{-4}$ M	3.05	3.09	>0.05	32	33	>0.05	1
Metiamide[g] $5x10^{-4}$ M	2.45 ± .23	2.05 ± .21	>0.05	30 ± 3	30 ± 6	>0.05	2

[a] Calculated from curves plotting % of maximum response in each curve.

[b] Maximum amount of histamine release relative to total histamine content.

[c] Significance of control vs. treated values.

[d] Number of observations.

[e] Tissues were taken from ovalbumin-sensitized animals pretreated with reserpine (5 mg/Kg i.p.) 16 to 24 hours previously.

[f] Tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to phenoxybenzamine.

[g] Tissues were taken from ovalbumin-sensitized animals.

FIGURE 5

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced heart in the absence and presence of indomethacin. All tissues are obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to phenoxybenzamine. The data taken from these curves are summarized in Table 3. Each curve represents the mean of 2 experiments. Vertical lines indicate S.E.M.

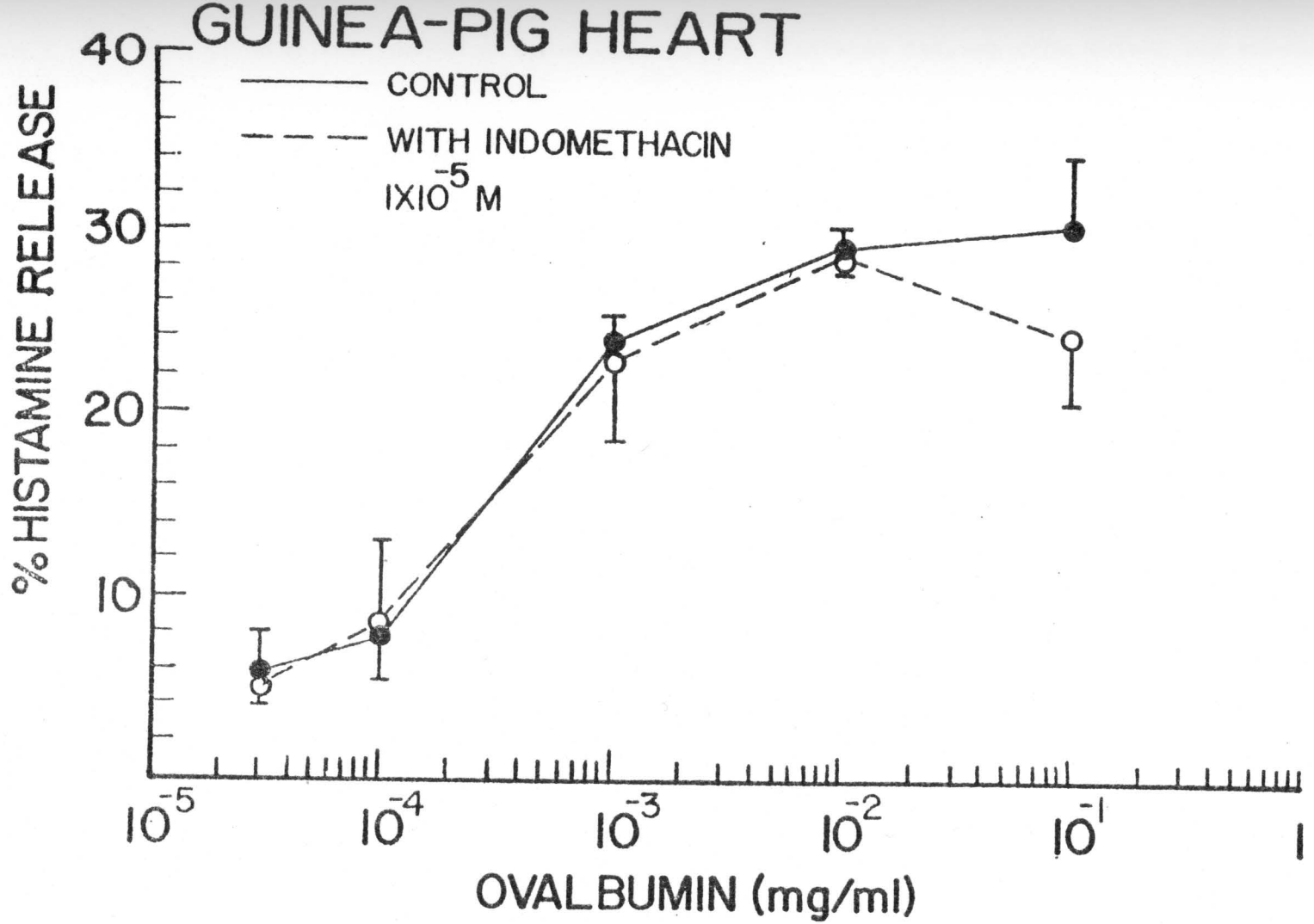

GUINEA-PIG HEART
CONTROL
WITH INDOMETHACIN
IXIO⁻⁵ M
% HISTAMINE RELEASE
40
30
20
10
10⁻⁵
10⁻⁴
10⁻³
10⁻²
10⁻¹
1
OVALBUMIN (mg/ml)

Table 4 - Effects of Drug Treatments on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.

Treatment	Ovalbumin -Log ED50[a] With S.E.M. Control	Ovalbumin -Log ED50[a] With S.E.M. Treated	p[c]	Maximum % Histamine Release[b] With S.E.M. Control	Maximum % Histamine Release[b] With S.E.M. Treated	p[c]	n[d]
Reserpine[e]	3.96 ± .14	3.76 ± .31	>.05	28 ± 7	29 ± 6	>.05	2
Phenoxybenzamine[e] 10^{-5}M	3.76 ± .31	3.51 ± .06	>.05	29 ± 6	32 ± 2	>.05	2
Indomethacin[f] 10^{-5}M	3.51 ± .06	3.59 ± .28	>.05	32 ± 2	29 ± 2	>.05	2

[a] Calculated from curves plotting % of maximum response in each curve.

[b] Maximum amount of histamine release relative to total histamine content.

[c] Significance of control vs. treated values.

[d] Number of observations.

[e] Tissues were taken from ovalbumin-sensitized animals pretreated with reserpine (5 mg/Kg i.p.) 16 to 24 hours previously.

[f] Tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to phenoxybenazmine.

absence of indomethacin, the -log ED50 values were 2.41 ± .02 (n=2) and 3.51 ± .06 (n=2), respectively. Therefore, there is a 4.1 fold difference in concentrations of ovalbumin to induce histamine release from guinea-pig lung and heart in the presence of indometachin; but a 12.2 fold difference in ovalbumin concentrations in its absence.

The maximum histamine release induced by ovalbumin in guinea-pig lung and heart were 43 ± 1% (n=41) and 29 ± 1% (n=25), respectively, in the presence of indomethacin; but were 35 ± .03% (n=2) and 32 ± 2% (n=2), respectively, in its absence. Concentrations of ovalbumin larger than 1 mg/ml in the lung and 10^{-1} mg/ml in the heart did not produce additional release regardless of experimental conditions. The histamine contents of the control, ovalbumin-sensitized guinea-pig lung and heart were 29 ± 3 µg/g (n=12) and 7.2 ± 0.8 µg/g (n=5) wet weight, respectively. These values were not altered by drug treatments (Table 20).

Figure 6 illustrates time-response curves for inhibition of ovalbumin-induced histamine release produced by selected concentrations of the beta receptor agonists in guinea-pig lung. Maximum inhibition of histamine release was attained at 15 minutes for (-)-isoproterenol,

FIGURE 6

Time-response curves for inhibition by (-)-isoproterenol, 10^{-5}M; (-)-soterenol, 10^{-4}M; (±)-sulfonterol, 10^{-4}M and (-)-H 80/62, 10^{-3}M, on histamine release from guinea-pig minced lung induced by ovalbumin, $3x10^{-3}$ mg/ml. All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Vertical lines indicate S.E.M. Each point represents the mean of three to four experiments.

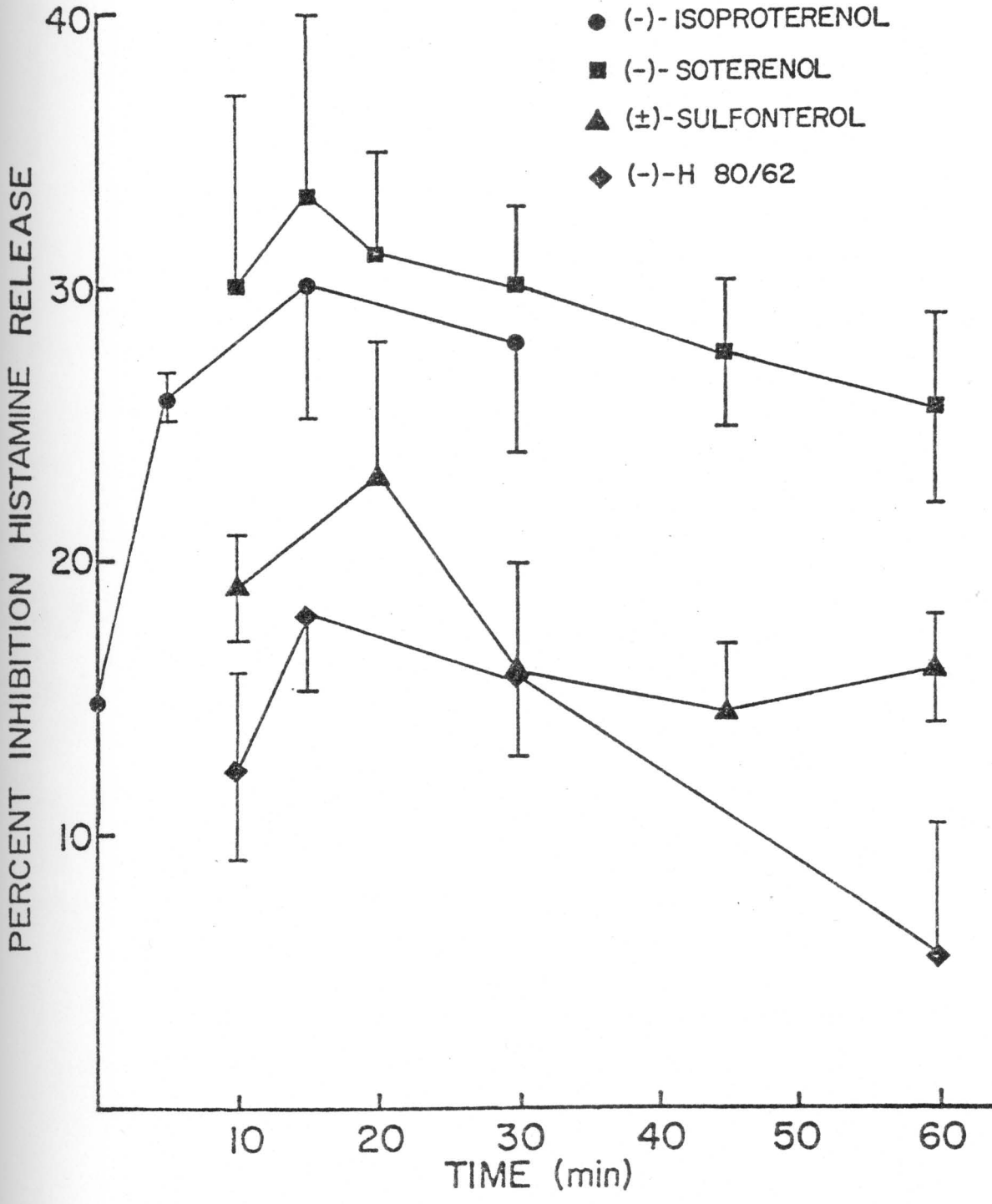

(-)- ISOPROTERENOL
(-)- SOTERENOL
(±)-SULFONTEROL
(-)-H 80/62
PERCENT INHIBITION HISTAMINE RELEASE
40
30
20
10
10
20
30
40
50
60
TIME (min)

Table 5 - Time Response for Inhibition by the Beta Agonists on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

Agonist	Per Cent Inhibition Histamine Release[b,c] Time (Min.) 0	5	10	15	20	30	45	60
(-)-Isoproterenol 10^{-5} M	15 ± 5	26 ± 1	-	30 ± 5	-	28 ± 4	-	-
(-)-Soterenol 10^{-4} M	-	-	30 ± 9	33 ± 7	31 ± 3	30 ± 3	28 ± 3	26 ± 4
(±)-Sulfonterol 10^{-4} M	-	-	19 ± 2	-	23 ± 5	16 ± 4	14 ± 3	16 ± 2
(-)-H 80/62 10^{-3} M	-	-	12 ± 3	18 ± 3	-	16 ± 3	-	6 ± 5

[a] Histamine release induced by ovalbumin (3×10^{-3} mg/ml) in the absence of the beta agonist is 31 ± 2% (n=12).

[b] Reduction of histamine release by the beta agonist is expressed as a percentage of the control histamine release.

[c] Mean ± S.E.M. of 3-4 observations for each time of incubation.

(-)-soterenol and (-)-H 80/62, and 20 minutes for (±)-sulfonterol (see Table 5). These respective time periods were used for incubation of tissue samples with the beta agonists prior to ovalbumin challenge.

Figure 7 illustrates the time-response curves for inhibition of ovalbumin-induced histamine release produced by selected concentrations of the beta receptor agonists in guinea-pig heart. For each agonist, maximum inhibition was attained at 10 minutes (see Table 6), and this time period was chosen for incubation of tissue samples with the beta agonist prior to ovalbumin challenge.

Effects of Beta Receptor Agonists on Histamine Release in Minced Lung.

Dose-response curves for ovalbumin-induced histamine release in guinea-pig minced lung in the absence and presence of different concentrations of (-)-isoproterenol, (-)-soterenol, (±)-sulfonterol and (-)-H 80/62 are illustrated in figures 8, 9, 10 and 11. The data taken from these curves are summarized in Tables 7, 8, 9 and 10.

The dose-response effects of ovalbumin were markedly inhibited by (-)-isoproterenol, a nonselective beta receptor agonist. The inhibition is characterized by a shift of the ovalbumin dose-response curve to the right and reduction of the maximum response. The maximum degree

FIGURE 7

Time-response curves for inhibition by (-)-isoproterenol, 10^{-5}M; (-)-soterenol, 10^{-4}M; (±)-sulfonterol, 10^{-4}M and (-)-H 80/62, 10^{-3}M, of histamine release from guinea-pig minced heart induced by ovalbumin, $3x10^{-4}$ mg/ml. All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Vertical lines indicate S.E.M. Each point represents the mean of four experiments.

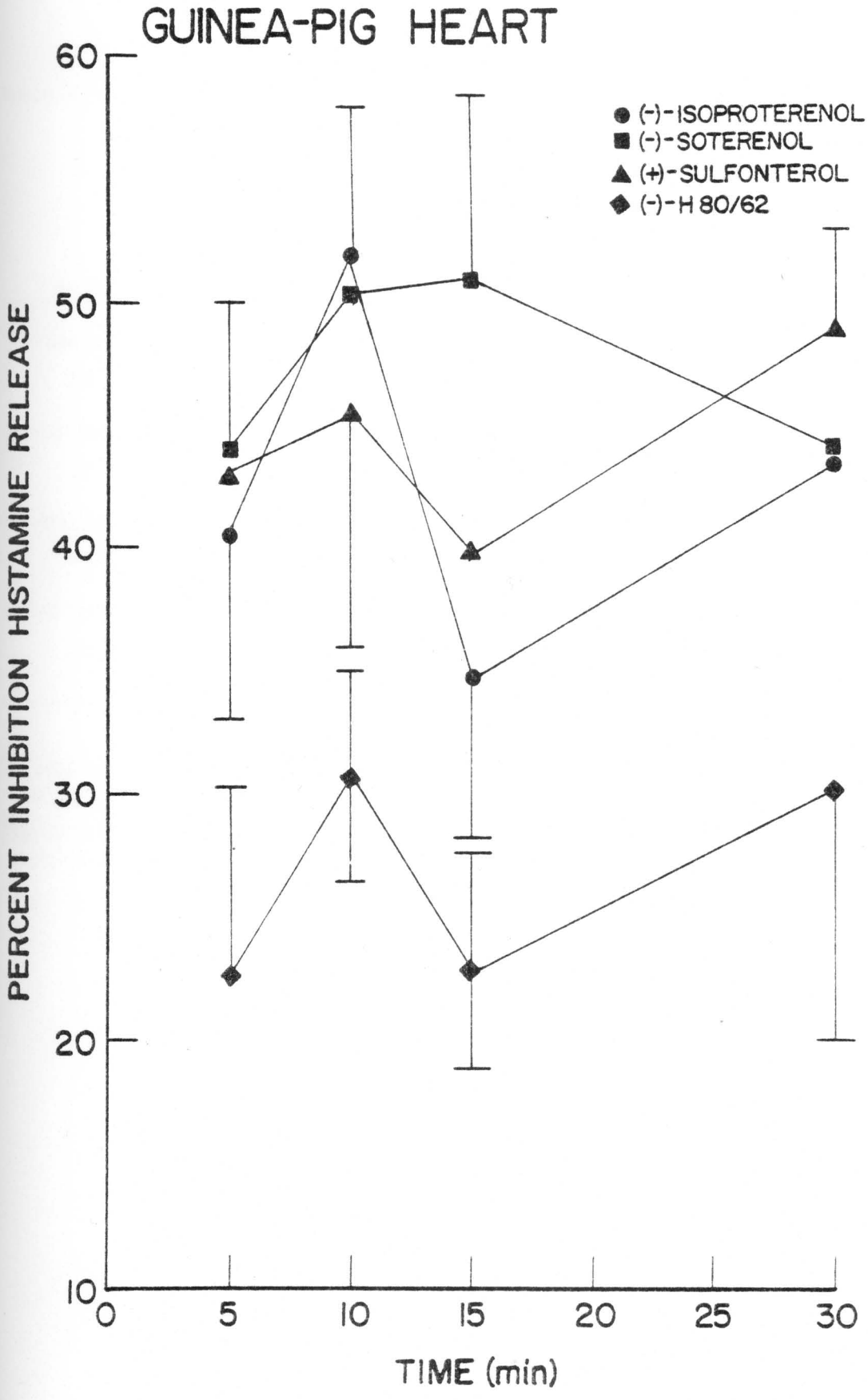

GUINEA-PIG HEART
(-)-ISOPROTERENOL
(-)-SOTERENOL
(+)-SULFONTEROL
(-)-H 80/62
PERCENT INHIBITION HISTAMINE RELEASE
60
50
40
30
20
10
0
5
10
15
20
25
30
TIME (min)

Table 6 - Time Response for Inhibition by the Beta Agonists on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.[a]

Agonist	Per Cent Inhibition Histamine Release[b,c] Time (Min.) 5	10	15	30
(-)-Isoproterenol 10^{-5} M	41 ± 8	52 ± 6	35 ± 6	44 ± 8
(-)-Soterenol 10^{-4} M	44 ± 6	51 ± 10	51 ± 7	44 ± 14
(±)-Sulfonterol 10^{-4} M	43 ± 7	46 ± 10	40 ± 9	49 ± 4
(-)-H 80/62 10^{-3} M	23 ± 8	31 ± 4	23 ± 5	30 ± 10

[a]Histamine release induced by ovalbumin (3×10^{-4} mg/ml) in the absence of the beta agonist is 18 ± 1% (n=16).

[b]Reduction of histamine release by the beta agonist is expressed as a percentage of the control histamine release.

[c]Mean ± S.E.M. is 4 observations for each time of incubation.

FIGURE 8

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced lung in the absence and presence of different concentrations of (-)-isoproterenol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 7. Each curve represents the mean of four to ten experiments. Vertical lines indicate S.E.M.

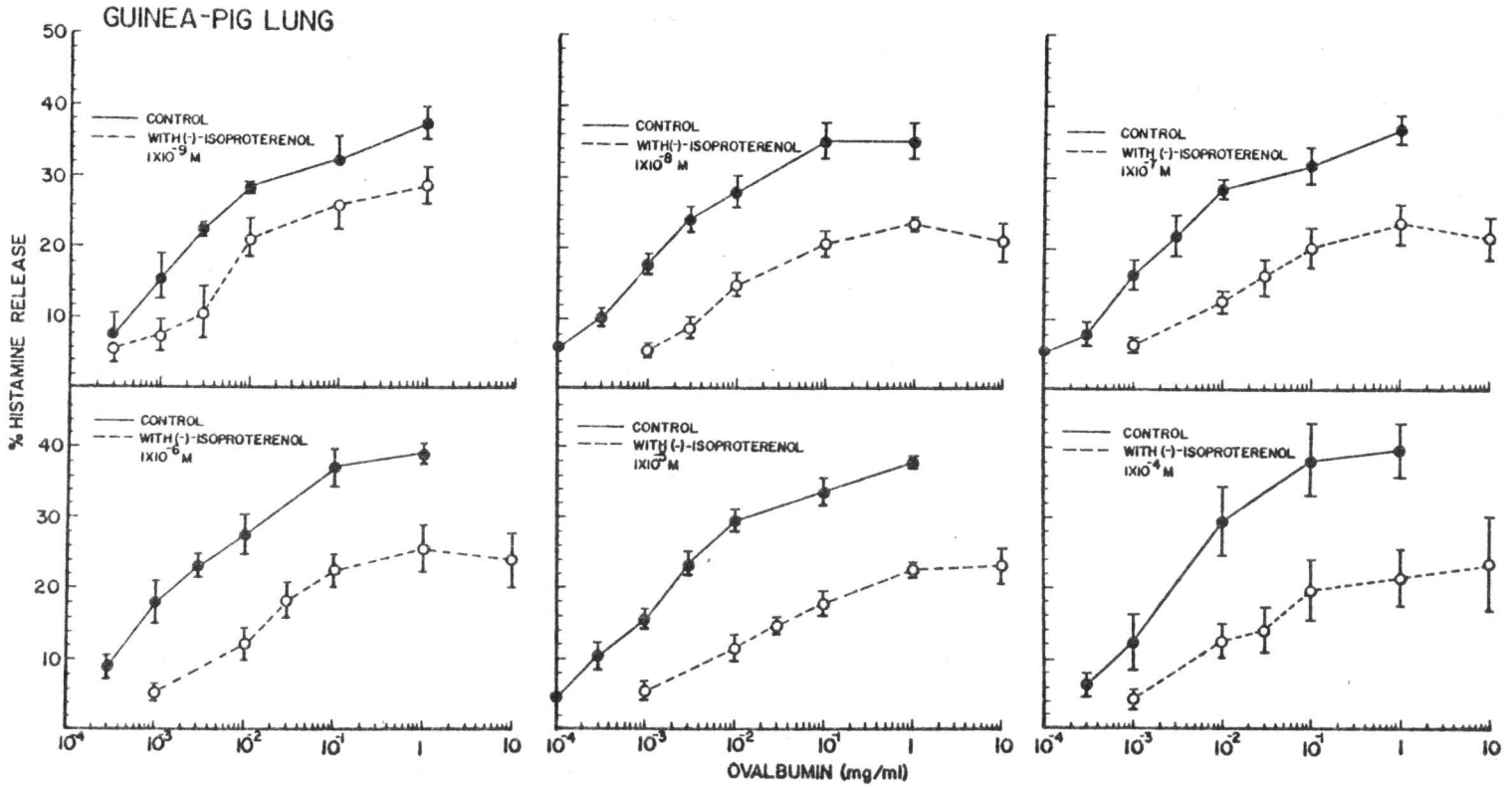

GUINEA-PIG LUNG
% HISTAMINE RELEASE
OVALBUMIN (mg/ml)
CONTROL
WITH (-)-ISOPROTERENOL
1X10⁻⁹ M
1X10⁻⁸ M
1X10⁻⁷ M
1X10⁻⁶ M
1X10⁻⁵ M
1X10⁻⁴ M

Table 7 - Inhibition by (-)-Isoproterenol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

(-)-Isoproterenol Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-9}$	2.82 ± .11	2.34 ± .11	3.1 ± .3	<0.01	38 ± 2	31 ± 3	82 ± 5	>0.05	4
$1x10^{-8}$	2.92 ± .07	2.18 ± .12	6.7 ± 1.5	<0.01	38 ± 2	26 ± 1	67 ± 2	<0.01	10
$1x10^{-7}$	2.85 ± .10	1.99 ± .12	8.1 ± 1.1	<0.01	37 ± 2	25 ± 3	66 ± 6	<0.01	10
$1x10^{-6}$	2.90 ± .08	1.88 ± .11	12.7 ± 3.4	<0.01	41 ± 2	27 ± 3	64 ± 5	<0.01	7
$1x10^{-5}$	2.91 ± .09	1.83 ± .13	14.6 ± 2.9	<0.01	38 ± 1	25 ± 2	66 ± 4	<0.01	10
$1x10^{-4}$	2.63 ± .08	1.73 ± .26	11.2 ± 4.5	<0.05	41 ± 4	27 ± 5	66 ±10	<0.05	4

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Isoporterenol was added to the tissues 15 minutes before ovalbumin challenge.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without isoproterenol) - (-log ED50 with isoproterenol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

FIGURE 9

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced lung in the absence and presence of different concentrations of (-)-soterenol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 8. Each curve represents the mean of four to six experiments. Vertical lines indicate S.E.M.

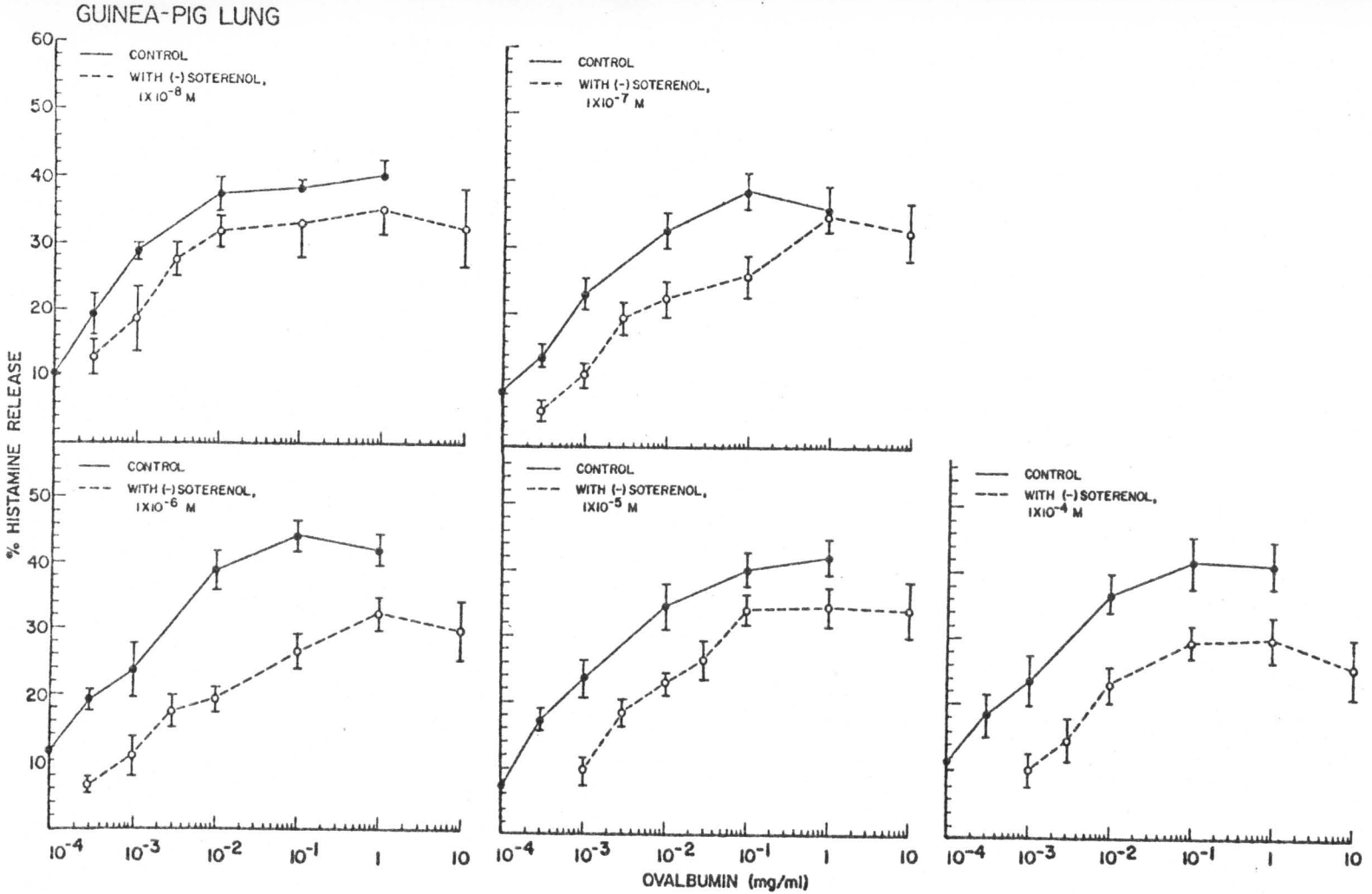

GUINEA-PIG LUNG
CONTROL
WITH (-) SOTERENOL, 1X10⁻⁸ M
CONTROL
WITH (-) SOTERENOL, 1X10⁻⁷ M
CONTROL
WITH (-) SOTERENOL, 1X10⁻⁶ M
CONTROL
WITH (-) SOTERENOL, 1X10⁻⁵ M
CONTROL
WITH (-) SOTERENOL, 1X10⁻⁴ M
% HISTAMINE RELEASE
OVALBUMIN (mg/ml)

Table 8 - Inhibition by (-)-Soterenol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

(-)-Soterenol Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-8}$	3.43 ± .12	3.14 ± .14	2.1 ± .5	>0.05	42 ± 2	37 ± 3	90 ± 8	>0.05	4
$1x10^{-7}$	3.14 ± .08	2.63 ± .09	3.4 ± .4	<0.01	40 ± 3	35 ± 3	90 ± 7	>0.05	6
$1x10^{-6}$	3.30 ± .07	2.58 ± .15	5.7 ± 1.0	<0.01	44 ± 2	34 ± 3	76 ± 6	<0.01	6
$1x10^{-5}$	3.21 ± .07	2.44 ± .11	6.8 ± 1.7	<0.01	43 ± 2	37 ± 3	86 ± 5	<0.05	6
$1x10^{-4}$	3.25 ± .14	2.43 ± .18	7.0 ± .8	<0.01	43 ± 3	34 ± 2	78 ± 4	<0.01	6

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Soterenol was added to the tissues 15 minutes before ovalbumin challenge.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without soterenol) - (-log ED50 with soterenol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

FIGURE 10

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced lung in the absence and presence of different concentrations of (±)-sulfonterol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 9. Each curve represents the mean of three to six experiments. Vertical lines indicate S.E.M.

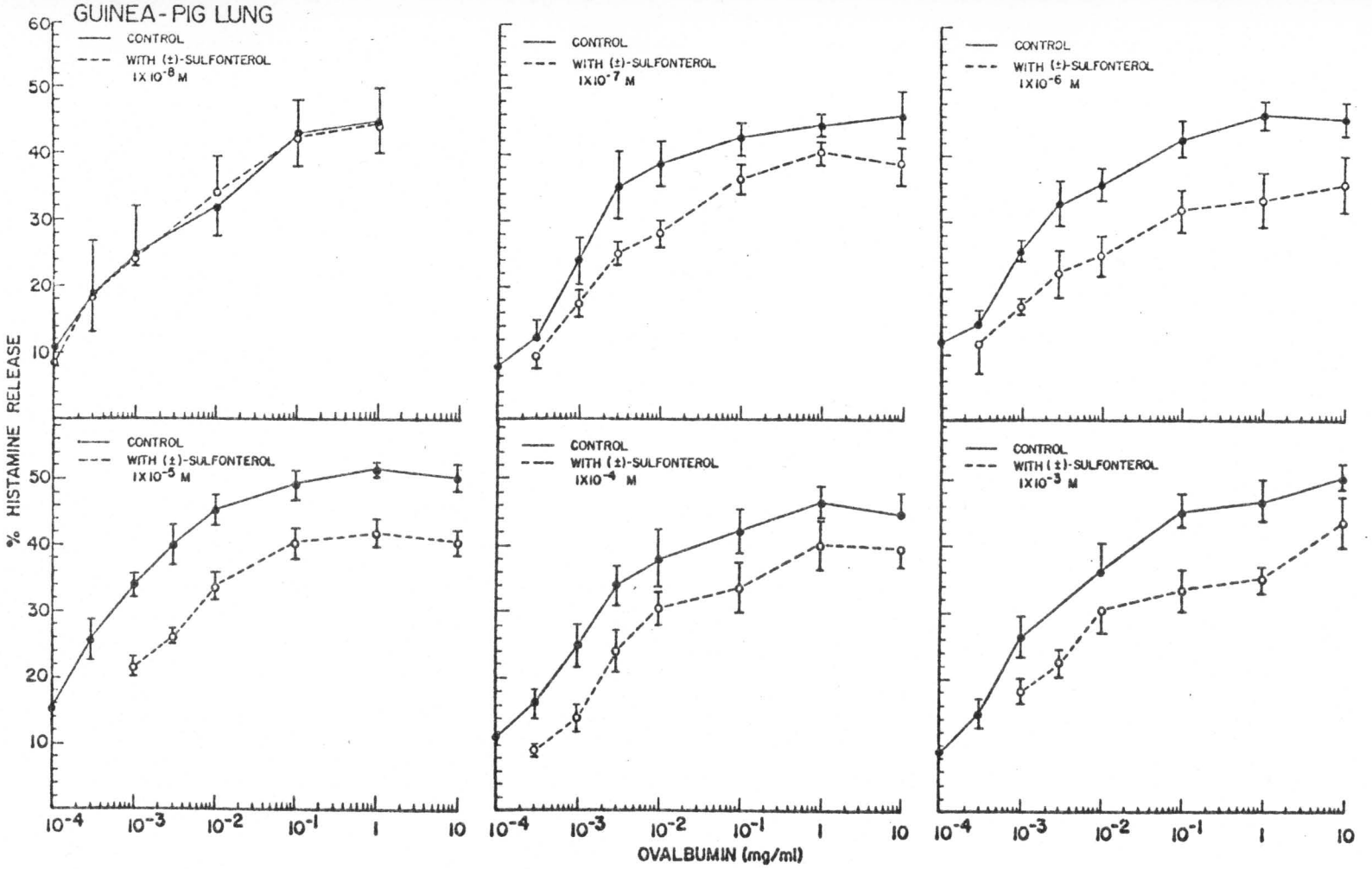

GUINEA-PIG LUNG
CONTROL
WITH (±)-SULFONTEROL
1X10⁻⁸ M
1X10⁻⁷ M
1X10⁻⁶ M
1X10⁻⁵ M
1X10⁻⁴ M
1X10⁻³ M
% HISTAMINE RELEASE
OVALBUMIN (mg/ml)
10⁻⁴
10⁻³
10⁻²
10⁻¹
1
10

Table 9 - Inhibition by (±)-Sulfonterol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

(±)-Sulfonterol Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-8}$	3.33 ± .25	3.17 ± .24	1.5 ± .3	>0.05	45 ± 5	44 ± 4	99 ± 8	>0.05	3
$1x10^{-7}$	3.09 ± .07	2.85 ± .09	1.8 ± .2	<0.01	46 ± 2	40 ± 2	88 ± 2	<0.01	6
$1x10^{-6}$	3.12 ± .07	2.78 ± .11	2.5 ± .5	<0.05	46 ± 2	38 ± 3	81 ± 6	<0.05	6
$1x10^{-5}$	3.48 ± .12	2.96 ± .07	3.6 ± 1.0	<0.01	52 ± 1	44 ± 1	86 ± 2	<0.01	4
$1x10^{-4}$	3.24 ± .06	2.65 ± .07	4.2 ± 1.0	<0.01	47 ± 2	42 ± 4	90 ± 4	<0.05	6
$1x10^{-3}$	3.09 ± .06	2.69 ± .08	3.1 ± 1.2	<0.05	48 ± 2	42 ± 4	88 ± 3	<0.05	5

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Sulfonterol was added to the tissues 20 minutes before ovalbumin challenge.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without sulfonterol) - (-log ED50 with sulfonterol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

FIGURE 11

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced lung in the absence and presence of different concentrations of (-)-H 80/62. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 9. Each curve represents the mean of four to six experiments. Vertical lines indicate S.E.M.

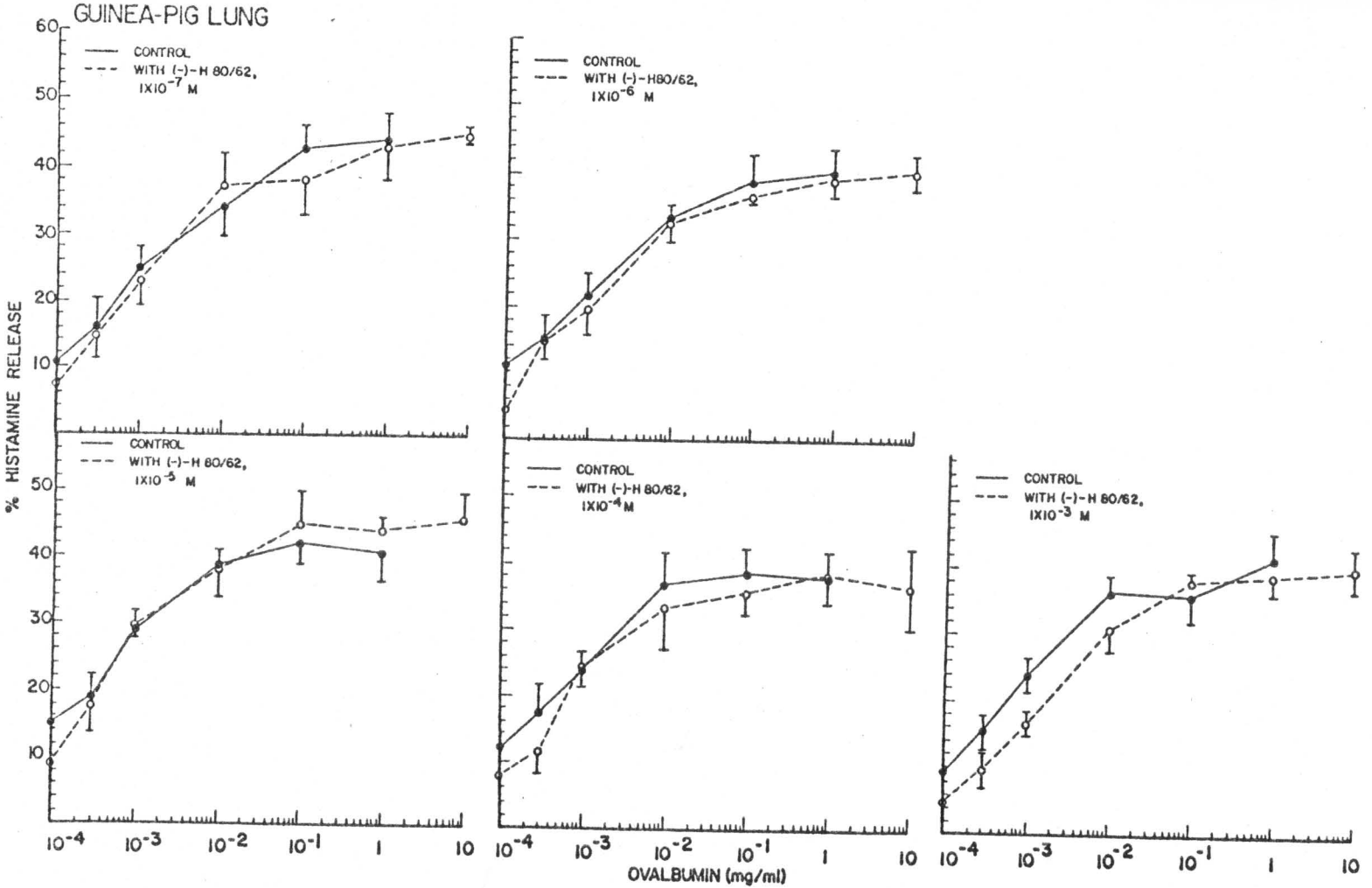

GUINEA-PIG LUNG
% HISTAMINE RELEASE
CONTROL
WITH (-)-H 80/62, 1X10⁻⁷ M
CONTROL
WITH (-)-H80/62, 1X10⁻⁶ M
CONTROL
WITH (-)-H 80/62, 1X10⁻⁵ M
CONTROL
WITH (-)-H 80/62, 1X10⁻⁴M
CONTROL
WITH (-)-H 80/62, 1X10⁻³ M
OVALBUMIN (mg/ml)

Table 10 - Inhibition by (-)-H 80/62 of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

(-)-H 80/62 Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-7}$	3.18 ± .13	3.08 ± .11	1.6 ± .7	>.05	46 ± 4	44 ± 5	95 ± 4	>.05	4
$1x10^{-6}$	3.11 ± .12	3.02 ± .18	1.4 ± .3	>.05	42 ± 4	41 ± 2	101 ± 5	>.05	4
$1x10^{-5}$	3.34 ± .13	3.26 ± .07	1.2 ± .2	>.05	45 ± 3	48 ± 3	107 ± 3	>.05	4
$1x10^{-4}$	3.30 ± .18	3.10 ± .16	1.7 ± .3	>.05	43 ± 3	42 ± 3	99 ± 3	>.05	4
$1x10^{-3}$	3.18 ± .14	2.75 ± .12	2.8 ± .4	<.05	43 ± 4	41 ± 3	97 ± 6	>.05	4

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. H 80/62 was added to the tissues 15 minutes before ovalbumin challence.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without H 80/62) - (-log ED50 with H 80/62)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

of shift to the right of the ovalbumin dose-response curve when measured at the ED50 level was 1.10 ± .09 log units. This was obtained in the presence of 10^{-5}M (-)-isoproterenol. The maximum reduction in maximum histamine release was 36 ± 5%, obtained at 10^{-6}M (-)-isoproterenol. Ovalbumin-induced histamine release was not completely abolished by concentrations of isoproterenol as large as 10^{-4}M.

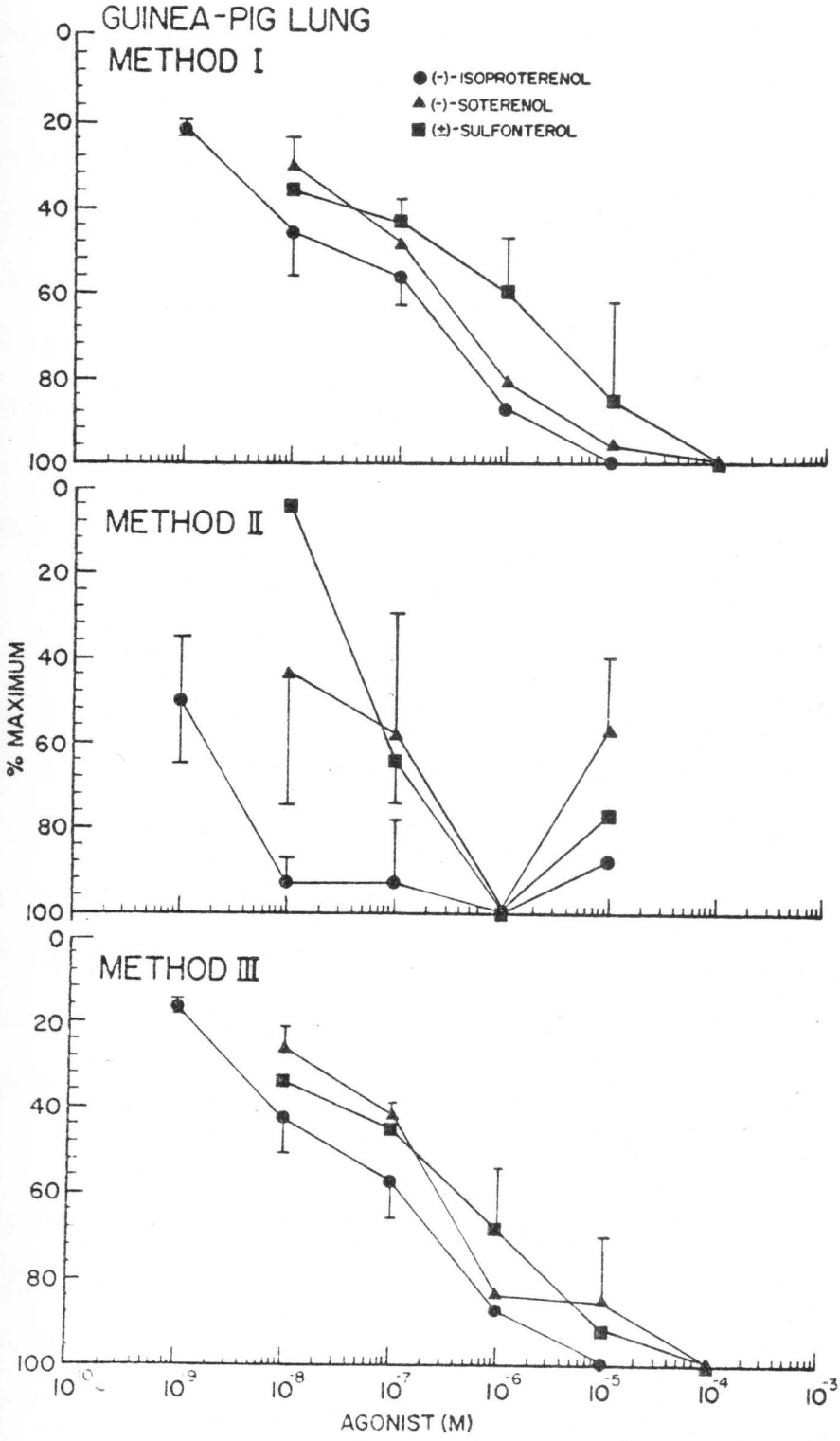
The dose-response effects of ovalbumin were inhibited in a similar manner by (-)-soterenol and (±)-sulfonterol, both of which are classified as bronchoselective beta receptor agonists. The inhibition produced by these agonists, however, was less than that of (-)-isoproterenol. The maximum degree of shift to the right of the ovalbumin dose-response curve when measured at the ED50 level was 0.82 ± .05 and 0.59 ± .08 log units, obtained at 10^{-4}M (-)-soterenol and 10^{-4}M (±)-sulfonterol, respectively. The maximum reduction in maximum histamine release were 24 ± 5% and 19 ± 6%, obtained at 10^{-6}M (-)-soterenol and 10^{-6}M (±)-sulfonterol, respectively. (-)-H 80/62, a cardioselective beta receptor agonist, did not significantly alter the ovalbumin dose-response curve at any concentrations below 10^{-3}M. In the presence of 10^{-3}M (-)-H 80/62, the ovalbumin dose-response curve was

significantly shifted 0.43 ± .08 log unit to the right, but the maximum degree of histamine release was not reduced.

Dose-response curves for inhibition by (-)-isoproterenol, (-)-soterenol and (±)-sulfonterol on ovalbumin-induced histamine release in guinea-pig minced lung are illustrated in figure 12 (see Table 22) and the data taken from these curves are summarized in Table 15. Dose-response curves for (-)-H 80/62 were not illustrated since it did not alter the ovalbumin effects except at the highest concentration used. The effects produced by the beta agonists on the ovalbumin dose-response curves were evaluated as described in "Methods". In general, the ED50 values determined by Method I and Method II were similar, but were 2.3 to 29 times larger than those estimated by Method II. Although the order of potencies for the beta agonists was the same in the three methods ((-)-isoproterenol > (-)-soterenol = (±)-sulfonterol >>> H-80/62), the relative potencies obtained in Method II differed from those obtained in Method I and Method III. As related to (-)-isoproterenol, the potencies of (-)-soterenol and (±)-sulfonterol were 3.5 and 9 times smaller, respectively, using Method I. The potencies were also 4 and 5 times smaller, respectively, using Method III, but were 25 and 67 times smaller, respectively, using Method II.

FIGURE 12

Log dose-response curves for inhibition by (-)-isoproterenol, (-)-soterenol and (±)-sulfonterol on ovalbumin-induced histamine release from guinea-pig minced lung. The response to each agonist concentration was calculated as a percentage of the maximum response produced by that agonist as described in "Methods". All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 15. Vertical lines indicate S.E.M.


GUINEA-PIG LUNG
METHOD I
(-)-ISOPROTERENOL
(-)-SOTERENOL
(±)-SULFONTEROL
METHOD II
METHOD III
% MAXIMUM
0
20
40
60
80
100
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
AGONIST (M)

Therefore, two different relative potencies were obtained for the beta receptor agonists in mediating the shift to the right of the ovalbumin dose-response curve and reduction of the maximum histamine release in guinea-pig minced lung.

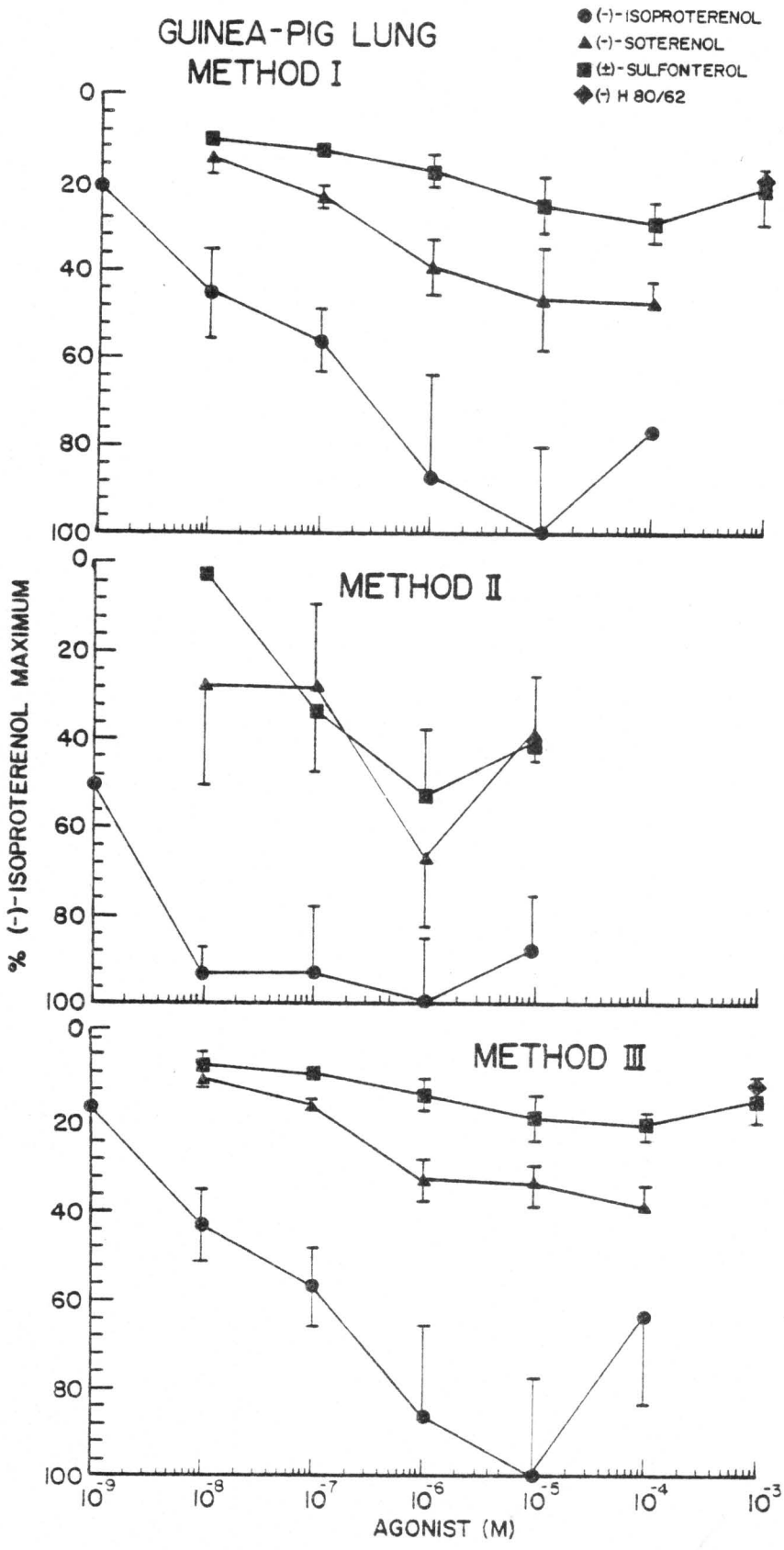
In the guinea-pig lung, beta receptor agonists such as (-)-soterenol and (±)-sulfonterol can be classfied as 'partial' agonists in relation to (-)-isoproterenol (figure 13, Table 23). As indicated in Table 23, the maximum inhibitory effects on histamine release produced by (-)-soterenol and (±)-sulfonterol were significantly less than that of (-)-isoproterenol as estimated in Method I and III; however, only the maximum inhibitory effect of (±)-sulfonterol was significantly different from that of (-)-isoproterenol using Method II. The results suggest that (-)-soterenol is a partial agonist relative to (-)-isoproterenol in shifting the ovalbumin dose-response curve, but is as effective as (-)-isoproterenol in reducing the maximum histamine release in guinea-pig lung.

Effects of Beta Receptor Agonists on Histamine Release in Minced Heart.

Dose-response curves for ovalbumin-induced histamine release in guinea-pig minced heart in the absence and

FIGURE 13

Log dose-response curves for inhibition by (-)-isoproterenol, (-)-soterenol, (±)-sulfonterol and (-)-H 80/62 on ovalbumin-induced histamine release from guinea-pig minced lung. The response to each agonist concentration was calculated as a percentage of the maximum response produced by (-)-isoproterenol as described in "Methods". All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Vertical lines indicate S.E.M.


GUINEA-PIG LUNG
METHOD I
(-)-ISOPROTERENOL
(-)-SOTERENOL
(±)-SULFONTEROL
(-) H 80/62
METHOD II
METHOD III
% (-)-ISOPROTERENOL MAXIMUM
0
20
40
60
80
100
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
AGONIST (M)

presence of different concentrations of (-)-isoproterenol, (-)-soterenol, (±)-sulfonterol and (-)-H 80/62 are illustrated in figure 14, 15, 16 and 17. The data taken from these curves are summarized in Tables 11, 12, 13 and 14.

The dose-response effects of ovalbumin in guinea-pig heart were inhibited by (-)-isoproterenol in a manner similar to that in the guinea-pig lung. Increasing concentrations of (-)-isoproterenol resulted in a shift of the ovalbumin dose-response curve to the right and reduction of the maximum histamine release. The maximum degree of shift to the right of the ovalbumin dose-response curve when measured at the ED50 level was 0.82 ± .08 log unit. This was obtained in the presence of 10^{-5}M (-)-isoproterenol. The maximum reduction in maximum histamine release was 34 ± 3.6% also obtained at 10^{-5}M (-)-isoproterenol. Therefore, (-)-isoproterenol was only 75% as effective in the heart in shifting the ovalbumin dose-response curve as in the lung, but was equally effective in reducing the maximum histamine release in both tissues.

(-)-Soterenol, however, was as effective as (-)-isoproterenol in inhibiting the ovalbumin-induced responses in the heart. The maximum degree of shift of the ovalbumin dose-response curve when measured at the ED50 level was 0.86 ± .10 log unit, obtained at 10^{-4}M

FIGURE 14

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced heart in the absence and presence of different concentrations of (-)-isoproterenol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 11. Each curve represents the mean of five to ten experiments. Vertical lines indicate S.E.M.

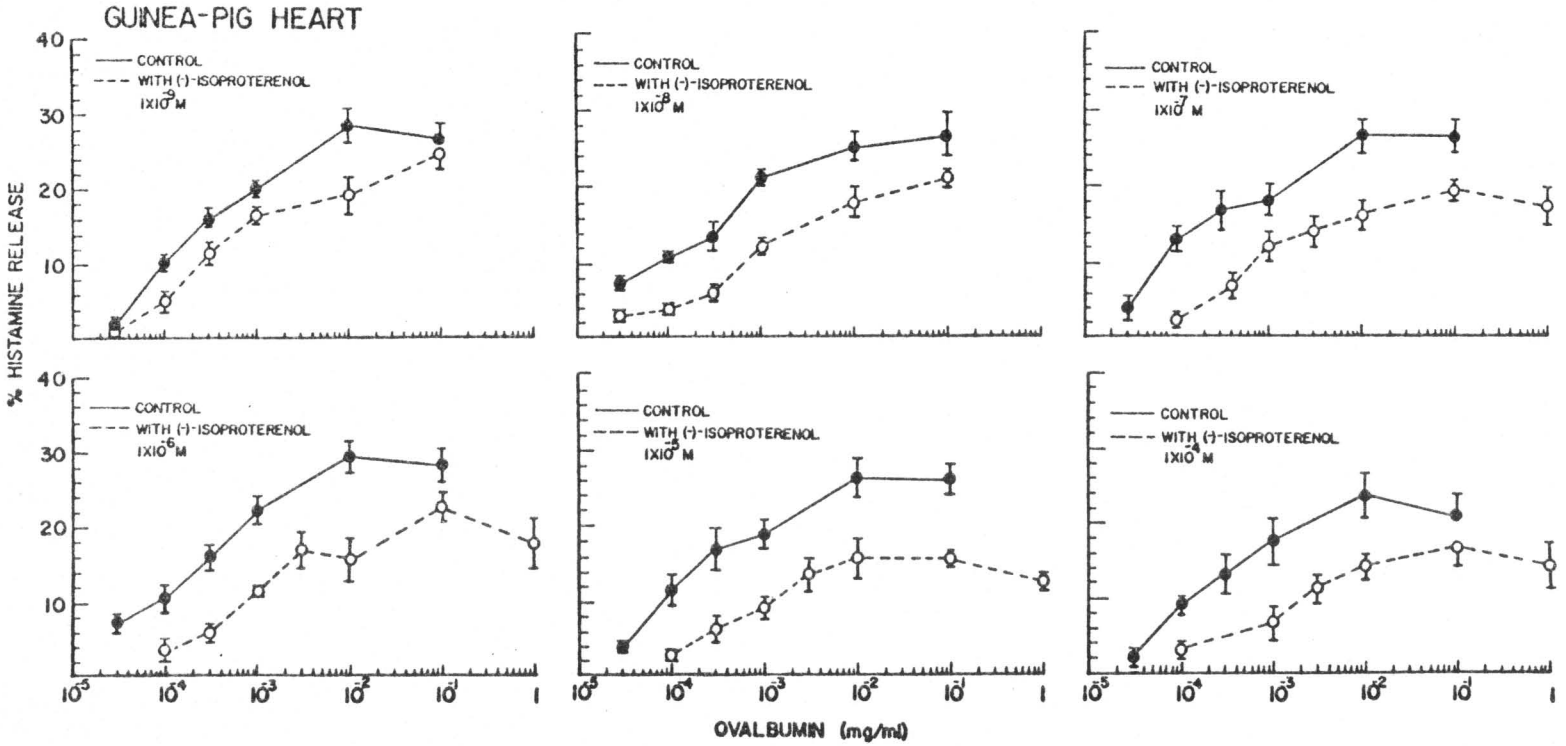

GUINEA-PIG HEART
% HISTAMINE RELEASE
OVALBUMIN (mg/ml)
CONTROL
WITH (-)-ISOPROTERENOL
1X10⁻⁹ M
1X10⁻⁸ M
1X10⁻⁷ M
1X10⁻⁶ M
1X10⁻⁵ M
1X10⁻⁴ M

Table 11 - Inhibition by (-)Isoproterenol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.[a]

(-)-Isoproterenol Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-9}$	3.61 ± .08	3.38 ± .08	1.7 ± .2	<0.05	29 ± 2	25 ± 2	83 ± 4	<0.05	5
$1x10^{-8}$	3.68 ± .06	3.20 ± .07	3.3 ± .6	<0.01	28 ± 2	21 ± 1	76 ± 4	<0.01	9
$1x10^{-7}$	3.80 ± .09	3.10 ± .11	5.5 ± 1.0	<0.01	28 ± 2	20 ± 1	73 ± 4	<0.01	9
$1x10^{-6}$	3.63 ± .04	2.92 ± .11	6.3 ± 1.8	<0.01	31 ± 2	23 ± 2	73 ± 3	<0.01	6
$1x10^{-5}$	3.72 ± .09	2.90 ± .12	7.5 ± 1.3	<0.01	27 ± 2	18 ± 2	66 ± 4	<0.01	10
$1x10^{-4}$	3.68 ± .07	2.89 ± .13	7.2 ± 1.7	<0.01	25 ± 3	17 ± 3	67 ± 5	<0.01	5

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Isoproterenol was added to the tissues 10 minutes before ovalbumin challenge.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without isoproterenol) - (-log ED50 with isoproterenol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control *vs.* treated values.

[g] Number of observations.

FIGURE 15

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced heart in the absence and presence of different concentrations of (-)-soterenol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 12. Each curve represents the mean of two to five experiments. Vertical lines indicate S.E.M.

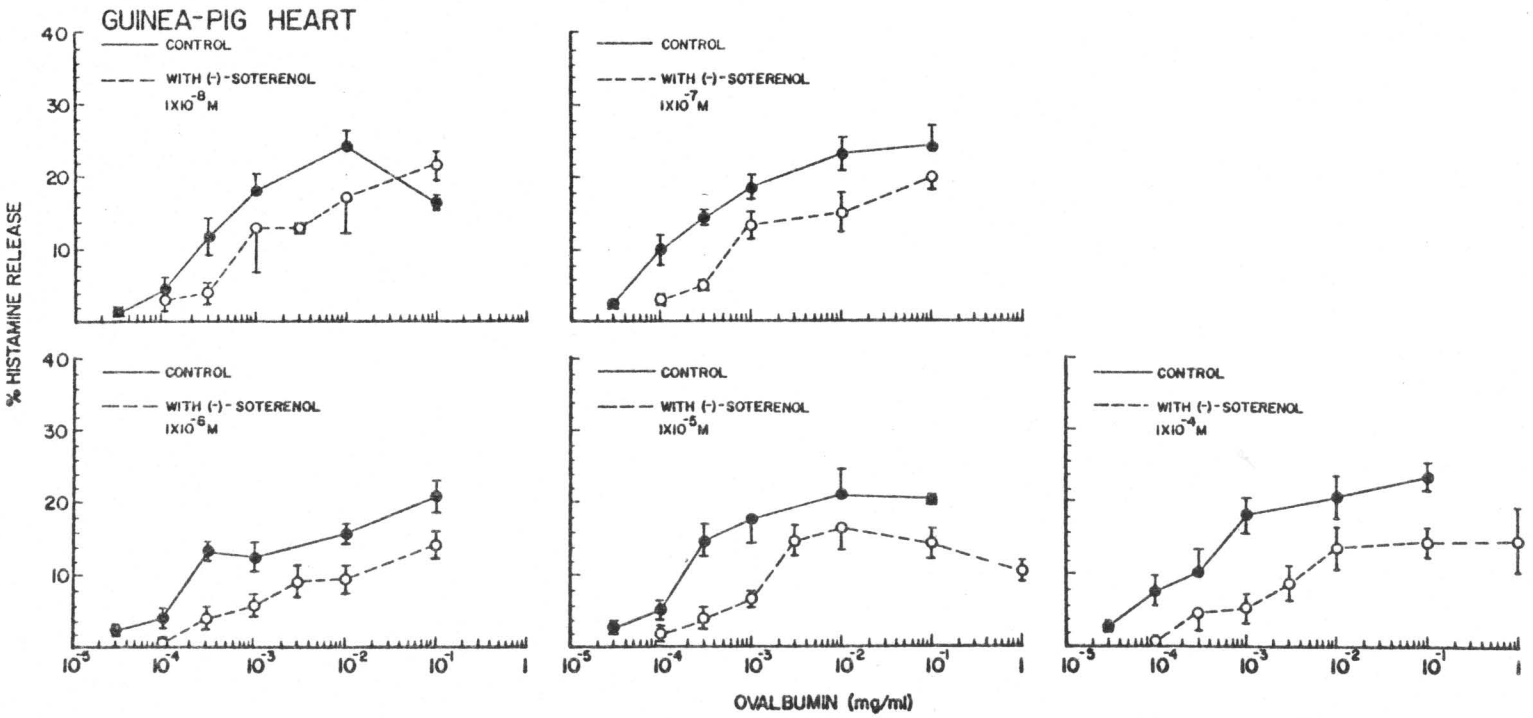

GUINEA-PIG HEART
CONTROL
WITH (-)-SOTERENOL
1X10⁻⁸M
1X10⁻⁷M
1X10⁻⁶M
1X10⁻⁵M
1X10⁻⁴M
% HISTAMINE RELEASE
OVALBUMIN (mg/ml)

Table 12 - Inhibition by (-)-Soterenol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.[a]

(-)-Soterenol Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-8}$	3.54 ± .07	3.11 ± .11	2.8 ± .3	<0.05	24 ± 1	22 ± 2	91 ± 7	>0.05	3
$1x10^{-7}$	3.68 ± .05	3.08 ± .09	4.0 ± .5	<0.01	26 ± 2	22 ± 2	82 ± 2	<0.01	5
$1x10^{-6}$	3.64 ± .10	2.88 ± .16	6.7 ± 1.6	<0.01	22 ± 2	15 ± 2	68 ± 5	<0.01	5
$1x10^{-5}$	3.65 ± .04	2.90 ± .12	6.5 ± 1.9	<0.01	24 ± 3	18 ± 2	76 ± 7	<0.05	5
$1x10^{-4}$	3.55 ± .12	2.69 ± .15	8.0 ± 1.5	<0.01	25 ± 2	16 ± 2	68 ±10	<0.05	5

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Soterenol was added to the tissues 10 minutes before ovalbumin challenge.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without soterenol) - (-log ED50 with soterenol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

FIGURE 16

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced heart in the absence and presence of different concentrations of (±)-sulfonterol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 13. Each curve represents the mean of three to five experiments. Vertical lines indicate S.E.M.

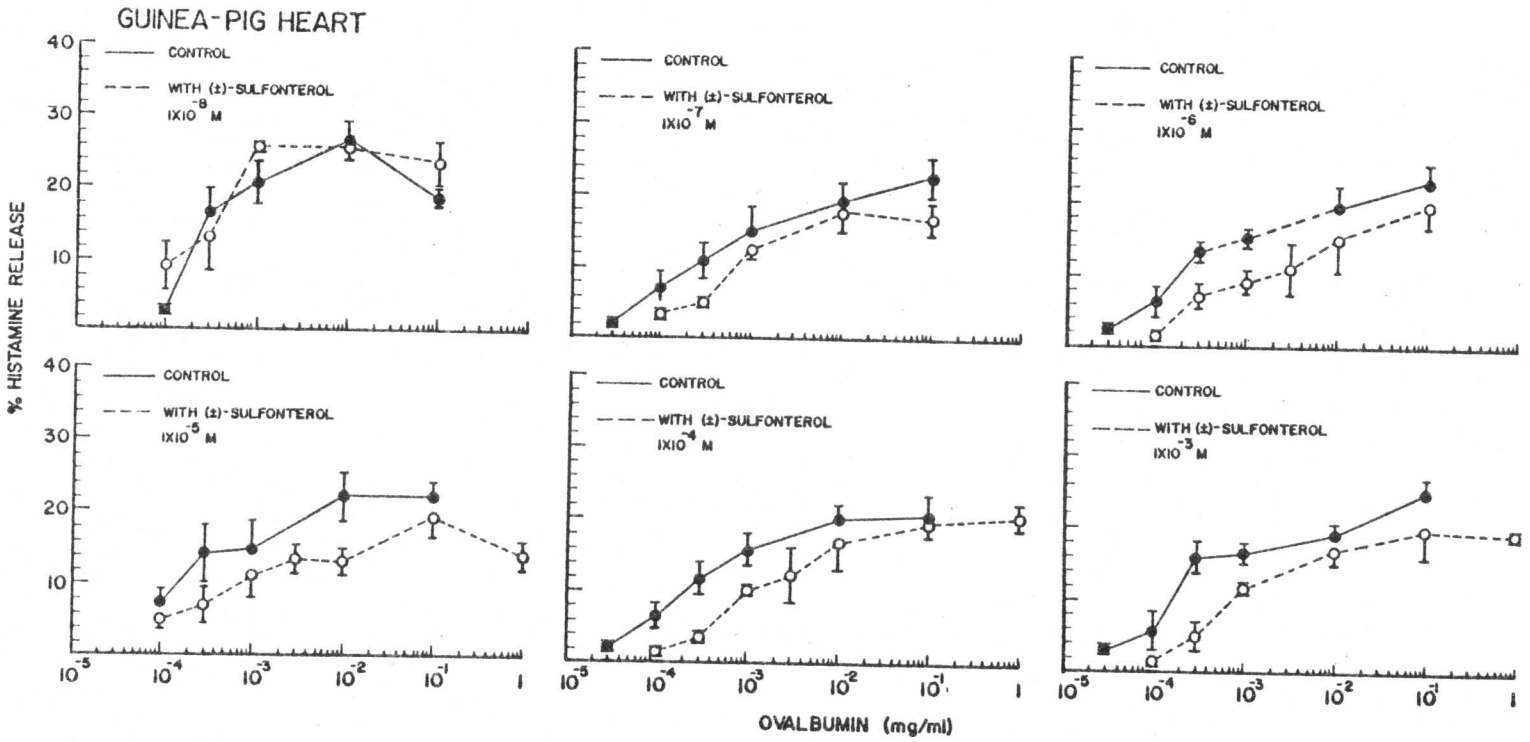

GUINEA-PIG HEART
% HISTAMINE RELEASE
OVALBUMIN (mg/ml)
CONTROL
WITH (±)-SULFONTEROL
1X10⁻⁸ M
1X10⁻⁷ M
1X10⁻⁶ M
1X10⁻⁵ M
1X10⁻⁴ M
1X10⁻³ M

Table 13 - Inhibition by (±)-Sulfonterol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.[a]

(±)-Sulfonterol Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-8}$	3.59 ± .08	3.63 ± .26	1.1 ± .5	>0.05	27 ± 3	27 ± 1	101 ± 5	>0.05	3
$1x10^{-7}$	3.52 ± .10	3.25 ± .10	2.1 ± .4	>0.05	25 ± 2	19 ± 2	76 ± 7	<0.05	5
$1x10^{-6}$	3.64 ± .10	3.10 ± .14	3.6 ± .4	<0.01	25 ± 2	18 ± 3	73 ± 9	<0.05	5
$1x10^{-5}$	3.67 ± .10	3.12 ± .13	4.2 ± 1.2	<0.05	25 ± 3	20 ± 2	81 ± 5	<0.05	4
$1x10^{-4}$	3.63 ± .07	2.95 ± .09	5.3 ± 1.1	<0.01	22 ± 2	20 ± 2	92 ± 4	>0.05	5
$1x10^{-3}$	3.69 ± .15	3.06 ± .18	4.3 ± .5	<0.05	25 ± 2	22 ± 3	85 ± 3	>0.05	2

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Sulfonterol was added tothe tissues 10 minutes before ovalbumin challenge.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without sulfonterol) - (-log ED50 with sulfonterol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

FIGURE 17

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced heart in the absence and presence of different concentrations of (-)-H 80/62. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 14. Each curve represents the mean of two to four experiments. Vertical lines indicate S.E.M.

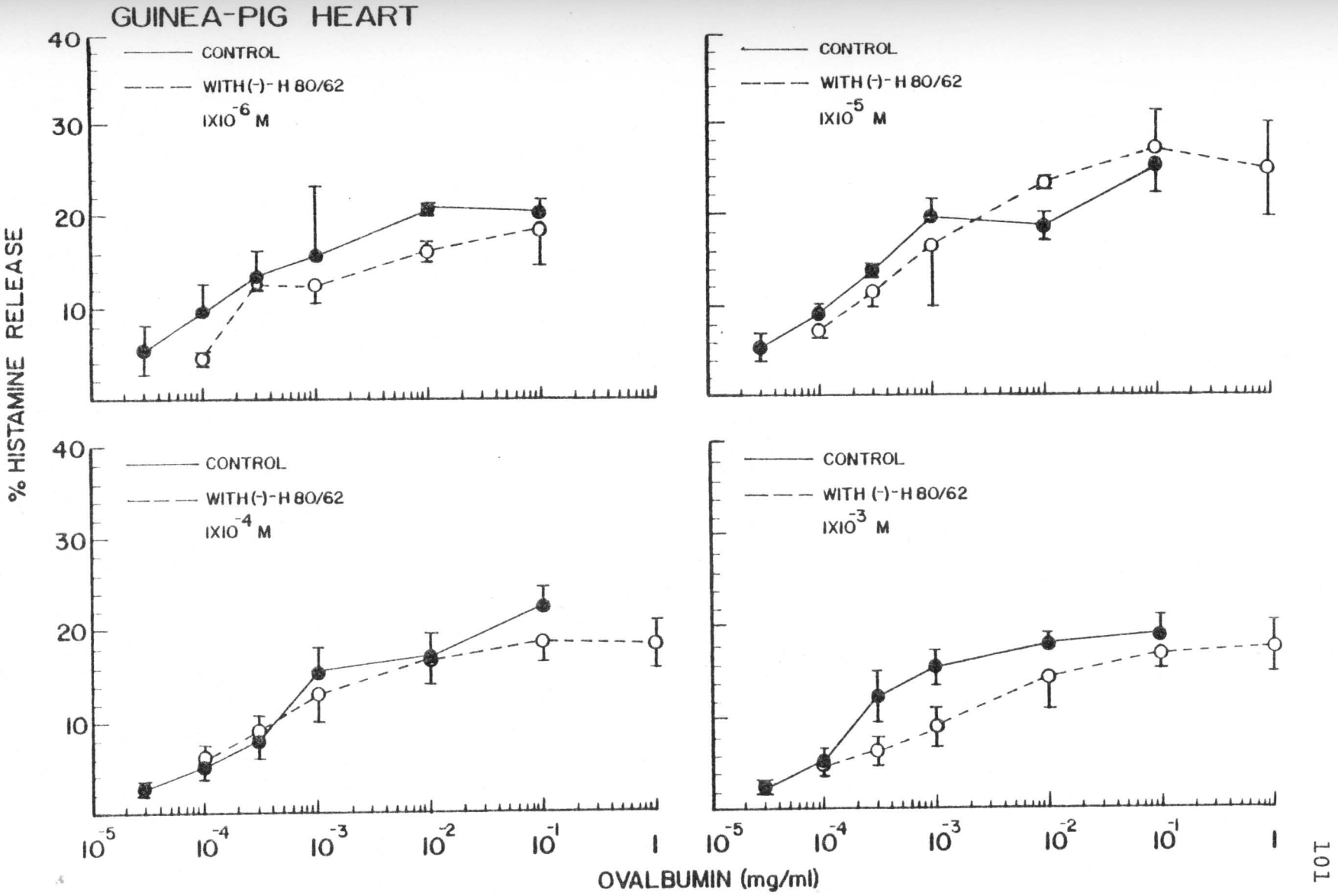

GUINEA-PIG HEART
CONTROL
WITH (-)-H 80/62
1X10⁻⁶ M
1X10⁻⁵ M
1X10⁻⁴ M
1X10⁻³ M
% HISTAMINE RELEASE
40
30
20
10
10⁻⁵
10⁻⁴
10⁻³
10⁻²
10⁻¹
1
OVALBUMIN (mg/ml)

Table 14 - Inhibition by (-)-H 80/62 of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.[a]

(-)-H 80/62 Concentration (M)	Ovalbumin[b] -log ED50 With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
$1x10^{-7}$	3.54	3.22	2.1	>0.05	27	26	98	>0.05	1
$1x10^{-6}$	3.86 ± .07	3.74 ± .06	1.4 ± .4	>0.05	22 ± 2	19 ± 4	85 ± 23	>0.05	2
$1x10^{-5}$	3.63 ± .12	3.50 ± .18	1.4 ± .2	>0.05	25 ± 3	27 ± 4	108 ± 4	>0.05	2
$1x10^{-4}$	3.49 ± .13	3.36 ± .17	1.4 ± .1	>0.05	23 ± 2	20 ± 2	87 ± 8	>0.05	4
$1x10^{-3}$	3.67 ± .10	3.01 ± .16	5.8 ± 2.2	<0.05	20 ± 1	18 ± 1	91 ± 4	>0.05	4

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. H 80/62 was added to the tissues 10 minutes before ovalbumin challenge.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without H 80/62) - (-log ED50 with H 80/62)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations

(-)-soterenol. The maximum reduction in maximum histamine release was 32 ± 5%, obtained at 10^{-6}M (-)-soterenol. Neither (-)-isoproterenol nor (-)-soterenol were capable of completely abolishing the ovalbumin dose-response curve.

As in the lung, (±)-sulfonterol was less effective than (-)-isoproterenol and (-)-soterenol, but still significantly altered the ovalbumin dose-response curve. The ovalbumin dose-response curve when measured at the ED50 level was maximally shifted 0.68 ± .10 log unit to the right in the presence of 10^{-4}M (±)-sulfonterol, and the maximum histamine release was maximally reduced by 27 ± 9% in the presence of 10^{-6}M (±)-sulfonterol.

The dose-response curve to ovalbumin was not significantly altered by (-)-H 80/62, except at 10^{-3}M. As in the lung, the ovalbumin dose-response curve was significantly shifted 0.66 ± .19 log unit to the right in the presence of 10^{-3}M H 80/62, but the maximum degree of histamine release was not altered.

Dose-response curves for (-)-isoproterenol, (-)-soterenol and (±)-sulfonterol in inhibiting ovalbumin-induced histamine release in guinea-pig minced heart are illustrated in figure 18 (see Table 24) and the data taken from these curves are summarized in Table 15.

FIGURE 18

Log dose-response curves for inhibition by (-)-isoproterenol, (-)-soterenol and (±)-sulfonterol on ovalbumin-induced histamine release from guinea-pig minced heart. The response to each agonist concentration was calculated as a percentage of the maximum response produced that agonist as described in "Methods". All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these are summarized in Table 15. Vertical lines indicate S.E.M.

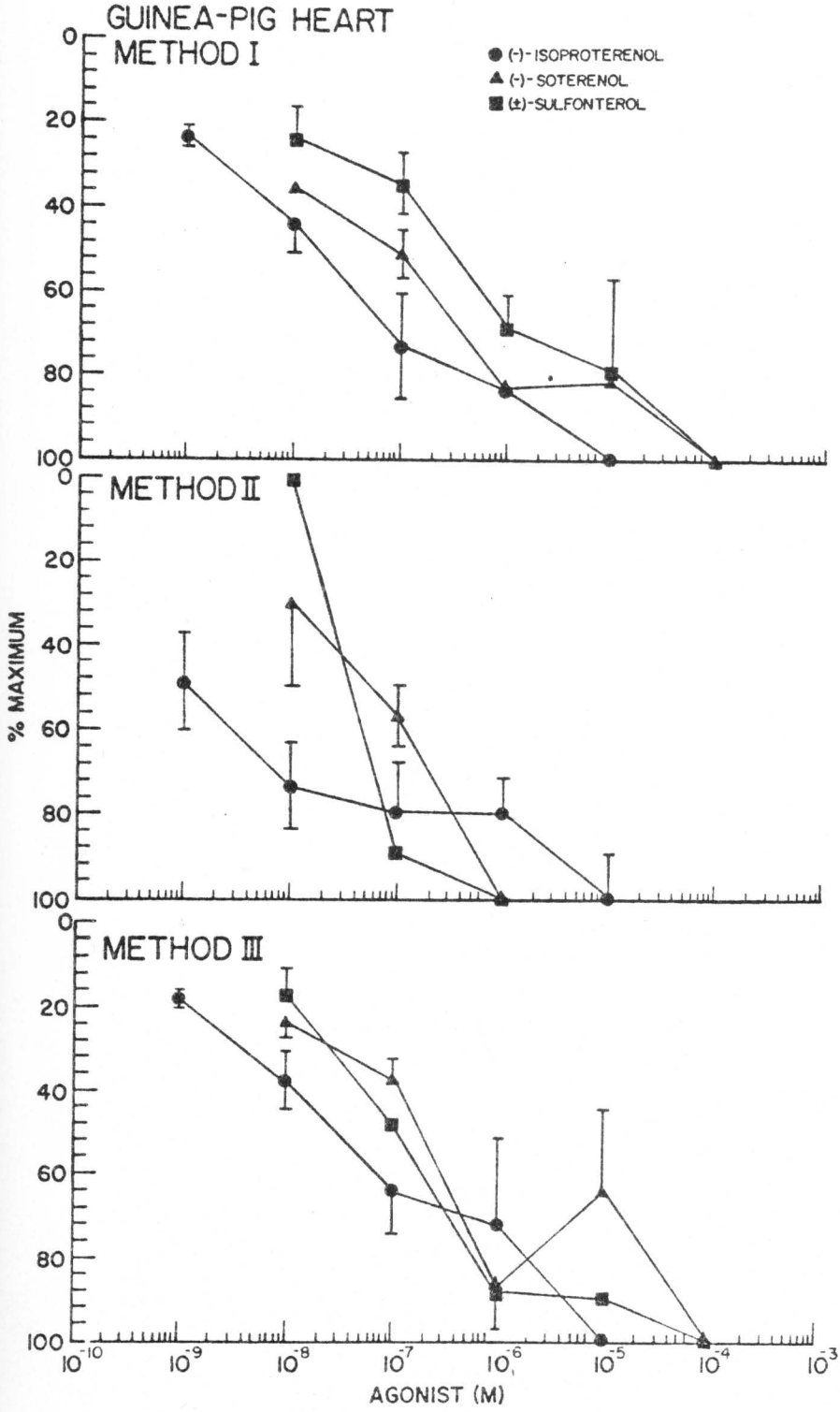

GUINEA-PIG HEART
METHOD I
(-)-ISOPROTERENOL
(-)-SOTERENOL
(±)-SULFONTEROL
METHOD II
METHOD III
% MAXIMUM
0
20
40
60
80
100
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
AGONIST (M)

Table 15 - Effects of Beta Adrenergic Receptor Agonists on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung and Heart.[a]

Agonist	Lung -Log Molar ED50[b] With S.E.M.	Lung Relative[c] Potencies	Lung N[d]	Heart -Log Molar ED50[b] With S.E.M.	Heart Relative[c] Potencies	Heart N[d]
Method I						
(-)-Isoproterenol	7.59 ± .44	1	31	7.77 ± .39	1	29
(-)-Soterenol	7.06 ± .36	.29	22	7.12 ± .41	.23	19
(±)-Sulfonterol	6.65 ± .61	.11	19	6.58 ± .20	.07	18
Method II						
(-)-Isoproterenol	9.00 ± .46	1	4	8.93 ± .42	1	23
(-)-Soterenol	7.60 ± .78	.04	10	7.37 ± .41	.027	9
(±)-Sulfonterol	7.17 ± .29	.015	9	7.28 ± .24	.023	5
Method III						
(-)-Isoproterenol	7.54 ± .38	1	31	7.60 ± .36	1	29
(-)-Soterenol	6.94 ± .39	.25	22	6.92 ± .38	.21	19
(±)-Sulfonterol	6.82 ± .47	.19	19	7.00 ± .31	.25	18

Table 15 continued

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[a]All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine.

[b]Negative log of the concentration of each agonist required in producing 50% of its own maximum effect. Curves were plotted as described in "Methods".

[c]Potency of each agonist relative to the potency of isoproterenol when it was taken as unity.

[d]N, the total number of tissue samples in the groups which, from the regression line, were expected to lie between profits 3.5 and 6.5 (Miller and Tainter, 1944).

Dose-response curves for (-)-H 80/62 were not illustrated since it did not alter the ovalbumin effects except at the highest concentration used. In comparison to the lung, similar ED50 values for the beta receptor agonists were obtained in the heart by three different methods. Similar orders of potencies for the beta agonists were also obtained in all three methods ((-)-isoproterenol > (-)-soterenol = (±)-sulfonterol >>> H-80/62). Thus, in both tissues, similar orders of potencies for the beta agonists existed for mediating the shift to the right of the ovalbumin dose-response curve and reduction of the maximum histamine release.

In addition, two different relative potencies for the beta agonists were revealed in the heart, as was the case in the lung, by three different methods of estimation. As related to (-)-isoproterenol, the potencies of (-)-soterenol and (±)-sulfonterol were 4.3 and 14.3 times smaller, respectively, using Method I. These potencies were also 4.8 and 4.0 times smaller, respectively, using Method III, but were 37.0 and 43.5 times smaller, respectively, using Method II. Therefore, within each tissue, there existed two distinctly different relative potencies for the beta receptor agonists in mediating the shift to the right of the ovalbumin dose-response curve and reduction of the maximum histamine release.

In contrast to the lung, both (-)-soterenol and (±)-sulfonterol produced same degree of effect relative to (-)-isoproterenol in the guinea-pig heart (figure 19, Table 25). As indicated in Table 25, the maximum inhibitory effects on histamine release produced by (-)-soterenol and (±)-sulfonterol were not significantly different from that of (-)-isoproterenol, as estimated by three different methods.

Effects of Enantiomers of Isoproterenol on Histamine Release from Minced Lung and Heart.

Dose-response curves for ovalbumin-induced histamine release in guinea-pig minced lung and heart in the absence and presence of different concentrations of (+)-isoproterenol are illustrated in figures 20 and 21. The data taken from these curves are summarized in Tables 16 and 17. In both tissues, dose-response curves to ovalbumin were inhibited by (+)-isoproterenol in a manner similar to its enantiomer. Both isomers were able to produce the same maximum degree of response pertaining to the shift to the right of the ovalbumin dose-response curve and reduction of the maximum histamine release. In both tissues, however, (+)-isoproterenol was unable to completely abolish the ovalbumin dose-response effects.

FIGURE 19

Log dose-response curves for inhibition by (-)-isoproterenol, (-)-soterenol, (±)-sulfonterol and (-)-H 80/62 on ovalbumin-induced histamine release from guinea-pig minced heart. The response to each agonist concentration was calculated as a percentage of the maximum response produced by (-)-isoproterenol as described in "Methods". All tissues were obtained from reserpine-pretreated animals and exposed to indomethacin and phenoxybenzamine. Vertical lines indicate S.E.M.

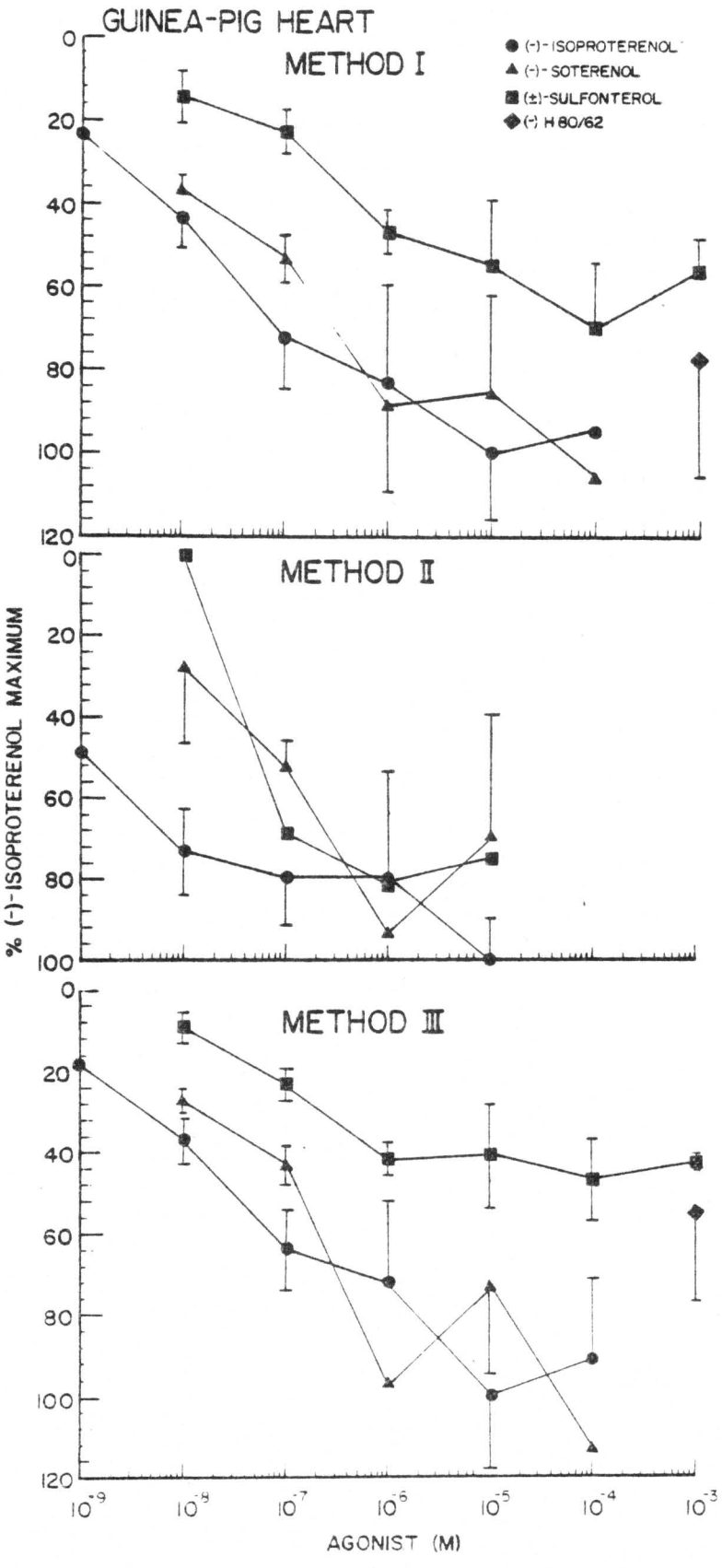
GUINEA-PIG HEART
METHOD I
METHOD II
METHOD III
(-)-ISOPROTERENOL
(-)-SOTERENOL
(±)-SULFONTEROL
(-) H 80/62
% (-)-ISOPROTERENOL MAXIMUM
0
20
40
60
80
100
120
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
AGONIST (M)

FIGURE 20

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced lung in the absence and presence of different concentrations of (+)-isoproterenol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 16. Each curve represents the mean of two to four experiments. Vertical lines indicate S.E.M.

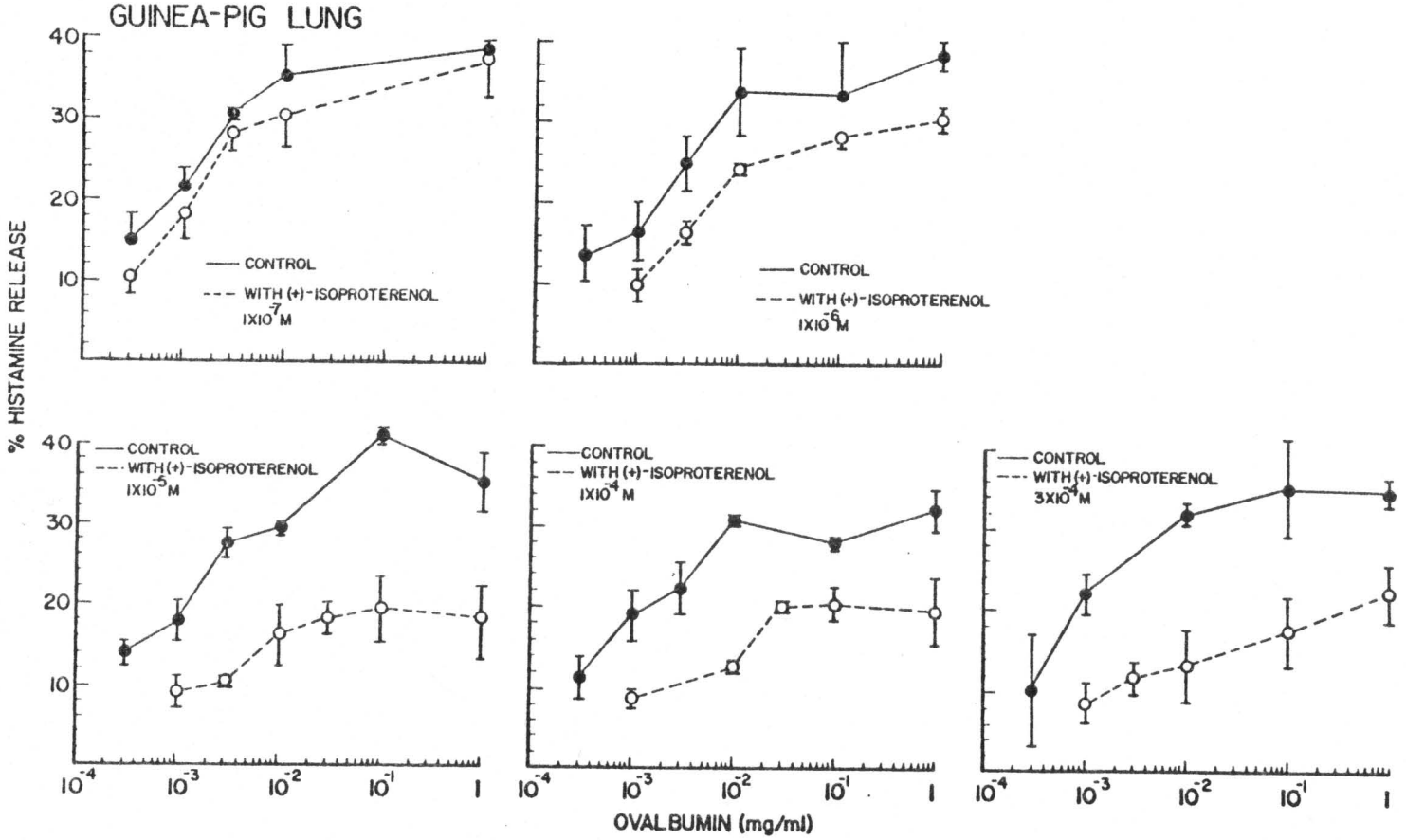
GUINEA-PIG LUNG
% HISTAMINE RELEASE
CONTROL
WITH (+)-ISOPROTERENOL
1X10⁻⁷M
1X10⁻⁶M
1X10⁻⁵M
1X10⁻⁴M
3X10⁻⁴M
OVALBUMIN (mg/ml)

Table 16 - Inhibition by (-)-Isoproterenol or (+)-Isoproterenol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

Agonist Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
(+)-Isoproterenol									
$1x10^{-7}$	3.23 ± .15	3.03 ± .19	1.6 ± .2	>0.05	40 ± 1	38 ± 5	93 ± 9	>0.05	3
$1x10^{-6}$	3.08 ± .19	2.61 ± .15	3.5 ± 1.0	<0.05	39 ± 2	31 ± 1	80 ± 4	<0.05	4
$1x10^{-5}$	3.10 ± .11	2.28 ± .15	6.7 ± .7	<0.01	39 ± 3	23 ± 3	61 ± 7	<0.05	4
$1x10^{-4}$	3.17 ± .18	2.22 ± .13	9.1 ± 1.3	<0.01	34 ± 2	23 ± 3	70 ± 8	<0.05	4
$3x10^{-4}$	3.33 ± .23	2.61 ± .01	6.1 ± 3.1	>0.05	37 ± 4	22 ± 4	60 ± 3	<0.05	2
(-)-Isoproterenol									
$1x10^{-8}$	3.16 ± .08	2.60 ± .07	4 ± 1.3	<0.05	38 ± 4	24 ± 1	65 ± 4	<0.05	3
$1x10^{-7}$	3.03 ± .27	2.25 ± .28	7.2 ± 2.4	<0.05	36 ± 3	27 ± 4	75 ± 8	>0.05	3
$1x10^{-5}$	3.28 ± .13	2.27 ± .07	11 ± 2.9	<0.05	40 ± 1	25 ± 3	64 ± 5	<0.05	3

Table 16 continued

---

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Either isomer of isoproterenol was added to the tissues 15 minutes before ovalbumin challenge. Data were obtained in "paired" experiments.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without isoproterenol) - (-log ED50 with isoproterenol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

FIGURE 21

Log dose-response curves for ovalbumin-induced histamine release from guinea-pig minced heart in the absence and presence of different concentrations of (+)-isoproterenol. All tissues were obtained from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. The data taken from these curves are summarized in Table 17. Each curve represents the mean of two to five experiments. Vertical lines indicate S.E.M.

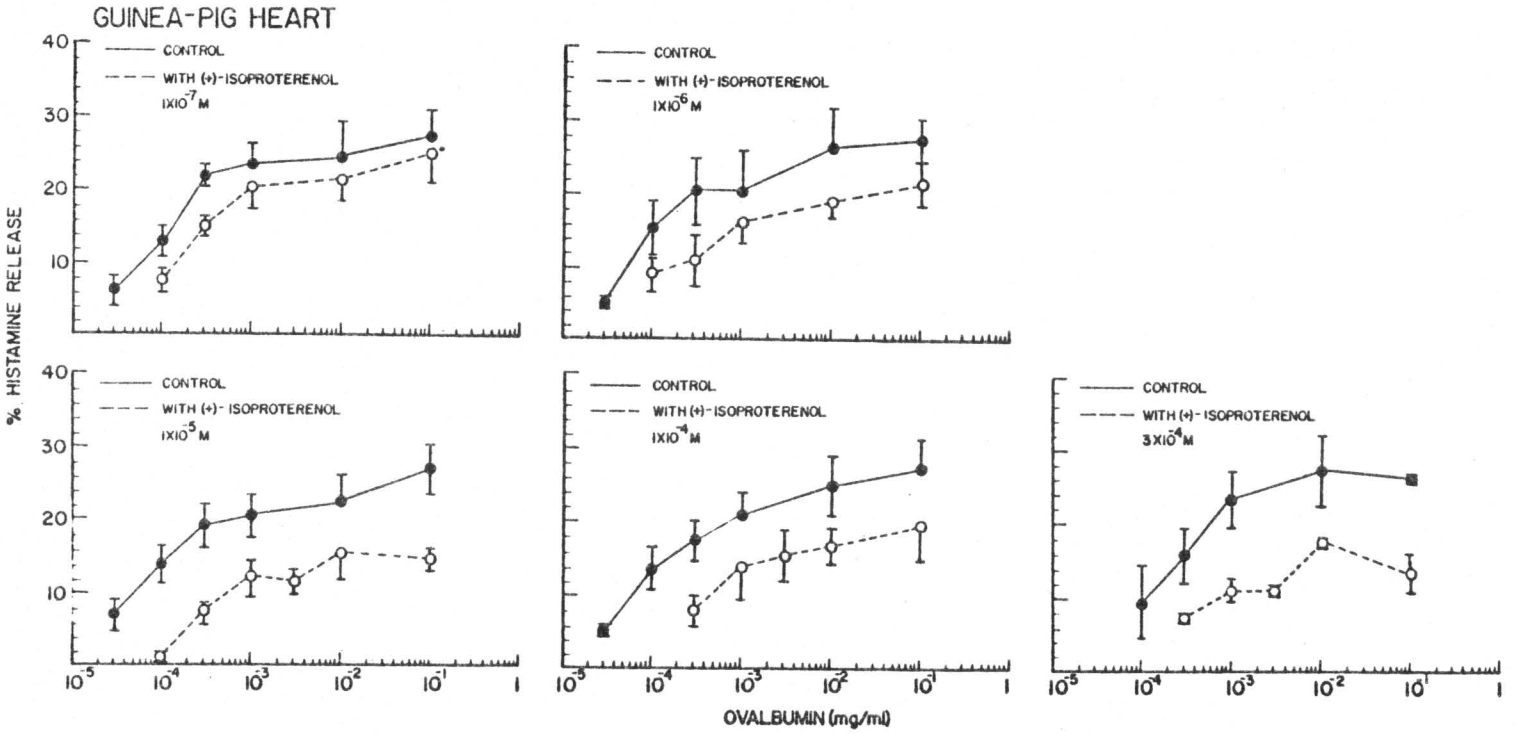
GUINEA-PIG HEART
% HISTAMINE RELEASE
CONTROL
WITH (+)-ISOPROTERENOL
1X10⁻⁷M
1X10⁻⁶M
1X10⁻⁵M
1X10⁻⁴M
3X10⁻⁴M
OVALBUMIN (mg/ml)

Table 17 - Inhibition by (-)-Isoproterenol or (+)-Isoproterenol of Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.[a]

Agonist Concentration (M)	Ovalbumin -log ED50[b] With S.E.M.		Dose Ratio[c] With S.E.M.	p[f]	Maximum % Histamine Release[d] With S.E.M.		Treated % of Control[e] With S.E.M.	p[f]	n[g]
	Control	Treated			Control	Treated			
(+)-Isoproterenol									
$1x10^{-7}$	3.83 ± .10	3.60 ± .10	2.1 ± .5	>0.05	30 ± 4	25 ± 4	89 ± 14	>0.05	4
$1x10^{-6}$	3.92 ± .09	3.55 ± .16	2.6 ± .7	<0.05	30 ± 5	22 ± 3	74 ± 9	>0.05	4
$1x10^{-5}$	3.87 ± .11	3.28 ± .08	4.0 ± .4	<0.01	29 ± 3	18 ± 3	61 ± 5	<0.01	5
$1x10^{-4}$	3.87 ± .08	3.15 ± .15	5.9 ± 1.3	<0.01	28 ± 4	21 ± 3	71 ± 7	<0.01	5
$3x10^{-4}$	3.81 ± .10	3.25 ± .18	4.6 ± 2.7	>0.05	30 ± 3	18 ± 1	60 ± 4	<0.05	2
(-)-Isoproterenol									
$1x10^{-8}$	3.79 ± .12	3.31 ± .14	3.1 ± .3	<0.01	28 ± 3	19 ± 1	72 ± 11	>0.05	3
$1x10^{-7}$	3.99 ± .10	3.35 ± .14	4.4 ± .5	<0.01	29 ± 4	22 ± 2	78 ± 6	>0.05	3
$1x10^{-5}$	3.90 ± .10	3.12 ± .19	6.5 ± 1.5	<0.01	31 ± 6	22 ± 4	72 ± 8	<0.05	4

Table 17 continued

---

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Either isomer of isoproterenol was added to the tissues 10 minutes before ovalbumin challenge. Data were obtained in "paired" experiments.

[b] Calculated from curves plotting % of maximum response in each tissue.

[c] Dose Ratio = antilog [(-log ED50 without isoproterenol) - (-log ED50 with isoproterenol)].

[d] Maximum amount of histamine release relative to total tissue histamine content.

[e] Maximum effect of the treated calculated as a percentage of the control maximum histamine release.

[f] Significance of control vs. treated values.

[g] Number of observations.

Figures 22 and 23 illustrate the dose-response curves to (-) and (+) isomers of isoproterenol in guinea-pig minced lung and heart as estimated by Methods I and III (also see Tables 26 and 27). Isomeric-potency-differences and S.E.M. were shown between the horizontal arrows connecting the curves. The data taken from these curves are summarized in Table 18. Similar curves could not be obtained by Method II since concentrations of (-)-isoproterenol lower than 10^{-8}M were not examined in these paired experiments. Nevertheless, ED50 values determined for (-)-isoproterenol in guinea-pig minced lung and heart by Method II of Table 15 were used and isomeric-potency-differences for isoproterenol in both tissues were calculated by Method II using these unpaired data. Regardless of the method of analysis, similar isomeric-potency-differences for isoproterenol were obtained in both tissues. Therefore, in both guinea-pig lung and heart, similar isomeric-potency-differences were obtained for mediating the shift to the right of the ovalbumin dose-response curve and reduction of the maximum histamine release.

Furthermore, isomeric-potency-differences for isoproterenol as calculated by Method I and Method III in both tissues were consistently smaller than those obtained by Method II. However, these values were

FIGURE 22

Log dose-response curves for inhibition by (-)- and (+)-isoproterenol on ovalbumin-induced histamine release in minced lung and heart taken from reserpine-pretreated, ovalbumin-sensitized guinea-pigs. Numbers between the horizontal arrows connecting the curves are isomeric-potency-differences in log units with S.E.M. The response to each isomer concentration was calculated as a percentage of the maximum shift of the ovalbumin dose-response curve to the right produced by that isomer (Method I). All curves were obtained in the presence of indomethacin and phenoxybenzamine. Vertical lines indicate S.E.M. and the data taken from these curves are summarized in Table 18.

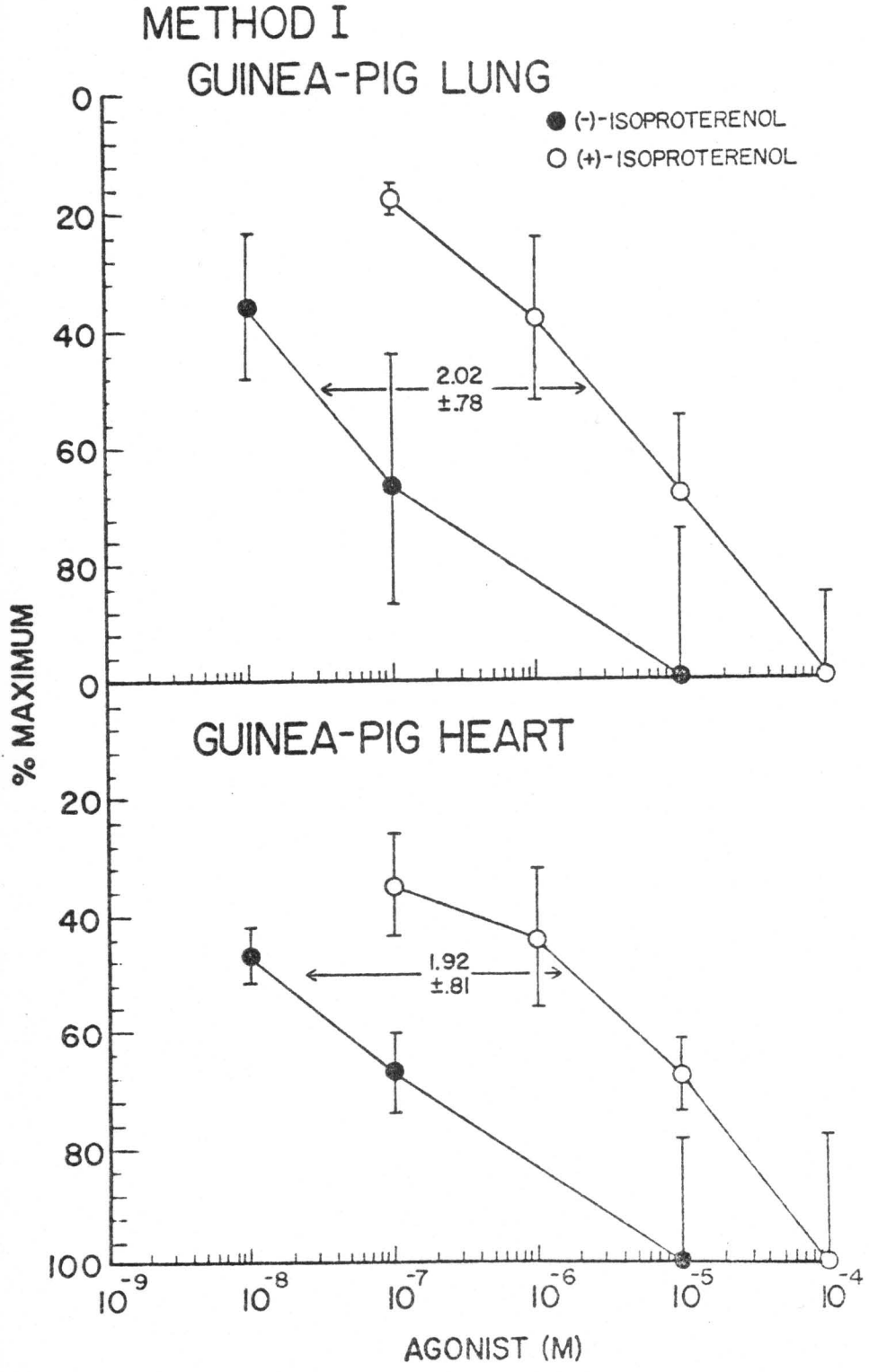
METHOD I
GUINEA-PIG LUNG
(-)-ISOPROTERENOL
(+)-ISOPROTERENOL
2.02
±.78
GUINEA-PIG HEART
1.92
±.81
% MAXIMUM
0
20
40
60
80
100
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
AGONIST (M)

FIGURE 23

Log dose-response curves for inhibition (-)- and (+)-isoproterenol on ovalbumin-induced histamine release in minced lung and heart taken from reserpine-pretreated, ovalbumin-sensitized guinea-pigs. Numbers between the horizontal arrows connecting the curves are isomeric-potency-differences in log units with S.E.M. The response to each isomer concentration was calculated as a percentage of the maximum dose ratio/% of control maximum response (Method III). All curves were obtained in the presence of indomethacin and phenoxybenzamine. Vertical lines indicate S.E.M. and the data taken from these curves are summarized in Table 18.

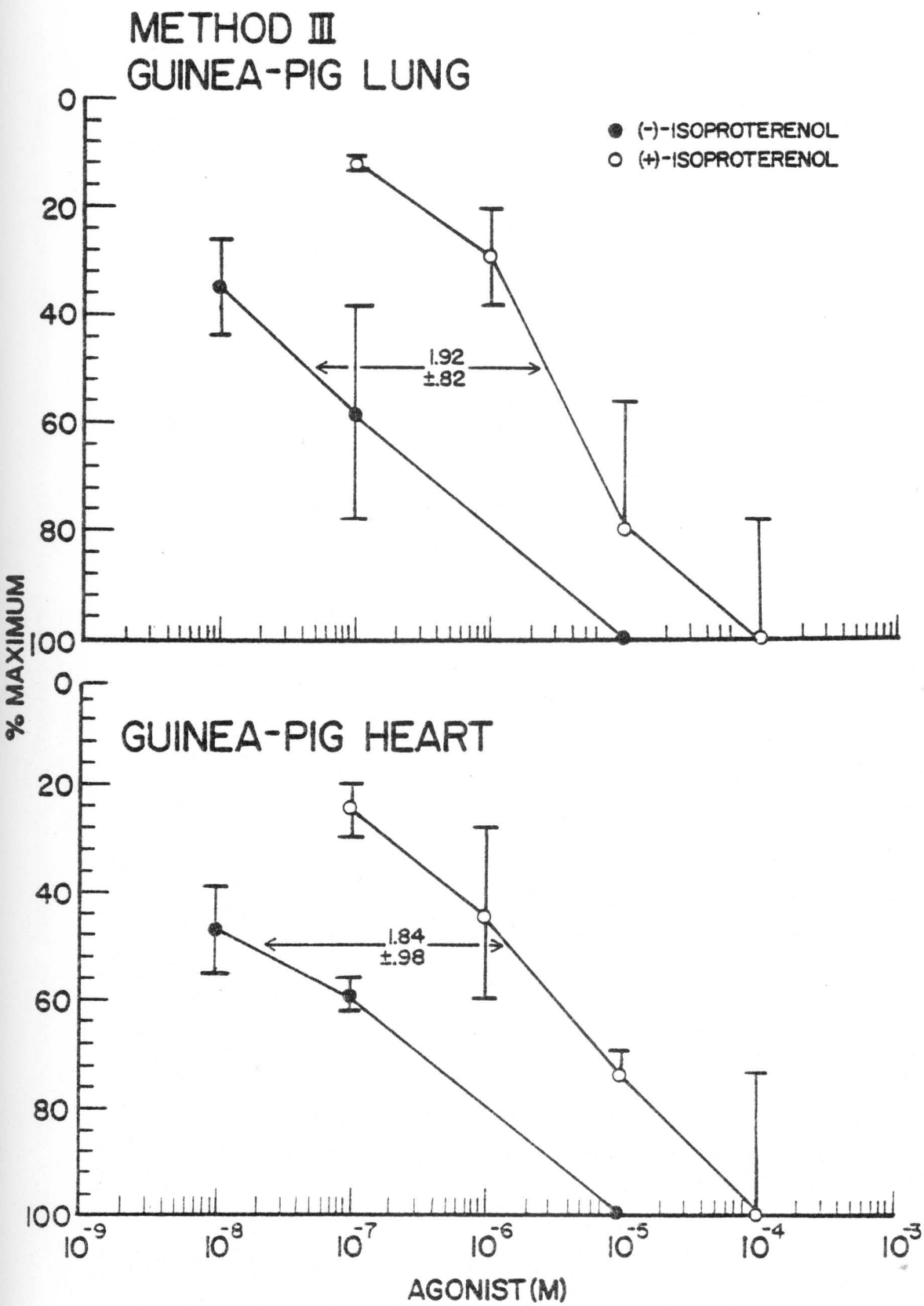
METHOD III
GUINEA-PIG LUNG
(-)-ISOPROTERENOL
(+)-ISOPROTERENOL
1.92
±.82
GUINEA-PIG HEART
1.84
±.98
% MAXIMUM
0
20
40
60
80
100
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
AGONIST (M)

Table 18 - Effects of Enantiomers of Isoproterenol on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung and Heart.[a]

Tissue	-Log Molar ED50 with S.E.M.[b] (-)-Isoproterenol[d]	N[e]	(+)-Isoproterenol	N[e]	Isomeric-Potency-[c] Differences with S.E.M.
Method I					
Lung	7.64 ± .60	6	5.62 ± .49	11	2.02 ± .78
Heart	7.81 ± .64	6	5.89 ± .50	13	1.92 ± .81
Method II					
Lung	9.00 ± .46	4	6.17 ± .48	7	2.83 ± .67
Heart	8.93 ± .42	23	6.47 ± .48	8	2.46 ± .64
Method III					
Lung	7.51 ± .68	6	5.59 ± .47	11	1.92 ± .82
Heart	7.77 ± .83	6	5.93 ± .51	13	1.84 ± .98

Table 18 continued

---

[a]All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine.

[b]Negative log of the concentration of each agonist in producing 50% of its own maximum effect. Curves were plotted as described in "Methods".

[c]Iosmeric-potency-difference = [(-log ED50 of (-)-isomer) - (-log ED50 of (+)-isomer)]. The S.E.M. of the isomeric potency difference is estimated by $[(\text{S.E.M. of (+)-isomer})^2 + (\text{S.E.M. of (-)-isomer})^2]^{1/2}$

[d]ED50 values of (-)-isoproterenol in both tissues in Method II were taken from Table 15. Data in Method II alone were obtained in "unpaired" experiments.

[e]N, the total number of tissue samples in the groups which, from the regression line, were expected to lie between profits 3.5 and 6.5 (Miller and Tainter, 1944).

associated with a large variability and were not statistically different from each other. Nonetheless, it tends to indicate that within guinea-pig lung or heart, there exists dissimilar isomeric-potency-differences for mediating the shift of the ovalbumin dose-response curve to the right and reduction of the maximum histamine release.

DISCUSSION

The results of the present study clearly demonstrate the inhibition by the beta receptor agonists of antigen-induced histamine release in guinea-pig heart and lung. Furthermore, the inhibitory effects of the beta receptor agonists on the antigen dose-response curve can be dissociated into two components: a shift of the curve to the right and reduction of the maximum histamine release.

The present data suggest the following:

1) The beta receptors mediating the shift to the right of the antigen dose-response curve in guinea-pig heart and lung are similar. Also, the beta receptors mediating the reduction of the maximum histamine release are similar in these tissues.
2) The beta receptors mediating the shift to the right of the antigen dose-response curve and reduction of the maximum histamine release are different in each tissue.

One of the most widely used procedures for investigating pharmacological receptors is the relative potencies of agonists. From studies using catecholamine agonists, the adrenergic receptors have been classified into alpha and beta (Ahlquist, 1948) and the beta receptors are later

subclassified into $\underline{\text{beta}}_1$ and $\underline{\text{beta}}_2$ (Lands et al., 1967a; b). Under proper experimental conditions, similar relative potencies for a series of agonists for eliciting a response in two different tissues would indicate that these tissues contain the same type of receptors. On the other hand, dissimilar relative potencies would indicate the presence of different types of receptors in these tissues.

Selective beta receptor agonists such as orciprenaline, terbutaline and soterenol have been demonstrated to inhibit antigen-induced histamine release from sensitized guinea-pig lung (Sorenby, 1974; Malta and Raper, 1975). These agents are generally less potent than isoproterenol. Studies using selective beta receptor agonists and antagonists to characterize beta receptors modulating antigen-induced histamine release in guinea-pig lung in comparison to those in guinea-pig atria ($\underline{\text{beta}}_1$) and those in the trachea ($\underline{\text{beta}}_2$) are inconsistent (Sorenby, 1974, Malta and Raper, 1975). These studies can be criticized on the basis that pharmacological environments were not properly controlled; therefore, factors such as neuronal and extraneuronal uptake, indirect sympathomimetic activity and possible stimulation of alpha receptors could alter the observed potencies of the adrenergic agonists. In the present study, these

interfering factors have been minimized by using reserpine-pretreated animals and phenoxybenzamine incubation. Under these controlled experimental conditions, similar relative potencies were obtained for the selected beta receptor agonists in mediating the shift to the right of the antigen dose-response curve and reduction of the maximum histamine release in guinea-pig heart and lung. This suggests that between these tissues, the beta receptors mediating these responses are of the same type.

Furthermore, in each tissue, different relative potencies were obtained for the beta receptor agonists in mediating the shift to the right of the antigen dose-response curve and reduction of the maximum histamine release. Despite a large variability associated with the data, it is suggested that there are different beta receptor types mediating these effects in guinea-pig heart and lung. This suggestion was based on the following observations:

1) The two effects produced by the beta agonists on the antigen dose-response curve (rightward shift and reduction of maximum histamine release) occurred at different agonist concentrations. This relationship was observed in the heart as well as the lung of the guinea pig.

2) Dissimilar orders of relative potencies of beta agonists were obtained for producing these two responses on the antigen dose-response curve. This was observed in both guinea-pig heart and lung.
3) Dissimilar isomeric-potency-differences were obtained for eliciting the shift to the right of the antigen dose-response curve and reduction of the maximum histamine release. This was also observed in both guinea-pig heart and lung.
4) In the guinea-pig lung, a selected concentration of a cardioselective beta receptor antagonist, practolol, was capable of antagonizing the ability of isoproterenol to shift the antigen dose-response curve without altering the ability to reduce the maximum histamine release (Buckner et al., in press). In the presence of practolol, the shift of the antigen dose-response curve produced by isoproterenol was reduced by 71%, whereas the reduction of the maximum histamine release was only inhibited by 25%.

5) In the guinea-pig lung, 10^{-4}M and 10^{-9}M isoproterenol produced the same degree of shift of the antigen dose-response curve (Buckner et al., in press). The shift of the antigen dose-response curve produced by 10^{-4}M isoproterenol was only 22% of the maximum shift obtained with isoproterenol. This suggested the possibility of the development of desensitization at large concentration of isoproterenol. However, desensitization did not occur on the level of reduction of maximum histamine release and 10^{-4}M isoproterenol was still able to produce 75% of the maximum effect obtained with isoproterenol on reduction of the maximum histamine release. In the present experiments, 10^{-4}M isoproterenol did not produce desensitization in its ability to shift the antigen dose-response curve. This might be due to the difference in experimental design and the fact that tissues used in the present experiments were taken from reserpine-pretreated animals and exposed to phenoxybenzamine and indomethacin.

Theoretically, if accurate dissociation constants for the agonist-receptor complexes could be determined for the agonists, they would be identical for identical receptors. Since it is generally agreed that dose-response curves produced by the agonists are not necessarily equivalent to the receptor-occupancy curves due to the possible presence of a receptor reserve (Furchgott, 1966; Stephenson, 1956; Ward, 1968), relative dissociation constants are a more sensitive criterion than the relative potencies of the agonists in differentiation of receptors. In the absence of an available irreversible competitive antagonist for the beta adrenergic receptors, it is difficult to estimate dissociation constants for beta receptor agonists. Only recently, other indirect pharmacological methods have been used to estimate dissociation constants for soterenol and isoproterenol on both mechanical and cyclic AMP responses in rat atria and guinea-pig trachea (Buckner and Saini, 1975; Buckner et al. 1978; Wong and Buckner, 1978).

In the guinea-pig heart and lung, isoproterenol is not capable of completely abolishing antigen-induced histamine release. Because an irreversible beta antagonist is not available, it is difficult to quantitate the receptor reserve for isoproterenol in producing the effect in these

tissues. However, lack of complete inhibition of antigen-induced histamine release by isoproterenol suggests the absence of a receptor reserve for isoproterenol in the guinea-pig heart and lung. Furthermore, other beta receptor agonists including soterenol and sulfonterol are being classified as 'partial agonists' relative to isoproterenol in the lung. Therefore, relative potencies for beta receptor agonists obtained in the guinea-pig lung represent relative dissociation constants for the agonist-receptor complexes.

In the guinea-pig heart, soterenol and sulfonterol are almost as effective as isoproterenol in modulating antigen-induced responses. However, if it is assumed that there is no 'receptor reserve' for isoproterenol due to its inability to totally abolish antigen effects, then in the guinea-pig heart, there is no receptor reserve for all the beta receptor agonists tested. It follows that, in the guinea-pig lung as well as in the heart, relative potencies for beta receptor agonists represent relative dissociation constants for the agonist-receptor complexes. This indicates that the relative potencies obtained in this study provide a useful criterion in differentiating beta adrenergic receptors.

Although the relative potencies of the beta receptor agonists for producing tracheal relaxation and cardiac stimulation are not obtained in the same study, we have attempted a rough classification of the anti-anaphylactic beta receptors of the guinea-pig heart and lung using data from the literature. Some of this data was obtained under improper experimental conditions or based on activity comparison of the agonists in intact animals (Table 19). Only the data from the guinea-pig lung was used for the purpose of comparison. For all four responses, isoproterenol was the most potent beta receptor agonist. Soterenol and sulfonterol, both of which are classified as bronchoselective beta receptor agonists, were more potent in producing tracheal relaxation than producing chronotropic effect in the atria. On the other hand, H 80/62, a cardioselective beta receptor agonist, had essentially zero activity in producing anti-anaphylactic responses in the lung and had not been tested on isolated guinea-pig trachea.

From this available data, a comparison of the relative potencies of the beta receptor agonists reveal two interesting observations:

Table 19 - The Effects of (-)-Isoproterenol, (-)-Soterenol, (±)-Sulfonterol and (-)-H 80/62 on Sensitized Guinea-Pig Lung, Isolated Guinea-Pig Trachea and Atria.

Agonist	Lung (Inhibition of Histamine Release)[a]				Trachea (Relaxation)		Atria (Chronotropic Effect)	
	Method I		Method II					
	-Log Molar ED50	Relative Potencies	-Log Molar ED50	Relative Potencies	-Log Molar ED50	Relative Potencies	-Log Molar[b] ED50	Relative Potencies
(-)-Isoproterenol	7.59	1	9.00	1	9.69[c]	1	8.70[d]	1
(-)-Soterenol	7.06	.29	7.60	.040	9.14[c]	.30	7.78[d]	.01
(±)-Sulfonterol	6.65	.11	7.17	.015	7.77[e]	.012	4.55[e]	.0001
(-)-H 80/62	N[e]	0	N[f]	0	--	--	--	.01[g]

[a] Data were taken from Tabel 15.

[b] For (±)-sulfonterol, value represented -Log ED_{25}.

[c] Buckner and Saini (1975). Data were obtained from tissues taken from reserpine-pretreated animals and exposed to tropolone and phentolamine.

[d] Buckner and Abel (1974). Data were obtained in the presence of tropolone and phentolamine.

[e] Kaiser et al., (1975).

[f] A meaningful ED50 value could not be otained.

[g] Data were obtained from anesthetized cats in vivo, Carlsson et al., (1977).

1) Similar relative potencies exist for the beta receptor agonists in producing the shift to the right of the antigen dose-response curve and tracheal relaxation. This suggests that the beta receptors mediating these responses are similar and may be classified as the $beta_2$ type.

2) Three distinctly different relative potencies for the beta receptor agonists are present suggesting that the beta receptors mediating reduction of the maximum histamine release in the lung have some different characteristics from those responsible for tracheal relaxation and for chronotropic response in the atria. Furthermore, it suggests that the beta receptors mediating reduction of the maximum histamine release in the lung cannot be classified according to the classical $beta_1$/ $beta_2$ receptor concept.

Additional support for the classification of the beta adrenergic receptors by the relative potencies of the beta agonists was provided by studies using optical isomers of isoproterenol. Stereochemical selectivity is suggested to be a useful experimental criterion in differentiation of

adrenergic receptors since it is assumed to reflect molecular configurations of the recognition sites of receptors involved. The use of enantiomers also provides a better control of the physiochemical factors determining accesses of different drug molecules to receptor sites (Patil, 1969). On the basis of similar isomeric-potency-differences between guinea-pig heart and lung for eliciting the shift to the right of the antigen dose-response curve and reduction of the maximum histamine release, it is suggested that similar beta receptors are present in these tissues mediating these responses. Furthermore, dissimilar isomeric-potency-differences for eliciting the shift of the antigen dose-response curve and reduction of maximum histamine release in guinea-pig heart and lung indicates that the beta receptors involved have different characteristics. However, it must be noted that the isomeric-potency-differences for these responses are associated with a large variability and are not statistically different from each other. Thus, the data may only represent an apparent difference and not a real difference between the receptors involved.

One assumption associated with the use of optical isomers in differentiation of adrenergic receptors is that the activity of the less potent (+)-isomer is not due to

contamination of the more potent (-)-isomer. In the absence of a pure standard for comparison, the degree of purity of a given sample of the (+)-isomer cannot be determined. However, absolute stereochemical purity, though desirable, is not essential in pharmacological experiments provided that the same sample of (+)-isoproterenol is used in all studies and the (+)-isomer does not have zero activity. This relationship is predicted by the Easson and Stedman (1933) hypothesis which states that the (+)-isomer of adrenergic compounds like isoproterenol are less potent than the (-)-isomers because their β-hydroxyl group, being oriented away from the receptor, do not contribute to the drug-receptor interaction. In such cases, the (+)-isomers should be equipotent to the corresponding desoxy derivatives in which their β-hydroxyl group is absent. It has been subsequently demonstrated that (+)-isomers are equal in potency to the corresponding desoxy derivatives in most cases (Patil *et al.*, 1970). In rat atria and uteri, the desoxy derivative of isoproterenol is equal in potency to (+)-isoproterenol for producing cardiac stimulation and uterine relaxation (Birnbaum *et al.*, 1975). This suggests that (+)-isoproterenol does indeed have its own activity. However, since the desoxy

derivative of isoproterenol has not been tested for inhibition of histamine release in the study, it is still not certain whether the effects produced by (+)-isoproterenol are elicited mainly by that isomer.

In view of the dissimilar relative potencies of the beta receptor agonists and dissimilar isomeric-potency-differences for isoproterenol, it seems possible that in the guinea-pig heart or lung there may exist two different beta receptor types responsible for eliciting different effects on the antigen dose-response curves. The beta receptors mediating the shift to the right of the antigen dose-response curve may be classified as the $beta_2$ type; whereas the beta receptors mediating the reduction of the maximum histamine release cannot be characterized by the classical $beta_1/beta_2$ receptor concept. This interpretation is in agreement with data from radioligand binding studies suggesting that at least two different types of beta adrenergic receptors are present in lung tissue (Barnett et al., 1978). Furthermore, it has been recently reported that the beta receptors involved in the inhibition of antigen-induced histamine release in the guinea-pig lung may be heterogeneous (Barrett-Bee and Lees, 1978). In that study, results obtained from using beta receptor agonists indicated a $beta_2$-receptor mediated

response, whereas those obtained with selective beta receptor antagonists suggested the response is mediated by $beta_1$ receptors.

If there exists two different types of beta receptors within guinea-pig heart or lung, these beta receptors may differ in function as well as anatomical distribution in each tissue. Owing to the heterogeneous cell populations, the beta receptors can be both present on the target cell, or one on the target cell and the other on another cell type, or both on another cell type. However, it is believed that the beta receptors which modulate histamine release are located on the mast cell membrane (Johnson et al., 1974; Pearce et al., 1977), then the latter possibility seems very unlikely. Regardless of the anatomical location, activation of the beta receptors on the other cell types can modulate antigen-induced histamine release by elaborating and releasing endogenous substances such as prostaglandins and adenosine, which is degraded from ATP. These substances then act on the mast cells in a 'negative feedback' mechanism to inhibit antigen-induced histamine release (Tauber et al., 1973).

The effects on antigen-induced histamine release resulting from activation of the beta receptors on the mast cells may either be a cyclic AMP-mediated or a non-cyclic AMP mediated event. Thus, it is possible that the

effects of one type of the beta receptor is cyclic AMP-mediated, whereas the effects of the other is non-cyclic AMP mediated. At present, we have no data to support this hypothesis. Some of the non-cyclic AMP mediated adrenergic effects on histamine release may result from actions on influx of calcium into the mast cells, calcium mobilization and utilization within the cells and the elaboration of endogenous substances such as prostaglandins which are capable of modulating anaphylactic release of histamine.

The presence of indomethacin in our experiment should not influence the relative potencies of the beta agonists nor the isomeric-potency-differences for isoproterenol. This is suggested by the fact that, in the absence of indomethacin, the antigen dose-response curve was reduced in a similar manner by isoproterenol in the guinea-pig lung (Buckner et al., in press). Although indomethacin in our lung experiments produced a potentiating effect over the whole range of antigen concentrations, the maximum degree of shift of the antigen dose-response curve and the maximum reduction of control release of histamine produced by isoproterenol were only slightly different. Therefore, data obtained in our study should remain the same regardless of the presence of indomethacin.

Enhancement of the antigen-induced histamine release by indomethacin in the guinea-pig lung is consistent with observations that this inhibitor of the cyclooxygenase pathway of arachidonate metabolism can augment the release of anaphylactic mediators (Adcock et al., 1978a; Engineer et al., 1978; Hitchcock, 1978). The precise mechanism for this effect is not known, but several explanations have been proposed:

1) By inhibiting cyclooxygenase indomethacin reduces the endogenous level of prostaglandins which may influence the release of anaphylactic mediators (Tauber et al., 1973).
2) Indomethacin may act preferentially to inhibit the formation of prostaglandins and to a lesser extent thromboxanes (Boot et al., 1977). It has been shown that thromboxane B_2 in the amount released during anaphylaxis from guinea-pig lung (Dawson et al., 1976) increased the amount of SRS-A release from guinea-pig chopped lung (Boot et al., 1977).
3) Inhibition of the cyclooxygenase pathway of arachidonate metabolism results in an increase in substrate available to the lipoxygenase pathway. Since SRS-A may be a metabolite of

arachidonate via this pathway (Jakschik et al., 1977; Adcock et al., 1978b), an increased supply of arachidonate would result in an increased release of mediators. This view is supported by the observations that inhibitors of both cyclooxygenase and lipoxygenase pathways of arachidonate metabolism such as 5,8,11,14-eicosatetraynoic acid did not alter release of anaphylactic mediators (Adcock et al., 1978a; Hitchcock, 1978).

It is interesting to observe that indomethacin did not alter antigen-induced histamine release in the guinea-pig heart. One possibility is that prostaglandins are not elaborated and released from the heart during anaphylaxis and thus exert no modulatory effect on histamine release. However, this seems unlikely because it has recently been reported that prostaglandins and thromboxanes were released from perfused sensitized guinea-pig heart after antigen challenge (Anhut et al., 1978). Another possibility is that the lipoxygenase pathway of the arachidonate metabolism is deficient in the heart. Subsequently, an increased supply of arachidonate due to inhibition of the cyclooxygenase does not result in an increased release of mediators. Finally, it is possible that the effects of

indomethacin on antigen-induced histamine release are tissue-dependent and may relate to the level of endogenous prostaglandins present in each tissue. Immunological release of mediators from guinea-pig lung has been suggested to be regulated in part by the de novo synthesis of prostaglandins (Hitchcock, 1978).

It has been suggested that histamine secreted from human peripheral basophilic leukocytes could inhibit its own release through stimulation of cyclic AMP increases via the H_2 receptors (Bourne et al., 1972). H_2 receptor antagonists have been shown to enhance reversed anaphylactic release of histamine from monkey skin (Yamamoto et al., 1976), but did not alter anaphylactic release of histamine from rat lung (Chakrin et al., 1974) or human lung (Platshon and Kaliner, 1978). The apparent enhancement of histamine release by H-2 antagonists may partly be a result of reduced histamine metabolism (Yamamoto et al., 1976). Our data demonstrating that metiamide did not alter antigen-induced histamine release in guinea-pig lung is consistent with the observations that H_2 antagonists produce no effects on anaphylactic release of histamine in lung tissues.

CONCLUSIONS

The results of the present study suggest the following:

1) the beta receptors mediating the shift to the right of the antigen dose-response curve in guinea-pig heart and lung are similar. Also, the beta receptors mediating the reduction of the maximum histamine release in these tissues are similar.
2) the beta receptors mediating the shift to the right of the antigen dose-response curve and reduction of the maximum histamine release are different in each tissue.

APPENDIXES

Table 20 - Effects of Drug Treatments on Histamine Contents of Guinea Pig Lung and Heart.

Treatment	Total Histamine Content (μg/g)[a] Lung	Heart
Control[c]	29 ± 3 (12)[b]	7.2 ± .8 (5)[b]
Reserpine[d]	22 ± 5 (3)	6.7 ± .6 (4)
Phenoxybenzamine[d] 10^{-5}M	24 ± 3 (6)	7.2 ± .8 (4)
Indomethacin[e] 10^{-5}M	29 ± 3 (7)	6.8 ± .6 (4)
Indomethacin, 10^{-5}M[e] + Metiamide, $5x10^{-4}$M	34 ± 2 (3)	
Metiamide[c] $5x10^{-4}$M	25 ± 3 (2)	

[a] Mean ± S.E.M. None of the treated values were significantly different ($P < .05$) from control value.

[b] Number in parentheses represents number of observations.

[c] Tissues were taken from ovalbumin-sensitized animals.

[d] Tissues were taken from ovalbumin-sensitized animals pretreated with reserpine (5 mg/Kg i.p.) 16-24 hours previously.

[e] Tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to phenoxybenzamine.

Table 21 - Effects of Inhibitors of Histamine Metabolism on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

Treatments	Ovalbumin -Log ED50[b] Control	Ovalbumin -Log ED50[b] Treated	Maximum % Histamine Release[c] Control	Maximum % Histamine Release[c] Treated	n[d]
Quinacrine 10^{-4} M	3.14	3.07	47	34	1
Aminoguanidine 10^{-4} M	3.14	3.00	32	24	1
Iproniazid 10^{-4} M	3.04	3.09	38	38	1
Quinacrine + Aminoguanidine + Iproniazid	3.11	2.45	32	23	1

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine.

[b] Calculated from curves plotting % of maximum response in each curve.

[c] Maximum amount of histamine release relative to total histamine content.

[d] Number of observations.

Table 22 - Effects of Beta Agonists on Ovalbumin-Induced Histamine Release from Guinea-Pig Minced Lung.[a]

Agonist	Per Cent Maximum With S.E.M.[b] Concentrations (M)						
	$1x10^{-9}$	$1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$1x10^{-3}$
Method I							
(-)-Isoproterenol	21 ± 2(4)[c]	46 ± 10(10)	56 ± 17(10)	87 ± 24(7)	100 ± 20(1)	76 ± 31(4)	-
(-)-Soterenol	-	30 ± 7(4)	48 ± 6(6)	81 ± 14(6)	96 ± 24(6)	100 ± 10(6)	-
(±)-Sulfonterol	-	36 ± 6(3)	43 ± 6(6)	60 ± 13(6)	86 ± 24(4)	100 ± 16(6)	74 ± 28(5)
Method II							
(-)-Isoproterenol	50 ± 15(4)	94 ± 6(10)	94 ± 15(10)	100 ± 14(7)	88 ± 12(10)	82 ± 18(4)	-
(-)-Soterenol	-	41 ± 33(4)	58 ± 29(6)	100 ± 23(6)	57 ± 19(6)	93 ± 17(6)	-
(±)-Sulfonterol	-	4 ± 20(3)	64 ± 9(6)	100 ± 29(6)	77 ± 9(4)	54 ± 2(6)	66 ± 17(5)
Method III							
(-)-Isoproterenol	17 ± 1(4)	43 ± 9(10)	57 ± 9(10)	87 ± 21(7)	100 ± 21(10)	65 ± 21(4)	-
(-)-Soterenol	-	26 ± 5(4)	42 ± 2(6)	84 ± 15(6)	86 ± 15(6)	100 ± 12(6)	-
(±)-Sulfonterol	-	34 ± 8(3)	45 ± 6(6)	68 ± 14(6)	92 ± 25(4)	100 ± 16(6)	74 ± 24(5)

[a] All tissues were taken from reserpine-pretreated ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine.

[b] The response to each agonist concentration was calculated as a percentage of the maximum response produced by that agonist.

[c] Number in parentheses represents number of observations.

Table 23 - Effects of Beta Agonists on Ovalbumin-Induced Histamine Release from Guinea-Pig Minced Lung.[a]

Agonist	Per Cent (-)-Isoproterenol Maximum with S.E.M.[b] Concentrations (M)						
	$1x10^{-9}$	$1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$1x10^{-3}$
Method I							
(-)-Isoproterenol	21 ± 2(4)[c]	46 ± 10(10)	56 ± 17(10)	87 ± 24(7)	100 ± 19(10)	76 ± 31(4)	-
(-)-Soterenol	-	15 ± 4(4)	23 ± 3(6)	39 ± 7(6)	46 ± 11(6)	47 ± 5[d](6)	
(±)-Sulfonterol	-	10 ± 2(3)	13 ± 2(6)	17 ± 4(6)	25 ± 7(4)	29 ± 5[d](6)	21 ± 8(5)
(-)-H 80/62	-	-	11 ± 4(4)	9 ± 2(4)	9 ± 1(4)	11 ± 2(4)	19 ± 3[d](4)
Method II							
(-)-Isoproterenol	50 ± 15(4)	94 ± 6(10)	94 ± 5(10)	100 ± 14(7)	88 ± 12(10)	82 ± 18(4)	-
(-)-Soterenol	-	28 ± 23(4)	28 ± 19(6)	68 ± 15(6)	39 ± 13(6)	63 ± 11(6)	-
(±)-Sulfonterol	-	3 ± 21(3)	34 ± 5(6)	53 ± 15[d](6)	41 ± 5(4)	28 ± 11(6)	35 ± 9(5)
(-)-H 80/62	-	-	13 ± 12(4)	4 ± 13(4)	20 ± 9(4)	3 ± 7(4)	9 ± 15(4)
Method III							
(-)-Isoproterenol	17 ± 1(4)	43 ± 9(10)	57 ± 9(10)	87 ± 21(7)	100 ± 21(10)	65 ± 21(4)	-
(-)-Soterenol	-	10 ± 2(4)	16 ± 1(6)	32 ± 6(6)	33 ± 1(6)	39 ± 5[d](6)	
(±)-Sulfonterol	-	7 ± 2(3)	9 ± 1(6)	14 ± 3(6)	19 ± 5(4)	20 ± 3[d](6)	15 ± 5(5)
(-)-H 80/62	-	-	8 ± 3(4)	6 ± 1(4)	5 ± 1(4)	7 ± 1(4)	12 ± 2[d](4)

Table 23 continued

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[a]All tissues were taken from reserpine-pretreated ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine.

[b]The response to each agonist concentration was calculated as a percentage of the maximum response produced by (-)-isoproterenol.

[c]Number in parentheses represents number of observations.

[d]Values significantly different from (-)-isoproterenol maximum ($P < .05$) as determined by Student's unpaired 't' test.

Table 24 - Effects of Beta Agonists on Ovalbumin-Induced Histamine Release from Guinea-Pig Minced Heart.[a]

Agonist	Per Cent Maximum with S.E.M.[b] Concentrations (M)						
	$1x10^{-9}$	$1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$1x10^{-3}$
Method I							
(-)-Isoproterenol	23 ± 3(5)[c]	43 ± 7(9)	73 ± 13(9)	83 ± 24(6)	100 ± 17(10)	95 ± 23(5)	-
(-)-Soterenol	-	35 ± 4(3)	51 ± 6(5)	83 ± 20(5)	81 ± 24(5)	100 ± 18(5)	-
(±)-Sulfonterol	-	24 ± 8(3)	34 ± 8(5)	68 ± 7(5)	79 ± 23(4)	100 ± 22(5)	81 ± 6(2)
Method II							
(-)-Isoproterenol	48 ± 12(5)	73 ± 11(9)	79 ± 12(9)	79 ± 8(6)	100 ± 11(10)	96 ± 16(5)	-
(-)-Soterenol	-	30 ± 20(3)	56 ± 7(5)	100 ± 14(5)	74 ± 21(5)	99 ± 31(5)	-
(±)-Sulfonterol	-	-	4 ± 23(3)	89 ± 26(5)	100 ± 33(5)	68 ± 22(4)	55 ± 15(2)
Method III							
(-)-Isoproterenol	18 ± 2(5)	38 ± 7(9)	64 ± 10(9)	72 ± 21(6)	100 ± 15(10)	91 ± 20(5)	-
(-)-Soterenol	-	23 ± 4(3)	37 ± 4(5)	87 ± 26(5)	64 ± 20(5)	100 ± 26(5)	-
(±)-Sulfonterol	-	19 ± 7(3)	48 ± 9(5)	89 ± 9(5)	89 ± 25(4)	100 ± 20(5)	90 ± 0(2)

[a] All tissues were taken from reserpine-pretreated ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine.

[b] The response to each agonist concentration was calculated as a percentage of the maximum response produced by that agonist.

[c] Number in parentheses represents number of observations.

Table 25 - Effects of Beta Agonists on Ovalbumin-Induced Histamine Release from Guinea-Pig Minced Heart.[a]

	Per Cent (-)-Isoproterenol Maximum with S.E.M.[b] Concentrations (M)						
Agonist	$1x10^{-9}$	$1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$1x10^{-3}$
Method I							
(-)-Isoproterenol	23 ± 3(5)[c]	43 ± 7(9)	73 ± 13(9)	83 ± 24(6)	100 ± 17(10)	95 ± 23(5)	-
(-)-Soterenol	-	37 ± 4(3)	54 ± 6(5)	89 ± 21(5)	86 ± 25(5)	106 ± 19[d](5)	-
(±)-Sulfonterol	-	14 ± 6(3)	23 ± 6(5)	47 ± 5(5)	55 ± 16(4)	70 ± 15[d](5)	57 ± 4(2)
(-)-H 80/62	-	-	-	19 ± 5(2)	18 ± 3(2)	18 ± 2(4)	78 ± 29[d](4)
Method II							
(-)-Isoproterenol	48 ± 12(5)	73 ± 11(9)	79 ± 12(9)	79 ± 8(6)	100 ± 11(10)	96 ± 16(5)	-
(-)-Soterenol	-	28 ± 19(3)	53 ± 7(5)	94 ± 13[d](5)	69 ± 19(5)	92 ± 29(5)	-
(±)-Sulfonterol	-	-3 ± 18(3)	68 ± 20(5)	80 ± 26[d](5)	75 ± 16(4)	23 ± 12(5)	43 ± 14(2)
(-)-H 80/62	-	-	-	40 ± 70(2)	23 ± 13(2)	37 ± 24[d](4)	26 ± 13(4)
Method III							
(-)-Isoproterenol	18 ± 2(5)	38 ± 7(9)	64 ± 10(9)	72 ± 21(6)	100 ± 18(10)	91 ± 20(5)	-
(-)-Soterenol	-	27 ± 4(3)	42 ± 5(5)	98 ± 29(5)	73 ± 23(5)	114 ± 26[d](5)	-
(±)-Sulfonterol	-	9 ± 4(3)	23 ± 4(5)	42 ± 4(5)	42 ± 12(4)	48 ± 10[d](5)	43 ± 0(2)
(-)-H 80/62	-	-	-	14 ± .2(2)	11 ± 1(2)	13 ± 2(4)	56 ± 23[d](4)

Table 25 continued

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[a] All tissues were taken from reserpine-pretreated ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine.

[b] The response to each agonist concentration was calculated as a percentage of the maximum response produced by (-)-isoproterenol.

[c] Number in parentheses represents number of observations.

[d] Values were not significantly different from (-)-isoproterenol maximum ($P < .05$) as determined by Student's unpaired 't' test.

Table 26 - Effects of Enantiomers of Isoproterenol on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Lung.[a]

Agonist	Per Cent Maximum with S.E.M.[b] Concentrations (M) $1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$3x10^{-4}$
Method I						
(+)-Isoproterenol	-	18 ± 3(3)[c]	39 ± 14(4)	68 ± 12(4)	100 ± 15(4)	66 ± 33(2)
(-)-Isoproterenol	37 ± 12(3)	66 ± 22(3)	-	100 ± 27(3)	-	-
Method II						
(+)-Isoproterenol	-	18 ± 17(3)	51 ± 10(4)	100 ± 19(4)	78 ± 21(4)	98 ± 8(2)
(-)-Isoproterenol	98 ± 12(3)	69 ± 23(3)	-	100 ± 14(3)	-	-
Method III						
(+)-Isoproterenol	-	12 ± 1(3)	29 ± 9(4)	79 ± 23(4)	100 ± 22(4)	74 ± 40(2)
(-)-Isoproterenol	35 ± 9(3)	58 ± 20(3)	-	100 ± 24(3)	-	-

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Data were obtained in "paired" experiments.

[b] The response to each isomer concentration was calculated as a percentage of the maximum response produced by that isomer.

[c] Number in parentheses represents number of observations.

Table 27 - Effects of Enantiomers of Isoproterenol on Ovalbumin-Induced Histamine Release from Guinea Pig Minced Heart.[a]

Agonist	Per Cent Maximum with S.E.M.[b] Concentrations (M) $1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$3x10^{-4}$
Method I						
(+)-Isoproterenol	-	35 ± 9(4)[c]	44 ± 12(4)	68 ± 6(5)	100 ± 23(5)	77 ± 45(2)
(-)-Isoproterenol	47 ± 5(3)	67 ± 7(3)	-	100 ± 23(4)	-	-
Method II						
(+)-Isoproterenol	-	29 ± 25(4)	67 ± 24(4)	100 ± 93(5)	74 ± 18(5)	99 ± 4(2)
(-)-Isoproterenol	98 ± 38(3)	77 ± 20(3)	-	100 ± 29(4)	-	-
Method III						
(+)-Isoproterenol	-	25 ± 5(4)	45 ± 16(4)	74 ± 4(5)	100 ± 27(5)	90 ± 56(2)
(-)-Isoproterenol	47 ± 8(3)	59 ± 3(3)	-	100 ± 27(4)	-	-

[a] All tissues were taken from reserpine-pretreated, ovalbumin-sensitized animals and exposed to indomethacin and phenoxybenzamine. Data were obtained in "paired" experiments.

[b] The response to each isomer concentration was calculated as a percentage of the maximum response produced by that isomer.

[c] Number in parentheses represents number of observations.

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