A STRUCTURAL CHARACTERIZATION OF

THE DOG MYOCARDIAL ADRENERGIC RECEPTORS

by

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ABSTRACT

The chronotropic and inotropic responses to isoprenaline and salbutamol were determined in the chloralose anaesthetized dog. The myocardium was denervated, sympathetically and parasympathetically to prevent direct neural influence on the heart rate and myocardial contractility. The heart rate was determined from the E.C.G. Myocardial contractility was indicated by the change in the maximum rate of rise of left ventricular pressure (dP/dt max) at a constant electrically paced heart rate.

The structure-activity relationships for salbutamol and isoprenaline were determined from dose-response curves and by plotting the change in contractility (ΔdP/dt max) against the change in heart rate (ΔHR). The data obtained from this series of experiments indicated that the only difference between the effects of the agonists on the inotropic and chronotropic responses of the myocardium was the lower affinity of salbutamol for the adrenergic receptor as indicated by the 100 times greater concentration required to produce the same response level.

Previously reported in vitro studies with the guinea pig atrium and dog papillary muscle had indicated that a smaller inotropic response to salbutamol should have been expected. To test this discrepancy between the present in vivo experimentation, and the previous in vitro work, studies were designed to test the guinea pig atrium and the dog papillary muscle in vitro.

The effects of the agonists were studied on the isolated guinea pig atrium in a manner that paralleled the

in vivo dog study. With the organ bath at 25°C, the chronotropic response, measured by the change in free contraction rate (ΔR), and the inotropic response, determined from the change in peak tension developed (ΔT) during electrical stimulation at 2 Hz, to a single randomly ordered dose of salbutamol or isoprenaline were determined. Salbutamol acted as a partial agonist, that is, had a lower efficacy than isoprenaline. However, the relative effect of each drug on the inotropic and chronotropic responses was almost identical.

In the isolated dog papillary muscle, salbutamol displayed a much lower efficacy, producing only 20% of the maximum isoprenaline increase in peak tension developed to the cummulative addition of agonist. The affinity of salbutamol for the adrenergic receptor in this preparation was much lower than that observed in vivo when compared with isoprenaline, 5,000:1 and 100:1 respectively.

The structure-activity relationships for salbutamol and isoprenaline showed that the relative effects of these agonists on the in vivo denervated dog myocardial inotropic and chronotropic responses were similar. This observation indicates that the adrenergic receptors of the dog myocardium mediating the inotropic and chronotropic responses are structurally similar at a site complementary to the phenyl ring of the agonist molecule. However, a definite conclusion regarding the adrenergic receptors responsible for the inotropic response cannot be made because of the unexplained difference in inotropic response observed with ventricular

muscle *in vivo* and *in vitro*. Examination of the structure-activity relationships for salbutamol and isoprenaline in the *in vitro* guinea pig atrium indicates that, in this preparation also, the adrenergic receptors involved in the two measured responses are probably structurally similar.

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The response observed in a physiological system to
an administered neurohumoral agent reflects the agonistic or
antagonistic activity of that agent. Within the sympathetic
nervous system, much experimentation has been carried out to
determine the relative activity of structurally related com-
pounds. Comparison of the structure-activity relationships
of these drugs provides information about the most favourable
molecular composition for response production or inhibition.
Sir Henry Dale (1906) first noted distinct response patterns
within the sympathetic nervous system to a single antagonist.
Since that time, many research workers have studied the
adrenergic receptors in pharmacological experiments and have
observed many different response patterns to the various
agonist and antagonist drugs administered. The observations
of these studies have led to the postulate of structurally
distinct receptor sites reflecting the structure-activity re-
lationships of the drug molecules. The research reported within
this thesis is an attempt to structurally classify the adrenergic
receptors of the myocardium, studying the positive inotropic
and chronotropic responses to two agonists in the _in vivo_
denervated dog heart.

In 1906, the classical paper on the selective antagonism
of adrenaline induced responses by ergot was published by Dale.
The conclusion reached from this study was that the motor or
excitatory responses to adrenaline administration were blocked
by ergot, while no effect was observed on the inhibitory actions
of adrenaline. Dale and Barger (1910) tested a series of
compounds structurally related to adrenaline to determine their

sympathomimetic activity. The observations of this work provided the first indication of various orders of potency for different sympathomimetic agents on systems controlled by sympathetic nervous activity. Cannon and Rosenblueth (1937) interpreted these results to support the existence of two different neurotransmitters, sympathin E and sympathin I. Sympathin E was the excitatory or motor neurotransmitter mediating vasoconstriction, pupil dilatation and the contraction of the ureter, the nictitating membrane and the pregnant uterus, as well as the inotropic and chronotropic responses of the heart. Sympathin I was the postulated transmitter for the inhibitory-type actions of vasodilatation, decreased intestinal motility, relaxation of the bronchi and relaxation of the non-pregnant uterus. However, this scheme was not entirely acceptable even in 1937 when it was first advanced, since it did not completely explain the action of ergot. The myocardium exhibited a motor response to adrenaline administration, but this excitatory action was not effectively blocked by ergot. It was this conflict which lead to the work of Youmans et al. (1940), von Euler (1946), and Ahlquist (1948).

Youmans et al. (1940) first proposed the existence of two receptive mechanisms, but were unable to identify the neurotransmitter agent involved. von Euler (1946) demonstrated that throughout the sympathetic nervous system, the natural neurotransmitter was noradrenaline. This substantiated the concept of two receptive mechanisms explaining the selective blockade by ergot. Further clarification of the proposal of Youmans et al. (1940) came from the work of Ahlquist (1948).

This classical study utilised a series of six amine and

α -carbon substituted (3,4 - dihydroxyphenyl) ethanolamines

to test the receptors of the sympathetic system. The data of

the experiments indicated two classes of adrenergic receptors,

the alpha (α) and beta (β). The α-adrenergic receptors

were stimulated primarily by noradrenaline and adrenaline,

and least by isoprenaline. However, isoprenaline was the

most potent agonist of the β-adrenergic receptor, with

noradrenaline the least potent.

According to the observations of Ahlquist (1948),

activation of the α-adrenergic receptor resulted in vaso-

constriction in the vessels of the viscera and skin, in

contraction of the nictitating membrane, the uterus, the

ureter and the dilator pupillae, and in relaxation of the

intestine. Stimulation of the β-adrenergic receptors re-

sulted in vasodilatation of the skeletal and coronary vessels,

in relaxation of the uterus and bronchi, and in increased rate

and force of contraction in the myocardium.

Within the β -class adrenergic receptors, Lands et al.

(1967) have shown the existence of a further subdivision.

Receptors subserving rate and force of myocardial contraction

and lipolytic responses have been differentiated pharmacolo-

gically from those receptors involved in vasodilatation and

bronchodilatation. The former receptor grouping received the

classification β_1 -adrenergic receptors, while the latter

division were termed β_2 -adrenergic receptors.

An investigation of the structure-activity relation-

ships of a series of α -carbon substituted sympathomimetic

amines led to the suggestion (Lands and Brown, 1964) that a further subdivision of the β_1-adrenergic receptors controlling the myocardium may exist. Their experiments indicated that an increase in the size of the α-carbon substituent led to a greater effect on myocardial contractility than on heart rate in the isolated perfused rabbit heart. The recently discovered sympathomimetic salbutamol (Brittain et al., 1968) (see fig. Ic) has been proposed (Brittain, 1971) to have a relatively greater effect on heart rate than myocardial contractility in the isolated atria of the rat and guinea pig. This was shown by comparing the drug dose of salbutamol to that of isoprenaline required to produce a response equivalent to 50% of the maximum response observed with isoprenaline. This was termed the DR_{50}, values being expressed with isoprenaline equivalent to one. Brittain (1971) reported DR_{50} values of 2500:1 and 500:1 for the effects of salbutamol compared to isoprenaline on myocardial contractility and heart rate respectively. Thus, two studies have shown possible variations in the structure of the receptors involved in the positive inotropic and chronotropic responses of the heart.

To date, only studies using the clinical dose range of salbutamol (0.5 - 2.0μg/kg) have reported the effects of this drug in the denervated dog myocardium (Nayler and McInnes, 1971). However, this small dose range of 0.5 to 2.0μg/ kg produced only very small increases in both heart rate and myocardial contractility and, therefore, did not allow a comparison of the relative effects of salbutamol and isoprenaline on the myocardial inotropic and chronotropic responses. The
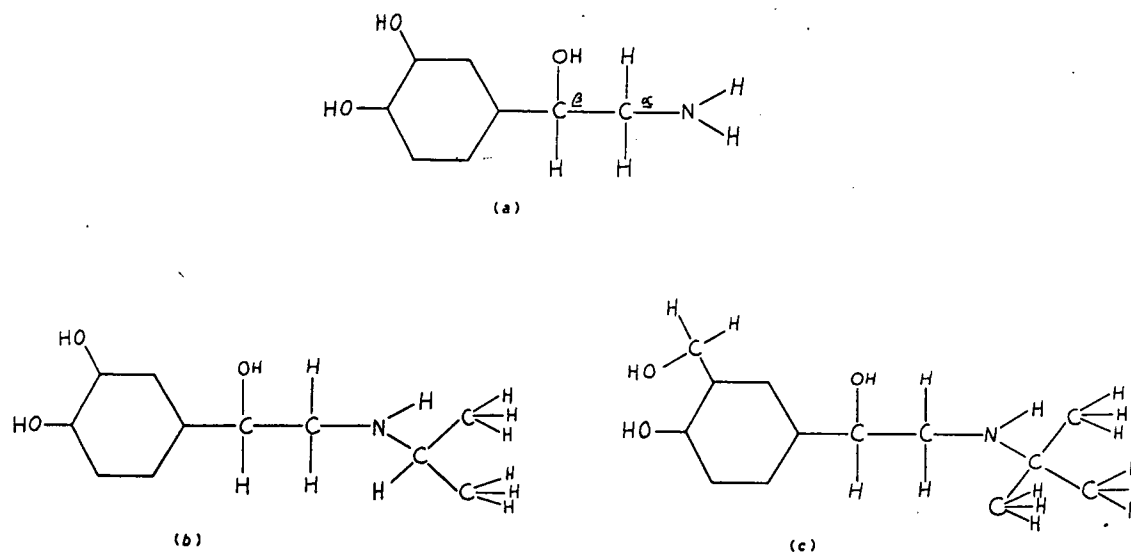
Figure I: Basic catecholamine structure demonstrated by noradrenaline (fig. Ia).
Structure of isoprenaline (fig. Ib) and of salbutamol (fig. Ic).

present study was designed to test the effects of salbutamol and isoprenaline (Fig. Ib and Ic) on the autonomically denervated myocardium of the dog over the full physiological response range. The results were then used to determine the relative effects of the two drugs on myocardial contractility and heart rate in an effort to delineate possible structural variations in the adrenergic receptors involved. Further tests of salbutamol and isoprenaline were carried out on the isolated guinea pig atrium and the isolated dog papillary muscle in an effort to explain the results of the in vivo denervated dog heart study which appeared contradictory to previously published reports.

<u>METHODS</u>

<u>Introduction.</u>

The experimental design incorporated three separate preparations in the study of the positive inotropic and chronotropic responses to salbutamol and isoprenaline. The first group of experiments were designed to test the effects of the two drugs on the inotropic and chronotropic responses of the denervated myocardium of the anaesthetized dog. In the next group, the positive inotropic and chronotropic responses in the isolated guinea pig atrium were assessed. Finally, in the last group, the papillary muscles of dogs, on which the response of the myocardium had been first tested <u>in</u> <u>vivo</u> by the above method, were examined for their positive inotropic responses to the two drugs in an isolated organ bath.

TABLE I

Experiments performed.

Experiment No.	Date	Sex (M or F)	Weight (kg)	Type of experiment
Dog 1	Oct.30/72	M	18	Preliminary
" 2	Nov. 6/72	M	16	"
" 3	Dec.14/72	M	21	
" 4	Jan.30/73	F	18	In vivo denervated dog study (data not included because of different infusion rate)
" 5	Feb. 6/73	M	18	"
" 6	Feb.13/73	M	17.5	In vivo denervated dog study
" 7	Feb.20/73	M	24	"
" 8	Feb.21/73	M	24	
" 9	Feb.28/73	M	29	
" 10	Mar.1 /73	M	28	
" 11	Mar. 6/73	M	26.5	
" 12	Mar. 7/73	M	20.5	
" 13	Mar.13/73	M	20	
Guinea pig 1	Apr. 5/73			Guinea pig atrium preliminary, rate only.
" 2	Apr. 6/73			"
" 3	Apr. 7/73			
" 4	Apr. 8/73			
" 5	Apr. 9/73			
" 6	Apr.10/73			Guinea pig atrium preparation
" 7	Apr.11/73			"
" 8	Apr.12/73			
" 9	Apr.13/73			
" 10	Apr.14/73			
" 11	Apr.16/73			
" 12	Apr.17/73			
" 13	Apr.19/73			
" 14	Apr.20/73			
" 15	Apr.25/73			
" 16	Apr.26/73			
" 17	Apr.27/73			
" 18	May 1/73			

TABLE I. Cont'd.

Experiment No.	Date	Sex (M or F)	Weight (kg)	Type of experiment
Guinea pig 19	May 2 /73			Guinea pig atrium preparation
" 20	May 4 /73			"
Dog 14	May 8 /73	M	14	In vivo denervated dog study, papillary muscle preliminary
" 15	May 18/73	M	16	"
" 16	May 23/73	M	14	In vivo denervated study, papillary muscle study
" 17	May 24/73	M	13	"
" 18	May 25/73	M	10	
" 19	May 28/73	F	11	In vivo data not used " because of low
" 20	May 31/73	M	12	In vivo denervated study "
" 21	June 1/73	M	14	"
" 22	June 4/73	F	14	
" 23	June 6/73	M	14	

Drugs.

Isoprenaline (isoprenaline salt sulfate, lot number 14068, K. & K. Laboratories, Plainview, N.Y.) and salbutamol (salbutamol sulphate, lot number 46257, Glaxo Canada Ltd.) were the drugs used in the comparison of the inotropic and chronotropic effects. Drugs for the in vivo dog preparation were made up in 0.9% NaCl solution in the concentrations, isoprenaline 1 μg/ml and salbutamol 100 μg/ml. For the in vitro isolated preparations, drugs were made up in the following concentrations: isoprenaline, 0.1 μg/ml, 1.0 μg/ml, 100 μg/ml and 1,000 μg/ml; salbutamol 1.0 μg/ml, 10 μg/ml, 100 μg/ml and 1,000 μg/ml, all in 0.9% NaCl. Drug solutions were made up just prior to use to prevent deterioration through oxidation.

In vivo denervated dog experiments:

a) Anaesthesia:

The dogs were subcutaneously injected with morphine sulphate (0.5 mg/kg) one hour prior to induction of anaesthesia. The anaesthetic, chloralose (British Drug Houses), was prepared in a solution of 0.9% NaCl at 60°C in a 1% (w/v) solution. This solution was filtered prior to use to remove undissolved particles. The chloralose was administered via a polyvinyl catheter placed in the saphenous vein, under local anaesthesia (carbocaine 1%), to the level of the inferior vena cava. Approximately 10 to 15 ml/kg was used as the initial dose. During the surgical preparation, additional chloralose (1-2 ml/kg) was given as required. Following the surgery, and at least one-half hour prior to the experimental period, a constant infusion of 0.5% solution of chloralose at a rate of 1.23 ml/min. was begun. This maintained the animal in a constant state of anaesthesia during the experiment.

b) Homeostatic maintenance:

Immediately following the start of surgery, dextran (Travenol, 6% Gentran 75 in 0.9% sodium chloride) infusion was started at a rate of approximately 5 ml/min. to give a total volume of 150 to 250 ml, to supplement fluid loss.

The animal was artificially respired with an oxygen-air mixture, containing approximately 40% oxygen, using a Harvard Respiratory Pump (Harvard Apparatus Co., Inc., Dover, Mass). Stroke volume was adjusted to maintain Pa CO_2 within

the range 35-45 mm Hg as determined by direct measurement.
Arterial blood was obtained from the subclavian artery and
analysed with an Instrumentation Laboratory Inc. pH-Blood Gas
Analyser model 113-51, with values displayed on an Instrumenta-
tion Laboratory Inc. Delta-matic pH/mv Electrometer model 245.
Corrections of blood gases, if necessary, were determined from
a Siggard-Anderson nomogram and made by alterations in the
stroke volume of the respiratory pump and/or infusion of
$NaHCO_3$ (1 N solution). Temperature of the animal was main-
tained between 37 and 38°C using a heated table controlled by
a thermistor probe inserted into the esophagus, activating a
Tele-thermometer control unit (Yellow Springs Instrument Co.,
Inc.)

Dogs number 11 and 19 received intravenous injections
of succinyl choline (1 mg/kg) to eliminate muscular twitching
which interfered with the recording of the E.C.G.

c) Experimental procedure:

Dogs used were mongrel dogs of either sex, 10 - 29 kg
(see Table I). Following administration of anaesthetic, the
trachea was immediately cannulated and the animal artificially
respired. Later, when the chest was opened, a variable re-
sistance in the outflow circuit was adjusted to provide a
3cm H_2O impedence to expiration. The left and right vagus
nerves and the external jugular vein were isolated in the neck.
The femoral artery and vein were isolated in the leg at this
time.

A mid-line incision was used to expose the heart. The

skin and muscle were divided using an electric cautery (Birtcher model 755 Electro-surgical Unit). A surgical saw was used to split the sternum. No sterile precautions were taken. Bleeding was kept to a minimum by tying the internal mammary arteries and by cauterizing the cut surfaces of the bone. The right and left ansae subclaviae were freed at their origins from the stellate ganglia, then crushed with clamps for ten minutes, thus sympathetically denervating the heart (Mizeres, 1955). The subclavian artery was cannulated using a metal cannula (2 mm inside dia.). The pericardium was split and sewn to the walls of the chest to form a pericardial cradle, which supported the heart during pressure recordings. Bipolar silver electrodes were sutured to the right atrium approximately 1 cm apart for pacing the heart, using a Grass model S8 stimulator. A metal cannula (2.0 mm inside dia.) was inserted through the apical dimple into the left ventricle and secured in place by a purse string suture. Both this cannula and the one placed in the subclavian artery were checked to ensure freedom from contact with the wall by withdrawing blood throughout the full cardiac cycle. At this point, cannulae were placed in the femoral vein, for the infusion of the drugs, and in the external jugular vein, for the infusion of chloralose, infusing in both cases with a constant infusion pump (Harvard Apparatus Co.,Inc., Millis, Mass.). The femoral artery, if large enough, was used to insert a small balloon to the level of the descending aorta, which could be inflated to maintain mean resistance to outflow (aortic pressure) constant. The left and right vagus nerves were sectioned

in the neck, completing the denervation of the heart.
The preparation was now ready for the infusion of the drugs.

Recordings made during the experimental period were
all recorded using a Honeywell model 1508 Visicorder (Figs.
II and III). End tidal PCO_2 was measured with a Beckman
Medical Gas Analyser model LB-1. Heart rate was monitored
by the E.C.G. obtained from a Grass model P15 A.C. preamplifier
and by a cardiotachometer (Honeywell Accudata 131 Cardiotacho-
meter. Aortic and ventricular pressure recordings were
obtained from Statham model P23 Gb pressure transducers, with
the signal amplified by Honeywell Accudata 113 bridge amplifiers.
The pressure records were calibrated in a step-wise manner using
a mercury monometer. Zero pressure was obtained post-mortem
with the cannulae tips free in the air. Mean aortic pressure
was determined electrically, using an R-C circuit built into
the Accudata 113 amplifier. Intraventricular pressure was
displayed on a low gain circuit (20 mm Hg/cm) as well as on a
high gain circuit (10 cm H_2O/cm) to determine the left ventri-
cular end diastolic pressure (L.V.E.D.P.). Additionally, the
ventricular pressure recording was differentiated using an
analogue differentiator and recorded as dP/dt, the rate of
change of left ventricular pressure. The differentiating
circuit was tested for linearity using a sine wave, of constant
amplitude, allowed to vary in frequency from 1 to 10 or 10 to
100 Hz at a constant rate with respect to time. In this
manner, the differentiating circuit was found linear from 1 to
greater than 75 Hz (Fig. IV). Calibration of the differentiator,
in mm Hg/sec., was accomplished with a variable frequency sine
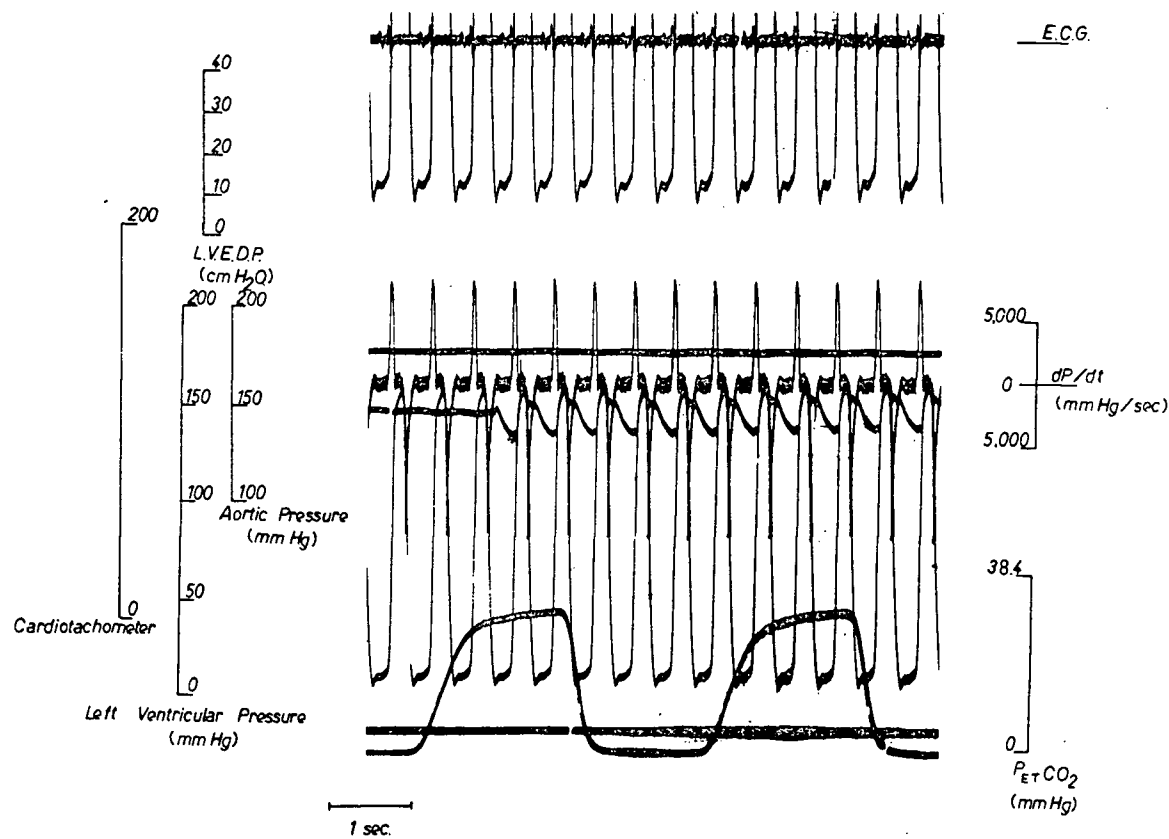
Figure II: The data obtained from the in vivo denervated dog preparation.
The figure shows, from top to bottom, the E.C.G., L.V.E.D.P., cardiotachometer,
dP/dt, aortic blood pressure and mean aortic blood pressure ($\overline{BP}$), left
ventricular pressure and end-tidal PCO_2 in the unpaced, pre-drug dog. Time
bar = 1 sec.
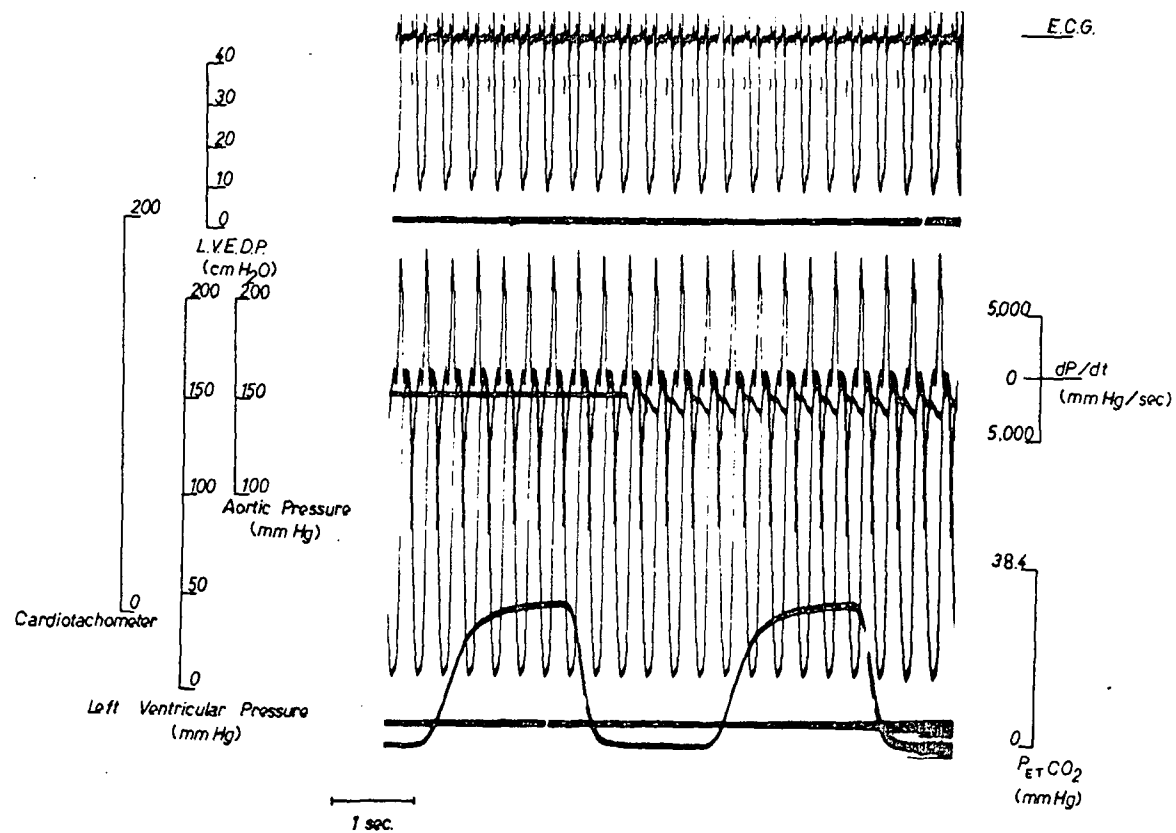
Figure III: The same data as presented in Figure II obtained during electrical pacing of the heart in the same pre-drug animal. Time bar = 1 sec.

wave generator, the output of which was set electrically equivalent to 50 mm Hg. With the output maintained equivalent to 50 mm Hg, the amplitude of the differentiated signal was measured at frequencies of 5 - 40 Hz, and the relationship of the signal to the input frequency determined from the mathematical relationship:

$$y = A \sin \omega t$$

$$dy/dt = A \omega \cos \omega t$$

where, dy/dt = any point on the differentiated sine wave, $A \sin \omega t$, at any time t.

A = amplitude of input signal (50 mm Hg)

$\omega = 2 \pi f$ (f= frequency)

The calibration plot has been presented in Fig. V.

The following protocol was observed for each experiment:

(1) a control record was obtained of the free heart rate, along with the other parameters, mean aortic pressure, end tidal PCO_2 and L.V.E.D.P.;

(2) the heart was then paced at a rate just below that which induced pulsus alternans and a control record of dP/dt max was made at this fixed heart rate. This fixed heart rate was used for all such tests in the same animal. Drug infusion was then started, using infusion rates of 0.123, 0.247, 0.494, 1.23, 2.47 and 4.94 ml/min. (except dogs 4 and 5, whose data was not used), with steps (1) and (2) being observed for each drug infusion rate. In all experiments, isoprenaline was studied first and salbutamol second because of the slow rate of removal of salbutamol from the circulation (Jack, 1971).
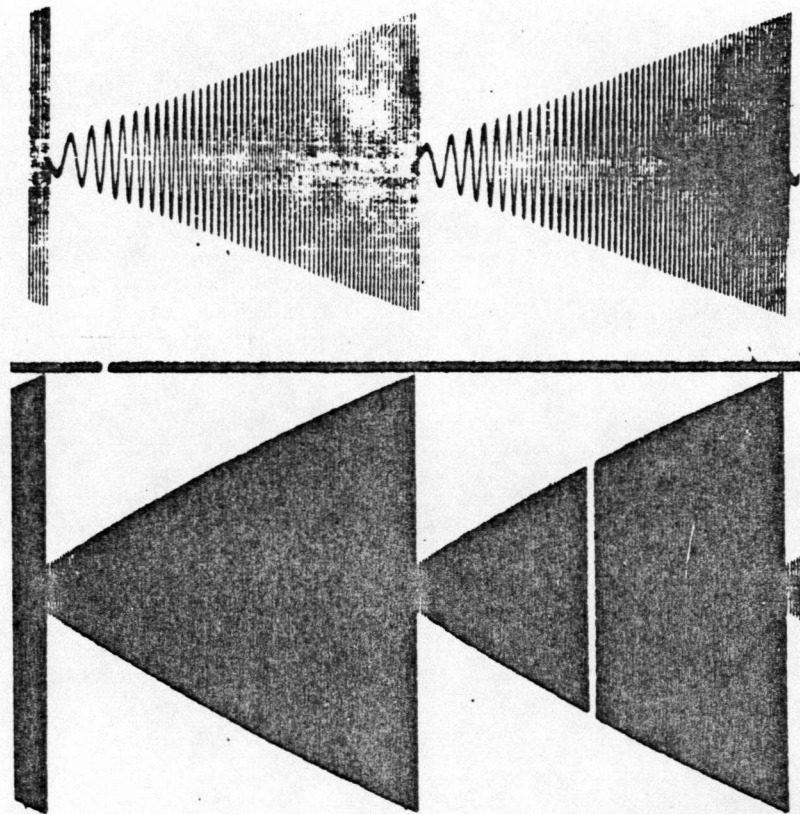
Figure IV: Upper trace: The differentiated signal of a constant amplitude sine wave sweeping at a constant rate with respect to time from 1 to 10 Hz.
Lower trace: The differentiated signal of a smaller constant amplitude sine wave sweeping at a constant rate with respect to time from 10 to 100 Hz.
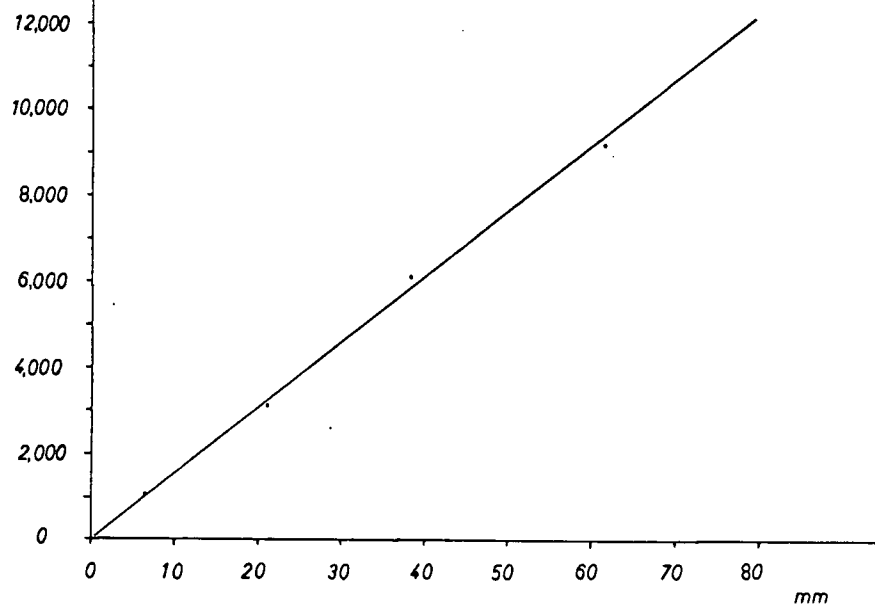
Figure V: Calibration plot of the differentiating circuit, showing relationship between recorded height of dP/dt in mm and amplitude of the signal in mm Hg/sec.

At each infusion rate, the drug was infused for three minutes,
then a record of the free heart rate and the dP/dt max. at
the constant paced rate used in that particular experiment was
obtained. Between the isoprenaline and salbutamol infusion, a
period was allowed long enough for both heart rate and dP/dt
max. to return to original control values. If at any point the
mean aortic pressure fell, the balloon in the descending aorta
was inflated to raise the pressure. This was not possible in
dogs 6, 18, 19 and 23, which were too small to allow the
balloon to be inserted into the femoral artery.

In vitro experiments:

  a) Preparation maintenance:

      In accord with the method of Reiter (1972), the salt
solution used in the isolated muscle preparations is expressed
in g/l quantities: NaCl 6.92, KCl 0.35, $CaCl_2$ 0.28, $MgSO_4$ 0.15,
$NaHCO_3$ 2.1 and glucose 2.0. Both the reservoir and the organ
bath were aerated with 95% O_2, 5% CO_2, which resulted in
conditions of pH 7.322, PCO_2 36.4 mm Hg and PO_2 211 mm Hg when
measured at $37^{\circ}C$ (Rahn and Baumgardner, 1972). The organ
bath was maintained at $25^{\circ}C$ by a water jacket in series with
a constant temperature bath.

  b) In vitro isolated guinea pig atria:

      Female guinea pigs (300 - 400g) were stunned by a
blow to the head. The heart was rapidly excised and placed
in cooled physiological salt solution, then the right atrium
was dissected free. The atrium was tied with silk thread at
both ends and suspended between a gold chain attached to a
Statham G7B 1.5 - 350 tension transducer and a lucite frame,
which also supported two large platinum plate electrodes used
to stimulate the preparation. The lucite frame was connected
to a micrometer for the precise maintenance of resting tension
at 0.5 g. The isolated organ bath contained 26.0 ml of
physiological salt solution at $25^{\circ}C$ as previously described.

      Recordings made of tension were recorded using an S.E.
Laboratory transducer/converter and an S.E. Laboratory model
3006 ultraviolet recorder. Calibration of tension, in grams,
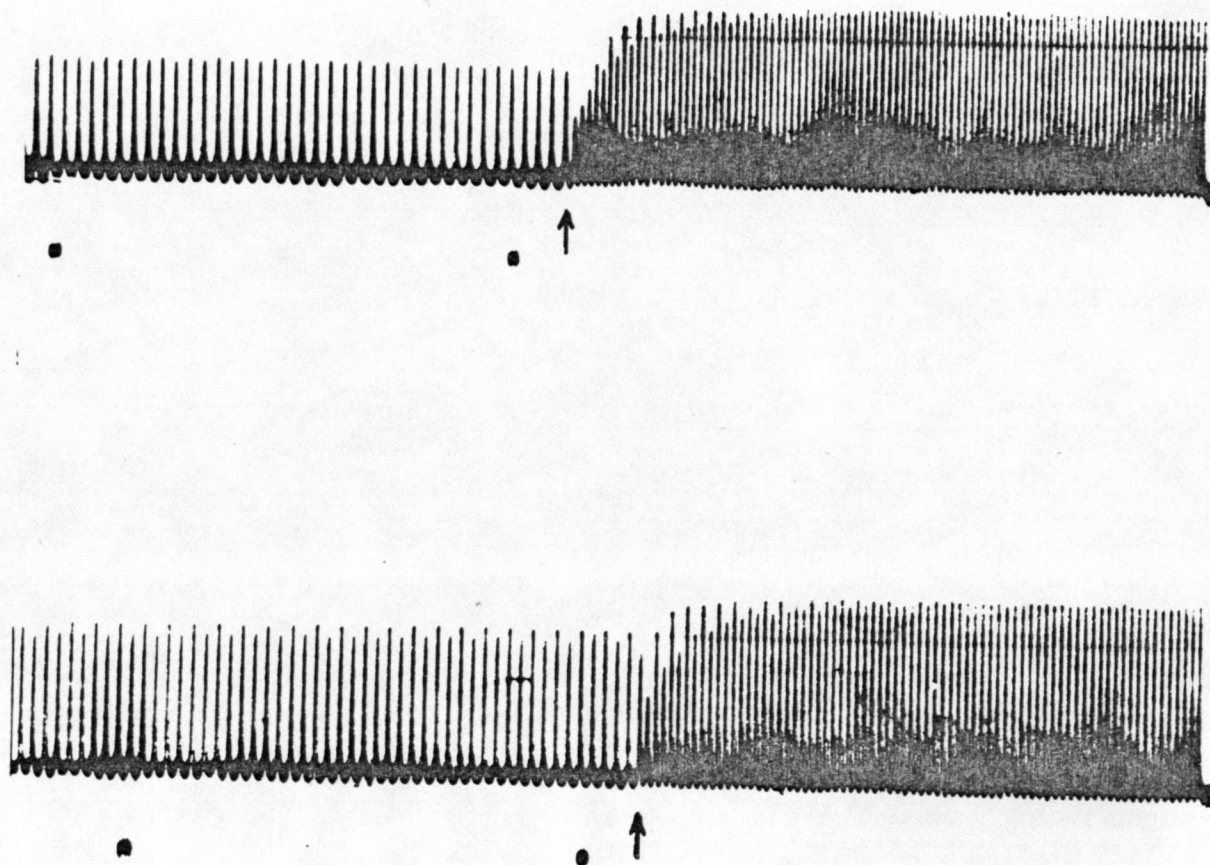
Figure VI: Upper trace: Pre-drug, control recording in the _in vitro_ isolated guinea pig right atrium of a 30 second period of free contraction rate, followed at the arrow by the commencement of electrical pacing for the measurement of peak tension developed at a constant frequency of contraction.

Lower trace: Same record as upper trace, showing the positive chronotropic and inotropic effect of isoprenaline (0.004 n mole/ml.)

was made using small weights.

The experimental protocol was designed to parallel the in vivo dog preparation. A control record of spontaneous rate of contraction was determined over a 30 sec. period. The preparation was then stimulated electrically to contract in most experiments at a frequency of 2 hz (120/min) by a Grass model S4 stimulator at a voltage 1.25 times that required to produce a maximal response. The peak tension developed was measured during electrical stimulation (see Fig. VI). Drugs were added to the bath in volume quantities of 1.0 ml or less in single doses. A period of three minutes was allowed for diffusion and equilibrium, then a 30 sec. record of free rate was measured. The electrical pacing of the preparation was started and a record made of peak tension developed. After observation of the drug effects, the preparation was double washed and a period of at least 20 minutes was allowed before the next drug dose was administered. The various drug concentrations were added in a random order.

c) In vitro isolated dog papillary muscle:

Eight dogs (10-14 kg) (Table I) were tested first with the previously described in vivo preparation; then were used in this portion of the study. Following the completion of the in vivo experiment, the hearts were removed and placed in physiological salt solution. Small papillary muscles were tied with silk thread and cut free of the heart. They were then attached to the same lucite holding device and recording equipment as previously described for the isolated guinea pig atria.
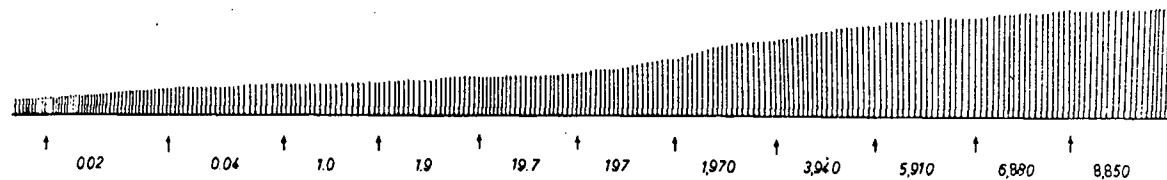
Figure VII: A tracing of an actual record of the tension developed by an
  isolated dog papillary muscle, stimulated to contract at a constant frequency
  of 0.2Hz, during cumulative addition of isoprenaline as indicated by arrows
  (cumulative dose in n mole/ml, shown below each section). Time bar = 1 min.
  (Some contractions omitted for clarity.)

In the bath, the muscle was set at an initial resting tension of 0.4 g, and stimulated at a frequency of 0.2 Hz with a voltage 1.25 times that required to produce a maximum response. A period of at least 60 - 90 min. was allowed prior to the start of the experimental period. Any preparations which developed ectopic foci during the experiment were discarded.

Drugs were added to the bath in cumulative doses, a continuous record being made so that, following the response plateau between approximately $2\frac{1}{2}$ - 3 min., the next addition could be made. The addition of drugs continued until no further increase in the maximal response plateau was observed over consecutive increases in drug concentration. (Fig. VII). To eliminate possible variations in response induced by deterioration of the preparation, four muscles were tested first with isoprenaline, then salbutamol, while the other four were tested in the reverse order. After attaining a maximum response to the first drug, the preparation was washed three to five times over the next 10 min., then allowed a further 45 - 60 min. to again attain a constant baseline value.

Evaluation of experimental techniques:

  a) In vivo denervated dog experiments:

The in vivo denervated dog heart preparation eliminates possible direct influences on the myocardium by the autonomic nervous system, particularly in response to the hypotension induced by the drugs. However, baroreceptor stimulation is still capable of releasing adrenal catecholamines, but the influence of this factor was minimised by the maintenance of mean aortic pressure using a balloon inflated in the descending aorta. Previous investigations of Kofi Ekue et al. (1971) and Daly et al. (1971) attempted to determine the relative influences of salbutamol and isoprenaline on heart rate in the neurally intact heart, thus observing the influence of both the drugs and the baroreceptor reflex. Determination of the positive chronotropic effects in the denervated myocardium can be seen as the true drug effects.

The maximum rate of rise of left ventricular pressure (dP/dt max.) was used as an index of contractility. The preparation used was similar to that described by Furnival et al. (1970), who evaluated dP/dt max. as the index of inotropic activity. dP/dt max. was shown by Furnival et al. (1970) to be sensitive to changes in the inotropic state induced by isoprenaline. dP/dt max. was shown to vary with afterload (aortic pressure) and heart rate, not with preload (left ventricular end diastolic pressure). Increases in left ventricular end diastolic volume do result in an increased strength of contraction of the heart producing a greater stroke volume. However, this is the Frank-Starling mechanism, which is a

reflection of the number of fibers contracting from the optimal length in their length-tension relationship (Yoran et al., 1973) and is not a result of an alteration in the inotropic state of the cardiac muscle (Sonnenblick, 1965). Other authors have not shown dP/dt max. to be independent of preload (Wallace et al., 1963, Taylor, 1970, Grossman et al., 1972 and Mirsky, 1972). However, all authors are agreed that any changes in the inotropic activity secondary to changes in L.V.E.D.P. are relatively small. The work of Wallace et al. (1963) indicated a rise of only 1,300 mm Hg/sec. for a 6 cm H_2O rise in L.V.E.D.P., and Furnival et al. (1970) described one dog in which L.V.E.D.P. increased from 2.9 to 16.8 cm H_2O, the stroke volume increased to 17.4 ml from 7.7 ml, but no change in dP/dt max. was observed. In the conditions of the present study, large changes in L.V.E.D.P. did not occur. An attempt was made to reduce changes in mean aortic pressure and all measurements of dP/dt max. were made at constant heart rate.

b) In vitro isolated guinea pig atria

The spontaneously contracting isolated right atrium has become a standard pharmacological preparation for the measurement of chronotropic alterations (Edinburgh.University, 1970, Brittain 1971).

The classical preparation for the study of inotropic interventions has been the electrically driven left atrium. The present study used the right atrium, stimulating it at a frequency slightly above the maximum rate obtained with drug addition. At this constant paced rate, the positive inotropic

effects could be assessed in a manner analogous to that used in the in vivo preparation. Criticism of the use of large platinum plate electrodes comes from Blinks and Koch-Weser (1963). However, since all measurements were carried out in a similar manner, the effects of massive stimulation affecting the results should be completely eliminated.

c) <u>In vitro isolated dog papillary muscle</u>:

The classical preparation for studying inotropic alterations in ventricular muscle is the isolated cat papillary muscle (e.g., Sonnenblick, 1965). Use of the dog papillary muscle has been limited because of the large size of these muscles in most dogs over 2 - 3 kg. Blinks and Koch-Weser (1963) used the Hill equation to predict the maximum effective distance for O_2 diffusion in the isolated working muscle. They found that 0.52 mm diameter was the greatest thickness permitting sufficient O_2 diffusion at 37°C. The use in this study of larger papillary muscles from dogs 10 - 14 kg may have led to inadequate diffusion of O_2 throughout the tissue. However, the order of drug administration was varied in each muscle, so that four were tested with isoprenaline first, the other four with salbutamol first, to eliminate possible variations in response due to preparation deterioration.

Maintaining the organ bath at 25°C resulted in a preparation described by Blinks and Koch-Weser (1963) as being more stable, however no evidence exists stating that the oxygen requirements are decreased at lower than physiological temper-

atures (Jewell and Blinks, 1968). The low bath temperature
results in a preparation producing stronger contractions
(Blinks and Koch-Weser, 1963). An additional factor of
great importance in this study is the increased tendency
toward oxidation of the drugs at 37°C (Blinks and Koch-Weser,
1963).

Mathematical Analysis

 a) In vivo denervated dog:

Data obtained from these experiments has been ex-
pressed as absolute changes from the pre-drug control values.
Changes in heart rate (Δ HR) and changes in contractility,
(Δ dP/dt max.) are displayed in Table III as the arithmetic
mean plus or minus the standard error of the mean for each drug
infusion rate.

Dose-response curves were plotted for the positive
chronotropic and inotropic responses by expressing the mean
Δ HR and Δ dP/dt max. at each infusion rate as a percent of
the maximum response to isoprenaline. DR_{50} values were esti-
mated from these dose-response curves. All dose ratios have
been expressed with the concentration of isoprenaline equivalent
to one.

To compare the relative effect of each drug on rate
and contractility, the Δ HR was plotted against the corres-
ponding Δ dP/dt max. at that infusion rate in Figs. X and XVI.
Linear regression equations for this data were calculated
from these points giving regression coefficients for each drug.

The influence of the two drugs on preload and after-
load was calculated by a paired t-test using the values of
Δ L.V.E.D.P. and Δ B.P. obtained from the highest infusion
rate and the control value in each dog (Table II).

 b) In vitro isolated guinea pig atria:

All changes in rate (Δ R) and in peak tension developed

(ΔT) were expressed as a percent change from the pre-drug control value (Table IV).

Dose-response curves of the chronotropic and inotropic responses were obtained from the geometric mean (Bliss, 1967) of values of ΔR and ΔT at each drug concentration, which were then expressed as a percent of the maximum response obtained with isoprenaline addition (Figs. XI and XII). DR values were estimated at the response level corresponding with 50% maximum response to isoprenaline.

The plotting of the geometric mean values of ΔR and ΔT at each drug infusion rate (Figure XIII) allows the appraisal of the relative effect of each drug on the rate and contractility. The regression coefficients for the drugs were determined by calculation of the linear regression equations from the mean values of ΔR and ΔT.

c) <u>In vitro isolated dog papillary muscle</u>:

The drug induced alterations in the inotropic state of the isolated papillary muscle preparation were expressed as a percent increase from control values. The geometric mean of the responses at each drug concentration (Table VI) were then expressed as a percentage of the maximum response to isoprenaline and plotted in Figure XVII. Estimation of a dose-ratio for Figure XVII included only the response range over which salbutamol produced a concentration related response.

RESULTS

a) In vivo denervated dog experiments

The data of dogs 4 - 18 and 20 - 23 was pooled to give a total of 17 experiments. This data has been reported in Tables II and III, and in Figures VIII, IX and X.

The effect of the highest infusion rates of isoprenaline and salbutamol on preload and afterload was small (Table II). Isoprenaline produced a mean increase in L.V.E.D.P. of 0.39 $\pm$ 0.53 cm H_2O, while salbutamol infusion resulted in almost no (0.00 $\pm$ 0.37 cm H_2O) mean increase in L.V.E.D.P. Statistical analysis of the differences in L.V.E.D.P., using a paired t-test for comparison of data at the highest drug infusion rate in each dog, showed further that no significant difference in the L.V.E.D.P. resulted from the highest infusion rates of each drug. Afterload, as indicated by mean aortic blood pressure, fell with infusion of both drugs despite attempts to regulate mean aortic pressure using a balloon in the descending aorta. Isoprenaline produced the smaller drop in pressure, -6.50 $\pm$ 1.87 mm Hg, while that produced by salbutamol, -10.64 $\pm$ 2.87 mm Hg, was significantly greater when analysed in terms of a paired date comparison ($P < 0.05$).

Results of the drug infusion on the positive inotropic and chronotropic responses (Table III) showed that isoprenaline and salbutamol both produced approximately the same effect. The highest rate of isoprenaline infusion resulted in a maximum increase in heart rate of 62.7 $\pm$ 6.5 beats/min. and

TABLE II

Effect of the highest infusion rate in the in vivo
preparation of isoprenaline and salbutamol
on preload and afterload.

	Change in preload Δ L.V.E.D.P.(cmH_2O)	Change in afterload Δ BP (mmHg)
Isoprenaline	0.39 ± 0.53* (Range - 5.0 to + 4.0)	-6.50 ± 1.87 (Range -21 to +2)
Salbutamol	0.00 ± 0.37 (Range - 5.0 to + 2.0)	-10.64 ± 2.87 (Range -28 to + 6)
* mean $\pm$ standard error of the mean		

Differences of mean analysed as paired data for the relative
effects of salbutamol

$$\text{mean} \left(\Delta \text{L.V.E.D.P.}_{iso} - \Delta \text{L.V.E.D.P.}_{sal} \right) = 0.40 \pm 0.83^{1}$$

$$\text{mean} \left(\Delta \overline{BP}_{iso} - \Delta \overline{BP}_{sal} \right) = 4.1 \pm 1.3^{2}$$

[1]not significant at $p < 0.05$

[2]significant at $p < 0.05$

TABLE III

Absolute changes in heart rate (Δ HR) and myocardial contractility (Δ dP/dt) at each infusion rate of isoprenaline and salbutamol in the _in_ _vivo_ preparation. (n = 17 dogs).

	Infusion Rate (μg/min)	Δ HR$_1$ (min^{-1})	Δ dP/dt max (mmHg/sec)
Isoprenaline	0.123	6.1 $\pm$ 2.1	600 $\pm$ 217
	0.247	11.6 $\pm$ 2.6	1488 $\pm$ 358
	0.494	27.2 $\pm$ 3.2	3068 $\pm$ 461
	1.23	48.4 $\pm$ 3.7	6239 $\pm$ 965
	2.47	62.7 $\pm$ 6.5	9840 $\pm$ 2227
Salbutamol	12.3	7.8 $\pm$ 3.2	908 $\pm$ 175
	24.7	17.5 $\pm$ 3.4	2752 $\pm$ 689
	49.4	31.2 $\pm$ 2.7	4829 $\pm$ 1034
	123.0	43.6 $\pm$ 4.1	7823 $\pm$ 1561
	247.0	51.7 $\pm$ 5.6	8650 $\pm$ 2658
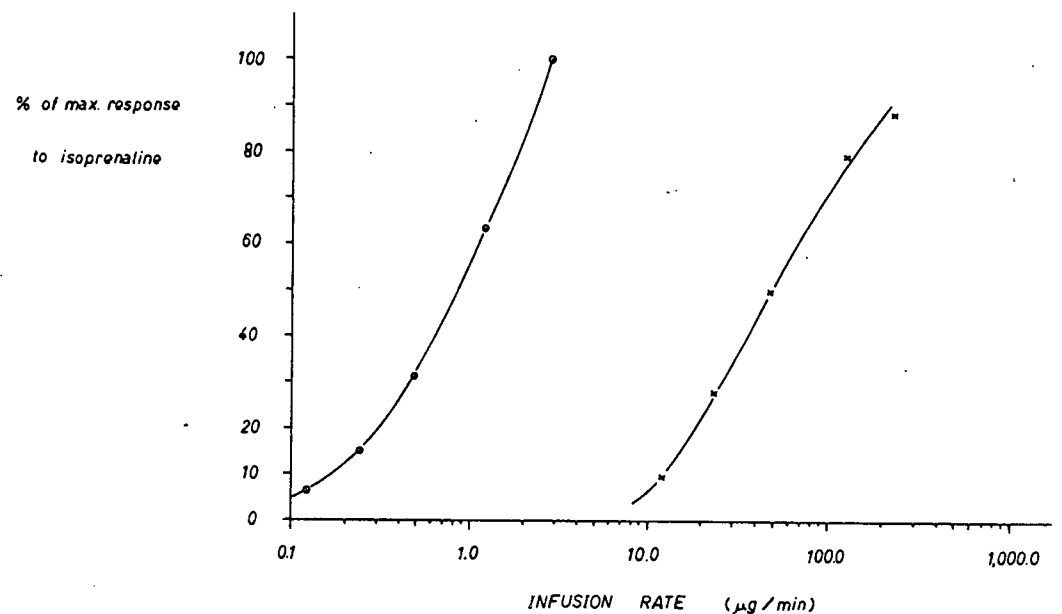
Figure VIII: The dose-response curves showing the positive inotropic effect of isoprenaline (⊙) and salbutamol (x) on the in vivo denervated dog myocardium (n= 17 dogs).

% of max. response
to isoprenaline

100

80

60

40

20

0

0.1   1.0   10.0   100.0   1,000.0

INFUSION   RATE   (μg / min)
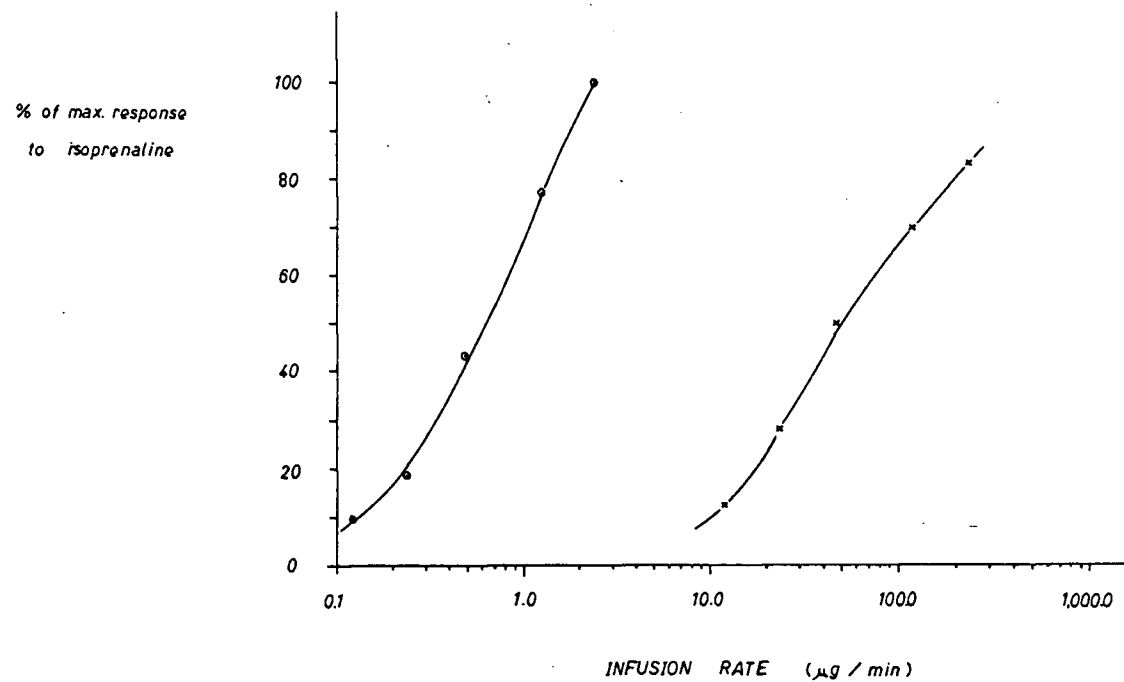
Figure IX: The dose-response curves showing the positive chronotropic effect of isoprenaline (⊙) and salbutamol (x) on the in vivo denervated dog myocardium (n= 17 dogs).
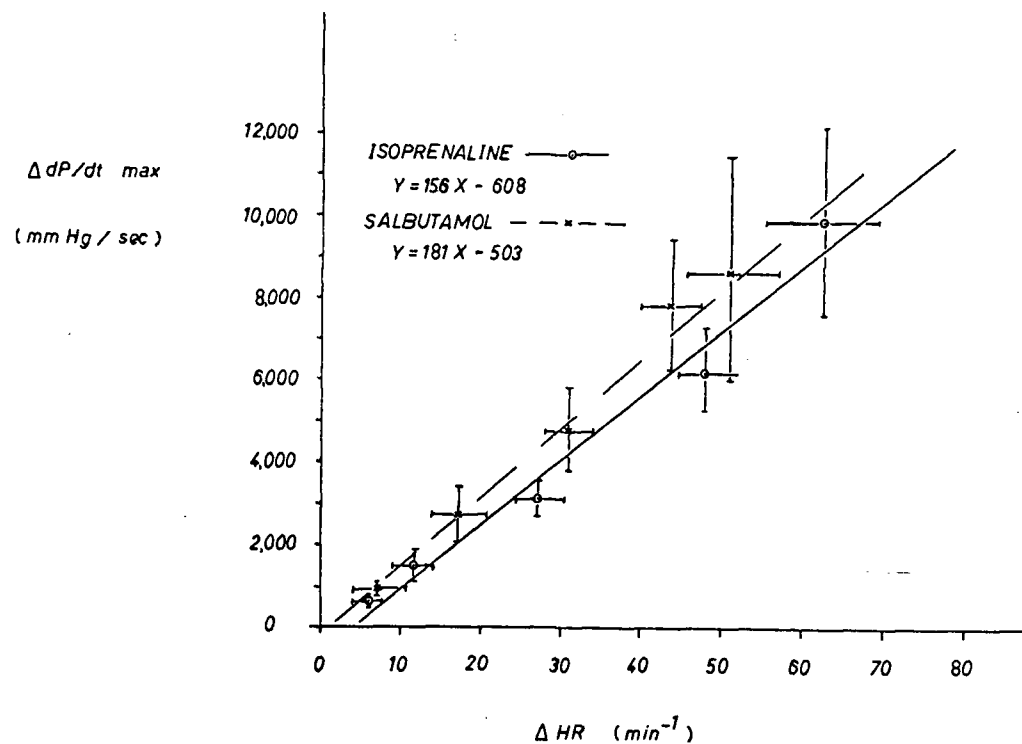
Figure X: Graph of the change in contractility (Δ dP/dt max) and change in heart rate (Δ HR) at the different drug infusion rates for isoprenaline and salbutamol in the in vivo denervated dog myocardium. Values are mean $\pm$ standard error of the mean for 17 dogs.

a maximum increase in dP/dt max. of 9,840 $\pm$ 2,227 mm Hg/sec.

The corresponding values for the highest rate of salbutamol in-

fusion were 51.7 $\pm$ 6.5 beats/min. and 8,650 $\pm$ 2,658 mm Hg/sec.

respectively. DR_{50} values estimated from the dose-response

curves were 100:1 for both inotropic and chronotropic responses.

A comparison of the relative effects of the drugs on

heart rate and myocardial contractility has been shown by

Fig. X. Regression coefficients for the linear regression

lines show a slightly greater effect on contractility than rate

produced by salbutamol compared with isoprenaline. The linear

regression equations were:

$$(\Delta \, dP/dt \, max) \; = \; 156 \, (\Delta HR) - 608 \text{ for isoprenaline}$$

and $\quad (\Delta \, dP/dt \, max) \; = \; 181 \, (\Delta HR) - 503 \text{ for salbutamol.}$

However, the large standard error of the mean points in Fig. X

eliminates possible conclusions regarding significance.

b) <u>In vitro isolated guinea pig atria</u>:

The inotropic and chronotropic responses to isoprenaline

and salbutamol have been summarized in Table IV. The maximum

increases in contractility (ΔT) and contraction rate (ΔR) were

71.6% and 63.0% respectively for isoprenaline. Salbutamol pro-

duced much smaller maximal responses, 46% and 44.5% for Δ T

and Δ R respectively. This partial agonist property of

salbutamol is further illustrated in Figures XI and XII, the

dose-response curves of the inotropic and chronotropic responses.

The slopes of the dose-response curves for salbutamol show a

variable pattern. This variation, plus the partial agonist

TABLE IV

Positive inotropic (Δ T) and chronotropic (Δ R)
effect on the _in vitro_ isolated guinea pig
atrium of isoprenaline and salbutamol.
(Geometric mean of % increase from control value
of 'n' observations at each concentration).

Drug Concentration (n. moles/ml)	n	Δ T	Δ R
Isoprenaline			
0.00197	4	5.9	7.4
0.00391	12	21.1	13.2
0.00981	14	23.5	27.2
0.0197	7	33.9	37.9
0.0981	10	45.5	61.5
0.197	3	71.6	63.0
Salbutamol			
0.171	8	9.7	2.6
0.342	9	12.5	20.8
0.855	9	19.6	26.0
1.71	5	30.4	29.1
8.55	11	34.1	38.9
17.1	3	46	44.5

Figure XI: The dose-response curves showing the positive inotropic effect
  of isoprenaline (⊙) and salbutamol (x) on the <u>in vitro</u> isolated electrically
  driven guinea pig atrium.
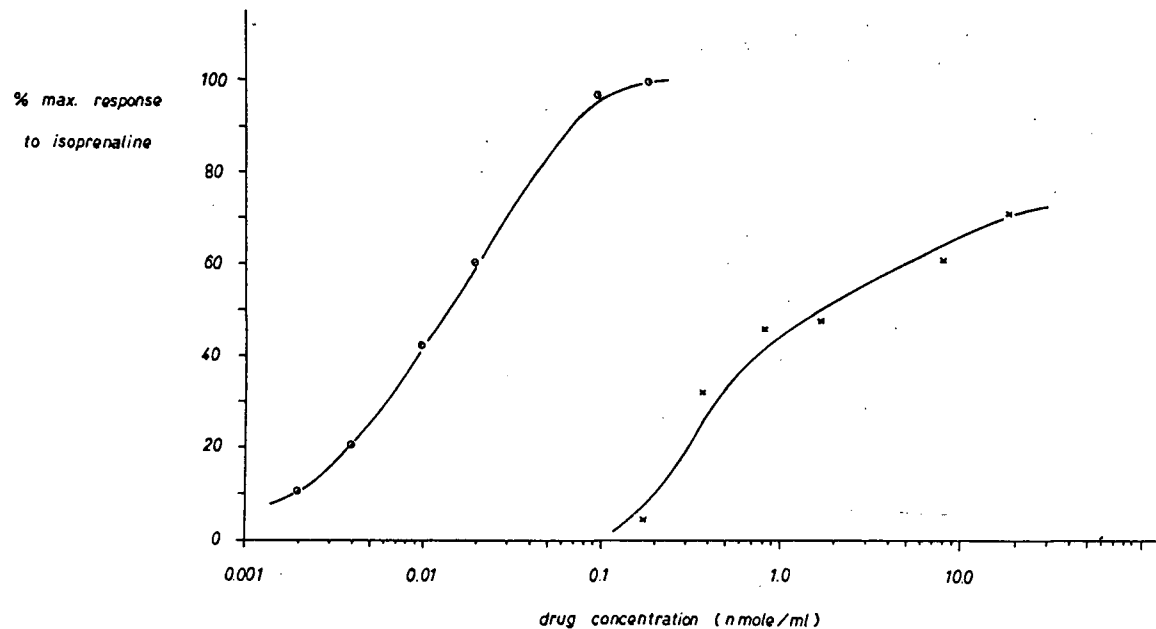
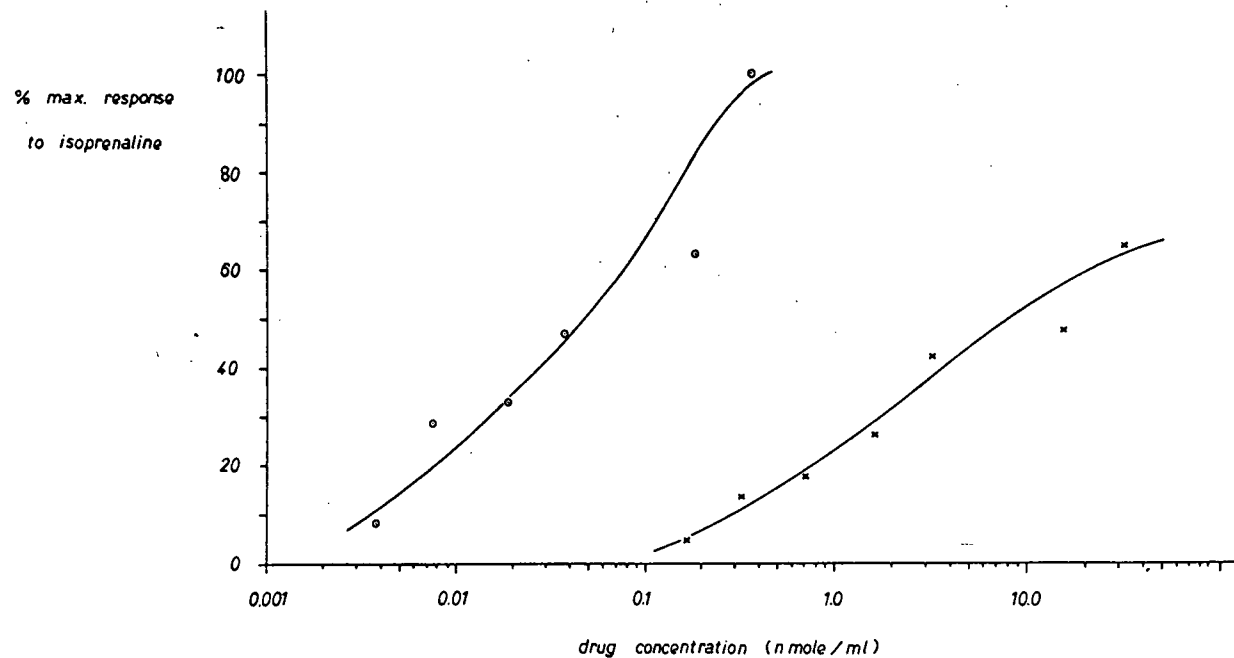Figure XII: The dose-response curves showing the positive chronotropic effect of isoprenaline (⊙) and salbutamol (x) on the <u>in</u> <u>vitro</u> isolated guinea pig atrium.
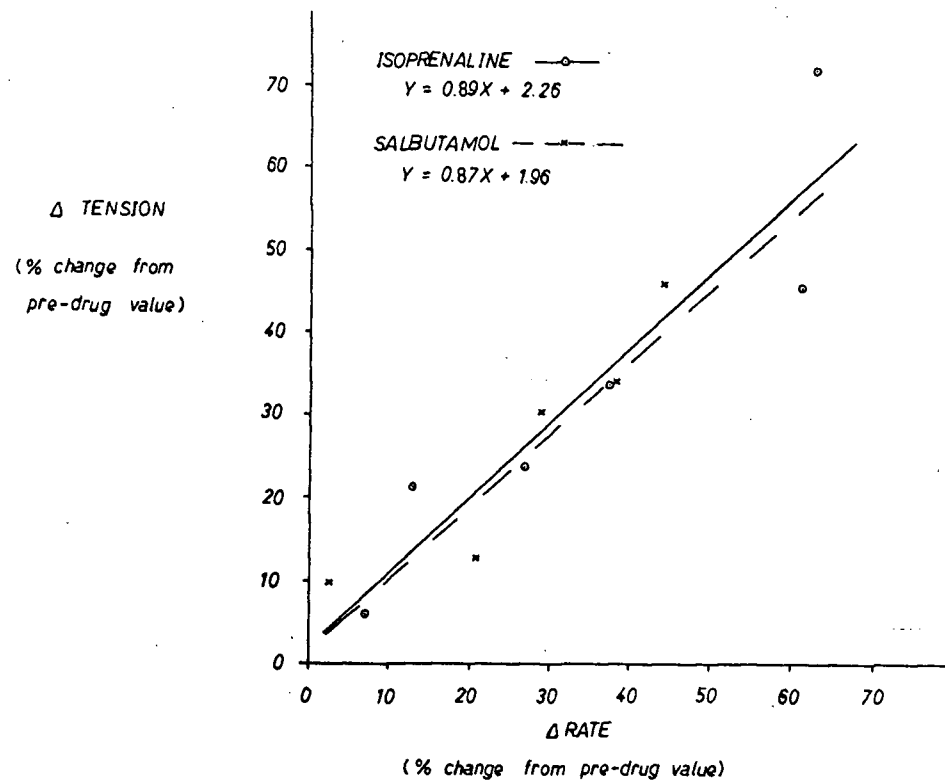
Figure XIII: Graph of the change in peak tension developed (Δ T) and the change in rate of contraction (Δ R) at the different drug concentrations of isoprenaline and salbutamol in the _in vitro_ isolated guinea pig atrium.

nature make the estimation of true DR_{50} values impossible.
However, a DR value at the 50% maximum isoprenaline response
line has been estimated, with values of 350:1 for both the
inotropic and chronotropic responses. The similarity of these
DR values is borne out by Figure XIII, which plots ΔT against
ΔR at each drug concentration. The regression coefficients
from the linear regression equations, $\Delta T = 0.87 (\Delta R) = 1.96$
for isoprenaline and $\Delta T = 0.89 (\Delta R) + 2.26$ for salbutamol,
indicate no difference in the relative effects of each drug
on contraction rate and contractility in the in vitro isolated
guinea pig atria.

c) In vitro isolated dog papillary muscle:

The data of the in vivo denervated myocardium studies
for the dogs used in this portion of the study have been pre-
sented separately in Table V and Figures XIV, XV and XVI. This
smaller sample size than that reported in section "a" of the
results indicated greater maximal inotropic and chronotropic
effects with salbutamol than isoprenaline. The mean maximum
increases in heart rate were 68.5 ± 4.5 and 64.3 ± 8.6 beats/
min. for salbutamol and isoprenaline respectively. Increases
in dP/dt max. of $16,550 \pm 6,800$ mm Hg/sec. with salbutamol
and $14,200 \pm 3,022$ mm Hg/sec. with isoprenaline were the cor-
responding maximum positive inotropic effects. These observa-
tions have been graphically illustrated in Figures XIV and XV.
Comparison of the relative effects of the two drugs on heart
rate and myocardial contractility in the ΔHR vs. $\Delta dP/dt$ max.

TABLE V

Absolute changes in heart rate (ΔHR) and myocardial contractility (Δ dP/dt max) at each infusion rate of isoprenaline and salbutamol in the in vivo preparation for the eight dogs used in the in vitro isolated papillary muscle study.

| Drug Infusion Rate (μ g/min)	n	Δ HR (min^{-1})	Δ dP/dt max (mm Hg/sec.)
Isoprenaline			
0.123	6	5.0 $\pm$ 1.7	700 $\pm$ 280
0.247	7	18.4 $\pm$ 4.1	2400 $\pm$ 679
0.494	7	36.1 $\pm$ 5.8	3778 $\pm$ 923
1.23	6	56.7 $\pm$ 6.4	5760 $\pm$ 2441
2.47	4	64.3 $\pm$ 8.6	14,200 $\pm$ 3022
Salbutamol			
12.3	7	8.1 $\pm$ 3.7	945 $\pm$ 198
24.7	7	25.1 $\pm$ 5.9	4,179 $\pm$ 1449
49.4	6	34.5 $\pm$ 3.2	7,425 $\pm$ 1637
123.0	4	42.5 $\pm$ 4.3	14,338 $\pm$ 2609
247.0	2	58.5 $\pm$ 3.5	16,200 $\pm$ 6500
494.0	2	68.5 $\pm$ 4.5	16,550 $\pm$ 6800
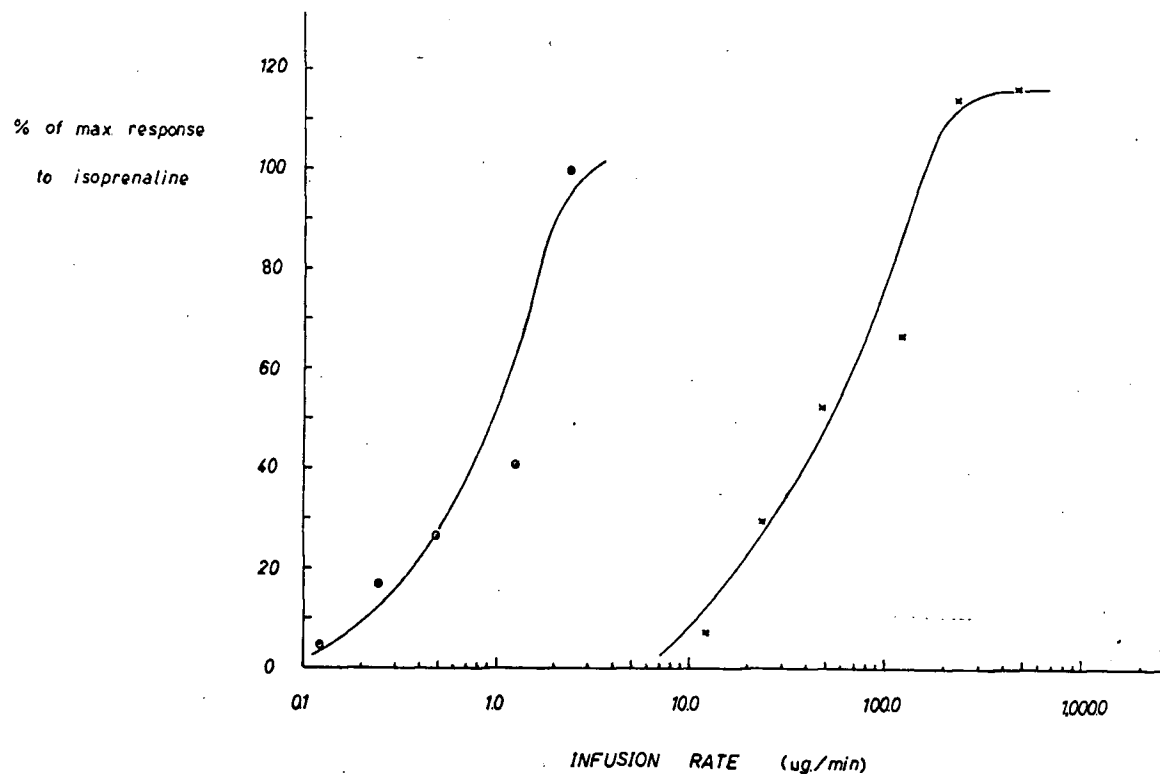
Figure XIV: The dose-response curves showing the positive inotropic effect of isoprenaline (o) and salbutamol (x) on the in vivo denervated dog myocardium (n= 7 dogs).

% of max. response

to isoprenaline

120

100

80

60

40

20

0

0.1   1.0   10.0   100.0   1,000.0

infusion rate (µg./min.)

Figure XV: The dose-response curves showing the positive chronotropic effect
of isoprenaline (⊙) and salbutamol (x) on the <u>in vivo</u> denervated dog
myocardium (n= 7 dogs).
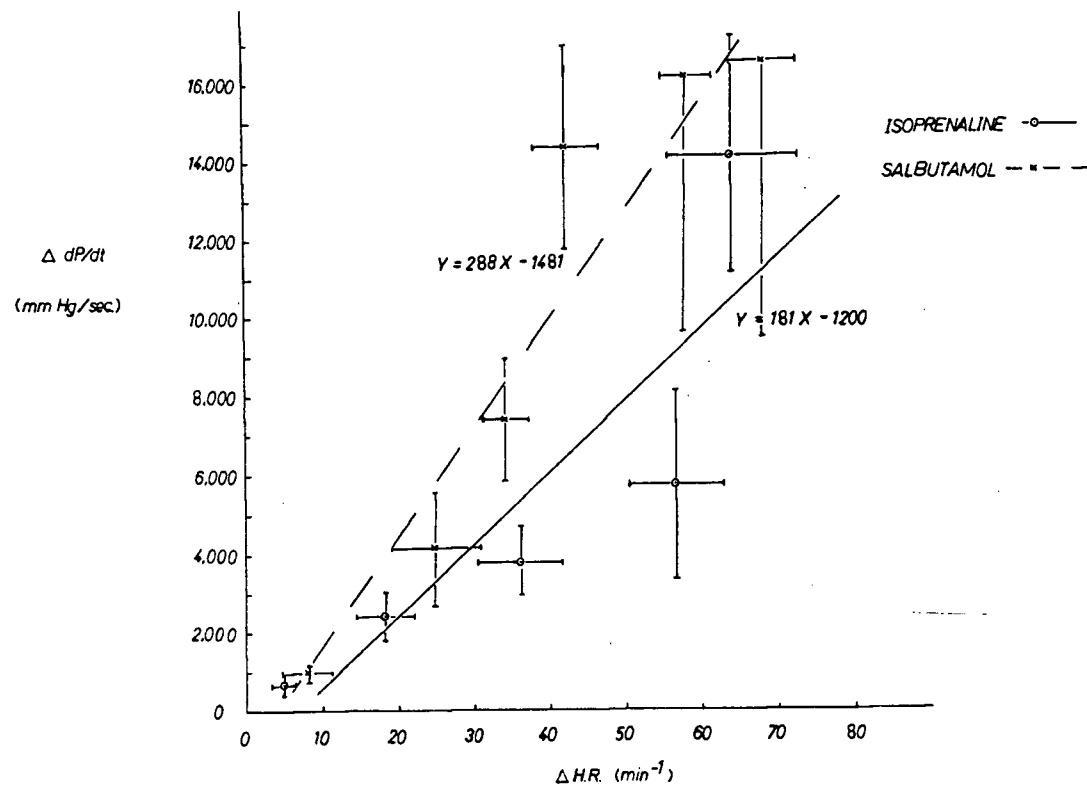
Figure XVI: Graph of the change in contractility (Δ dP/dt max) and change in heart rate (Δ HR) at the different drug infusion rates for isoprenaline and salbutamol in the in vivo denervated dog myocardium. Values are mean $\pm$ standard error of the mean for 7 dogs.

plot (Figure XVI) showed that, for this series of dogs, sal-
butamol produced a slightly greater effect on myocardial con-
tractility relative to heart rate than isoprenaline. The
large error bars prevented a conclusion regarding significance
of the difference between the regression coefficients from the
linear regression equations $(\Delta dP/dt \max) = 181 \ (\Delta HR) - 1200$
for isoprenaline and $(\Delta dP/dt \max) = 288 \ (\Delta HR) - 1481$ for
salbutamol.

In the _in vitro_ isolated papillary muscle preparation,
salbutamol acted as a very weak partial agonist. The data of
Table VI and Figure XVII indicated that salbutamol was capable
of producing only 20% of the maximum response obtained with
isoprenaline. A dose ratio estimated from the dose-response
curve was difficult to obtain because of the differing slopes
for the two drugs. However, a value obtained at the 20%
maximum isoprenaline response was approximately 5,000:1.

TABLE VI

Positive inotropic effect (ΔT) on the <u>in vitro</u>
isolated dog papillary muscle of isoprenaline and
salbutamol. (Geometric mean of % increase from
control value of 'n) observations at each concen-
tration.)

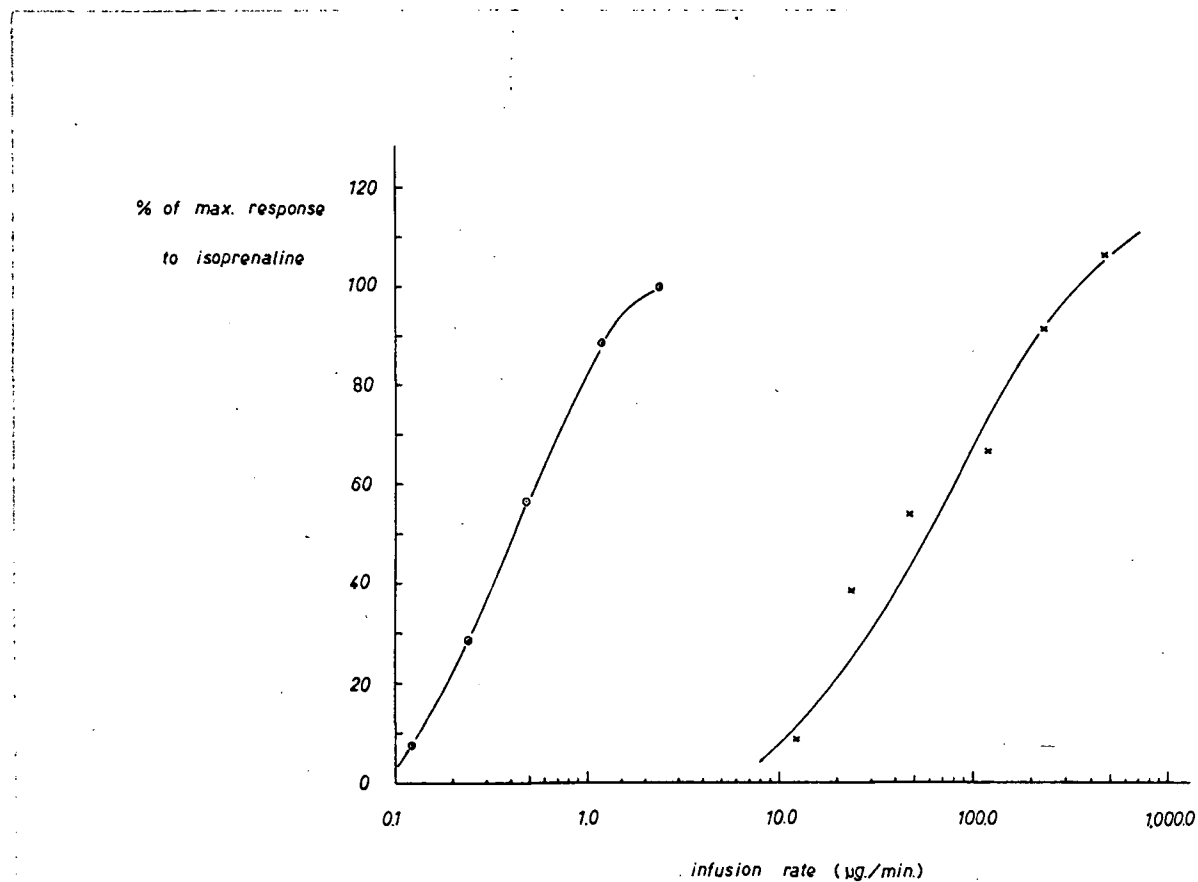| Drug Concentration (n mole/l)	n	Δ T
Isoprenaline		
0.0197	8	9.9
0.0394	7	14.7
0.0981	8	24.3
9.81	5	63.7
19.7	8	74.0
197.0	8	93.5
394.0	8	100.
Salbutamol		
0.171	4	3.9
1.71	7	5.4
17.1	7	16.3
34.2	7	20.5
171.0	8	26.7
342.0	8	28.7
1,710.0	8	28.7
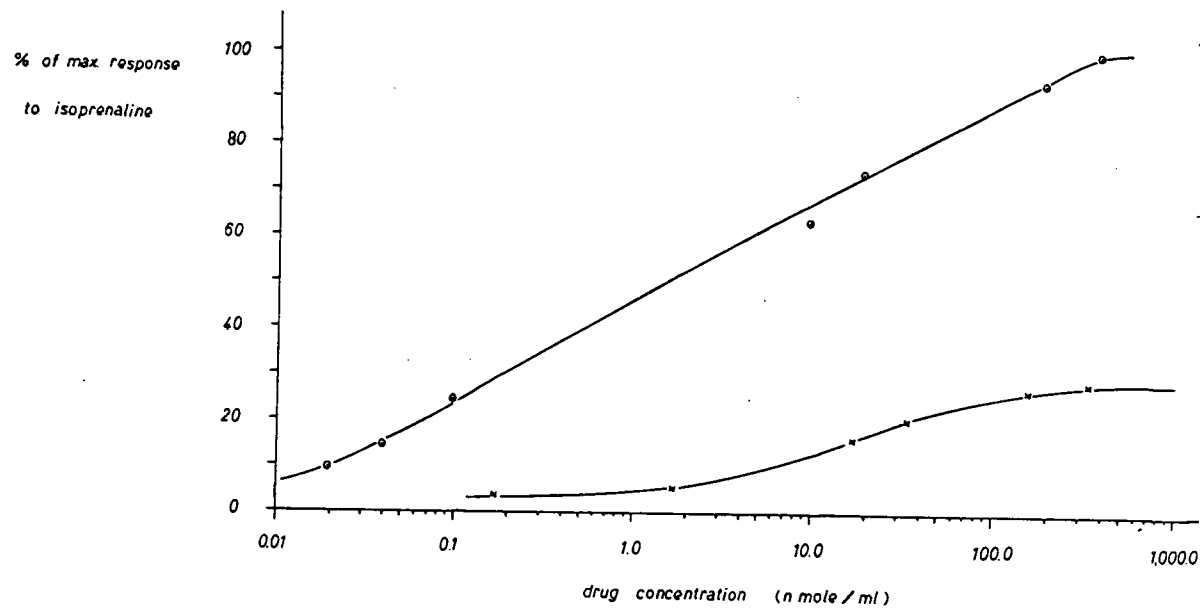
Figure XVII: The dose-response curve showing the positive inotropic effect of isoprenaline (o) and salbutamol (x) on the electrically driven in vitro isolated dog papillary muscle (n= 8 dogs).

DISCUSSION

Receptor identification by means of pharmacological studies has, as was stated in the Introduction, been attempted since the experiments of Sir Henry Dale in 1906. One of the first proposals of adrenergic receptor structure was the Easson-Stedman concept (Easson and Stedman, 1933). This theory postulated the existence of a receptor with three specific binding sites. Receptor areas complimentary to the amine and phenyl groups of the catecholamine agonist (see Figure I) were viewed as essential binding points, with a third site corresponding with the position of the β-carbon hydroxyl group of the agonist acting as a binding site to provide a better fit for the R-stereoisomer. However, this early receptor theory needed to be modified following the work of von Euler (1946) and Ahlquist (1948) to account for the structurally different α- and β- adrenergic receptors. In an attempt to explain drug-receptor interactions, three different molecular level theories were advanced in the mid-1960's. The theories of Belleau (1965), Paton and Rang (1966), and Bloom and Goldman (1966) provide a valuable basis on which the concept of structure of the receptors can be based.

The conformational perturbation theory of Belleau (1965) explained response activation by an alteration in the molecular structure of the receptor following combination with the agonist molecule. The receptor, by this structural alteration, then became an active drug-receptor complex capable of combining with a substrate molecule to yield an activated drug-receptor-

substrate complex which then produced the response. The limiting step in response production was the rate at which the drug molecule could combine with the receptor to form an activated drug-receptor complex. Substrate combination was viewed as immediate. After the response was initiated from the drug-receptor-substrate complex, the drug-receptor combination had to dissociate before another substrate molecule could be acted upon.

Paton and Rang (1966) proposed the limiting factor in the production of a response was the rate of drug-receptor complex formation and dissociation. Unlike Belleau's theory, however, no structural alteration of the receptor was necessary. The receptor was seen as a combination of receptor plus substrate, with the substrate taking no part in the binding of the drug molecule. Under this theory, the drug-receptor complex had to dissociate, as in the conformational perturbation theory (Belleau, 1965), but it had to do so to form another complex of receptor-substrate in preparation for activation by the next drug-receptor interaction.

The dynamic receptor theory (Bloom and Goldman, 1966) was similar to Paton and Rang's theory but differed in that the substrate combined with the "receptor" and played an actual physical role in the binding of the drug molecule. The "receptor" of this theory was actually termed an enzyme by Bloom and Goldman. Following the initiation of a response, the drug-enzyme complex was required to dissociate prior to the formation of a new receptor, that is, an enzyme-substrate complex.

Bloom and Goldman (1966) went further in their characterization of the adrenergic receptor. Since Ahlquist (1948) had first demonstrated the existence of α- and β-adrenoceptors, more had been discovered about the difference in these receptors. It was realized that the primary structural variation in the activating amines was the presence of a highly polar amine group in the α-agonists and a large non-polar amine substituent in the β-agonists. Further, cellular studies had revealed the β-adrenergic responses were mediated by cyclic 3', 5' - adenosine monophosphate (cAMP) formed from adenosine triphosphate (ATP) (Sutherland et al. 1964), and that α- adrenergic responses required the hydrolysis of ATP for the release of energy. The obvious similarity between these responses was the presence of ATP as the initial substrate of each reaction. Bloom and Goldman demonstrated how the catecholamine structure could bind with the ATP molecule in both the Mg^{++} - ATPase - ATP α- adrenoceptor and the adenyl cyclase - ATP β-adrenoceptor, allowing for the bulk and electrostatic differences of the selective agonists.

More recent views of molecular structure of the receptor complex have revised the theory of Bloom and Goldman (1966). Investigations of adenyl cyclase have revealed it to be bound to the inner surface of the membrane (Robison et al., 1971) and activated through either a polar discontinuity (Watkins, 1965) or an allosteric communication (Robison et al., 1971). The adenyl cyclase enzyme has been shown to be an integral part of the β-adrenergic receptor. Microsomal fractions (78,000 xg) of the canine ventricle, which contain complete

adenyl cyclase activity, have been shown to bind catecholamines with the same affinity series seen in the heart either in vivo or in vitro using papillary muscles or isolated perfused hearts (Lefkowitz et al., 1973).

Study of the β-adrenergic receptor was further complicated by the demonstration of at least two sub-groups, the β_1 - and the β_2 - adrenergic receptors (Lands et al., 1967). Burges and Blackburn (1972) have confirmed that these receptors retain their specificity in activating adenyl cyclase from tissues activated in vivo by agonist action at the β_1 - and β_2 - adrenergic receptors. Thus, the β -adrenergic receptor must now be viewed as, 1) consisting of at least two distinct surface binding conformations, 2) possessing a connection between the surface site and the enzyme, either polar or allosteric and 3) acting through the activation of adenyl cyclase.

A molecular approach to the structure of the β -adrenergic receptor has been attempted by Smythies (1972) using Corey-Pauling-Kaltun molecular models. In this manner, models of the β -adrenergic receptor have been constructed which allow for electrostatic binding of the catecholamine and are structurally different from models made of α -adrenergic receptors. Binding in these molecules occurs between polar groups of the amino acids composing the receptor site and the polar groups of the drug molecule. The proposed difference between the α - and β -adrenergic receptor is the presence in the β - adrenergic receptor and the absence in the α-adrenergic receptor of prostaglandins. Modulation of the adrenergic receptor has been shown to occur in the blood vessels of a dog,

with the substance responsible being cited as either a
prostaglandin or a factor causing the production of a prosta-
glandin (Szentivanyi et al., 1970). Although none of these
reports suggest the structural variation between β_1- and
β_2-adrenergic receptors to be due to the specific prosta-
glandin involved, this is a possible explanation.

Characterization of β-adrenergic receptors has also
indicated possible structural variations in the receptors
responsible for the myocardial inotropic and chronotropic re-
sponses. Lands and Brown (1964), using a series of α-carbon
substituted catecholamines, showed that increasing the size of
this substituent resulted in the production of a greater myo-
cardial contractile response than a heart rate response. The
opposite situation of a greater effect on heart rate than
myocardial contractility, when compared with isoprenaline,
has been reported for salbutamol in the isolated atria of the
guinea pig and rat (Brittain, 1971).

As previously described, the present study attempted to
confirm the findings of Lands and Brown (1964) and Brittain
(1971) using the in vivo denervated dog heart. The choice of
the dog as the experimental model was based on the fact that
most cardiovascular studies designed for human physiological
applicability have been conducted in this animal. This was the
case in the studies which demonstrated that stimulation, by
stretch, of the left atrial-pulmonary vein junction resulted in
an increase in heart rate (Ledsome and Linden, 1964 and 1967),
but no increase in cardiac contractility (Furnival et al., 1971).
These observations indicated that two distinct physiological

control systems may exist in the dog mediating the inotropic
and chronotropic responses, with this difference in control
pathways possibly being reflected in the structure of the
adrenergic receptors involved.

The use of dP/dt max as a contractile index has been
discussed earlier. Under the conditions of this study, this
index can be said to be valid. This statement of validity
has been based on the measurement of preload, L.V.E.D.P. and
afterload, $\overline{BP}$. The existence of a controversy regarding the
sensitivity of dP/dt max to preload (compare Furnival et al.,
1970 and Wallace et al., 1963) prompted the measurement of
L.V.E.D.P. The difference in L.V.E.D.P. during the infusion
of either isoprenaline or salbutamol in the same dog was not
significant as determined by a t-test for paired data (p >0.05).
Neither drug produced a significant alteration in the preload
from the control to the experimental period (Isoprenaline
+0.24 $\pm$ 0.49 cm H_2O, and salbutamol 0.00 $\pm$ 0.37 cm H_2O) indi-
cating that any changes in dP/dt max were not due to variations
in preload.

Increases in afterload ($\overline{BP}$) produce increases in con-
tractility reflected in dP/dt max. In this study, decreases in
$\overline{BP}$ occurred indicating that dP/dt max values would be lower
than actually expected if afterload had remained constant.
Results indicate that salbutamol tended to produce a slightly
greater rise in dP/dt max, which was accompanied by a greater
fall in $\overline{BP}$, -10.1 $\pm$ 2.5 mm Hg compared to -7.7 $\pm$ 1.9 mm Hg
with isoprenaline. This difference in the induced fall in blood
pressure was significant at p <0.05. Consequently, it can be

seen that the actual increase in dP/dt max produced by sal-
butamol would actually be expected to be a little higher than
that measured. However, the additional value of dP/dt max
would not be significant because Furnival et al., (1970) demon-
strated only a 120 mm Hg/sec rise in dP/dt max for every 10 mm
Hg rise in $\overline{BP}$. The conclusion is, therefore, that dP/dt max
provided a sensitive index of inotropic interventions in the
present study.

The chronotropic influences of salbutamol or isoprenaline
were assessed by counting the QRS complexes of the E.C.G. Since
the myocardium was sympathetically and parasympathetically
denervated, with only the possible influence of circulating
adrenal catecholamines, the response measured by this method is
a true indication of the chronotropic effects of the two agonists.

The relative influences of salbutamol and isoprenaline
on the inotropic and chronotropic responses are indicated by the
regression coefficients 181 for salbutamol and 156 for isoprena-
line. Examination of Figure X, from which these coefficients
were obtained indicates that, despite the difference calculated,
no really significant trend can be observed concerning the
degree of activation of the inotropic and chronotropic responses
by the two drugs. This point is demonstrated further by the
shape of the dose-response curves for the inotropic and chrono-
tropic responses. The slopes of the curves are almost identical,
and both drugs act as total agonists in this preparation. DR_{50}
values for the inotropic and chronotropic responses are both
100:1.

The in vivo denervated dog heart studied only the normal physiological range of increased heart rate. The maximum heart rate observed in this study was limited to a rate just below that which induced pulsus alternans. It was quite possible, therefore, that if the preparation had allowed higher heart rates to be attained, a difference in either or both the inotropic and chronotropic responses may have been noted. Since that was not possible, analysis of the data available indicated no significant difference in the relative effects of salbutamol and isoprenaline on the inotropic and chronotropic responses. The absence of a significant difference in response precludes the description of two structurally distinct receptors mediating the inotropic and chronotropic responses.

The previously reported work of Brittain (1971) had indicated that salbutamol would be expected to produce a relatively greater effect on heart rate than myocardial contractility when compared to isoprenaline. These observations were reported for the guinea pig and rat isolated atria. The present study demonstrated an opposite trend to what was anticipated by projecting Brittain's data onto the dog. Other studies with the dog had indicated that salbutamol would not be expected to produce the same response as isoprenaline. Nayler and McInnes (1971) tested doses of 0.5 - 2.0 μg/kg, much lower than the present study, in the in vivo denervated dog heart and observed very significantly smaller responses of both heart rate and myocardial wall tension measured with a strain gauge arch than the corresponding responses observed with 0.5 μg/kg isoprenaline. Visual observation of the small dose

range tested in their study did not allow projection of the total response. The isolated dog papillary muscle (Nayler, 1971) had demonstrated a very significantly smaller response with salbutamol compared to isoprenaline over a large pharmacological dose range 0.0005 μg/ml to 50.0 μg/ml, a greater range than that tested in the in vivo denervated heart preparation.

The observations of the present study were based on a large sample population (n=10 for the first series of dogs, n=17 for the total project), implying high statistical significance. Because the data obtained was significant and because it reflected a trend definitely in opposition to that anticipated from previous work with salbutamol and isoprenaline (Brittain, 1971, Nayler, 1971 and Nayler and McInnes, 1971), the two in vitro preparations, the isolated guinea pig atrium and the isolated dog papillary muscle, were tested to confirm the previous observations and to attempt to discover a possible explanation why the data of the in vivo denervated dog heart preparation did not support the hypothesis of two structurally different receptor sites.

The in vitro isolated guinea pig atrial preparation confirmed the previous observation (Brittain, 1971) that salbutamol is a partial agonist. However, the differing effect of salbutamol on rate of contraction and strength of contraction when compared with isoprenaline proposed by Brittain (1971) was not supported. Instead, salbutamol produced chronotropic and inotropic effects in a manner that paralleled the effects of isoprenaline on these parameters, as can be seen in Figure XIII. A possible explanation for this discrepancy may be that Brittain

determined the positive inotropic effects of the two drugs on the electrically driven left atrium, rather than the right atrium as in the present study. It is felt that the large number of observations of the present study justify the conclusion made regarding the similarity of the response to isoprenaline and salbutamol.

Describing the relative activity of salbutamol and isoprenaline at the adrenergic receptor can be accomplished using the terminology affinity (Ariëns, 1954) and efficacy (Stephenson, 1956). Clark (1937) advanced the first basic receptor theory, with the concept that all responses occurred in relation to the concentration of the drug at the receptor site. However, it soon became apparent that not all drugs produced the same maximum response, nor the same shape dose-response curve. Ariëns (1954) showed that various agonist compounds differed in their degree of attraction to the receptor site, exhibiting, therefore, variable affinity. Ariëns (1954) also proposed the term intrinsic activity to account for the degree of activation produced by a drug-receptor interaction. Stephenson (1956) advanced this concept to include not only partial agonists, but those drugs which could elicit a maximal response at a concentration which did not saturate the receptors, and applied the term efficacy to this property of activation. Salbutamol can, therefore, be described in the isolated guinea pig atrium as having a lower affinity for the receptor site than isoprenaline, as seen by the right-shift of the dose-response curve and also a lower efficacy than isoprenaline, evidenced by the less steep slope of the dose-response curve and by the lower maximum response.

In previous tests of salbutamol on the isolated dog papillary muscle, it appeared that salbutamol was a very weak agonist, producing a maximum positive inotropic effect less than one-ninth the response during maximal stimulation with isoprenaline (Nayler, 1971). This observation was a direct contradiction to the results of the _in vivo_ denervated dog myocardium experiments of the present study. The discrepancy between these experiments was investigated by testing the responsiveness of a papillary muscle removed from a dog, which had just been tested in the previously discussed _in vivo_ denervated myocardium preparation, _in vitro_, in an isolated organ bath during electrical stimulation. This method allowed comparison of the responsiveness of the same ventricular muscle _in vivo_ and _in vitro_.

The data of the isolated papillary muscle showed a response pattern similar to that published by Nayler (1971). Salbutamol acted as a partial agonist producing only a 20% maximum contractile response compared with isoprenaline. The slopes of the dose-response curves were different, with the slope of the salbutamol curve being much less steep. The conclusion reached from these results is that salbutamol affects contractility differently in the _in vitro_ isolated papillary muscle than the _in vivo_ denervated myocardium. This difference is not only in the slope of the dose response curves or the maximum response observed, but even more significantly in the dose ratio of salbutamol compared to isoprenaline required to produce the same percent response. In the _in vitro_ isolated

papillary muscle preparation, the estimated dose ratio was 5,000:1 over the 0 to 20% response range; however, the in vivo denervated myocardium exhibited a DR_{50} of 100:1. The results of the in vitro papillary muscle verified the previous work of Nayler (1971) regarding the marked difference in response of this preparation to isoprenaline and salbutamol and also confirmed the postulate of a discrepancy existing between the responses of the in vivo denervated myocardium and the in vitro isolated papillary muscle.

Explanations of the dissimilarity observed between the two preparations have been attempted, looking first at the method of response measurement, then at the components of the system involved in the production of the response as a physiological "black-box" problem.

The active state of the cardiac muscle is defined by Blinks and Koch-Weser (1963) as the state "in which the contractile component ... either shortens or develops tension."[1] The present study has measured alterations in the active state induced by the drugs. In the in vivo denervated myocardium of the dog, dP/dt max, the contractile index, reflects rate of tension development in the ventricular muscle. Here, measured pressure is related to wall tension for the Laplacian expression T=PR (T= tension, P= pressure, R= radius), an approximation based on the assumption of spherical cardiac chambers. There-

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[1]Blinks and Koch-Weser, "Physical factors in the analysis of the actions of drugs on myocardial contractility," Pharmacol. Rev. 15:(1963), p. 538.

fore, dP/dt reflects dT/dt. The peak tension measurement employed in examining the in vitro isolated dog papillary muscle and guinea pig atrium does not reflect rate of tension development (dT/dt), but instead measures the combined effects of rate of development of tension and the duration of tension development. It is theoretically possible to observe an increase or decrease in rate of tension development without changes in the peak tension developed if the duration of the active state is decreased or increased accordingly.

A comparison on the two indices of change in contractility used in the present study can be obtained from the data of the in vivo denervated dog myocardium and the in vitro guinea pig atrium. The inotropic response to salbutamol and isoprenaline measured by peak tension development in the guinea pig atrium paralleled the chronotropic response observed to the two drugs in a manner similar to the inotropic response, as measured by dP/dt max, and the chronotropic response in the in vivo denervated dog. This means that, for the in vitro guinea pig preparation, the two drugs probably produce their inotropic response in the same way, through an identical degree of variation in the intensity and duration of the active state. Therefore, it would not be expected that the difference in inotropic response observed in the in vitro isolated dog papillary muscle would be due to a different mechanism of alteration of the active state of the muscle by the two drugs, but rather to some other direct or indirect difference in the inotropic state induced by the drugs.

The first step examined within the black box was the interaction of the drug with the receptor. It is doubtful that concentration differences between the in vivo and in vitro preparations existed between the two drugs at the receptor sites. Both the in vivo denervated heart and the in vitro papillary muscle required diffusion of the drug molecule, in the first case from the blood to the extracellular fluid and in the second from the bathing solution to the extracellular fluid. Diffusion time differences would have been expected to be very small, as predicted from Graham's Law of Diffusion which relates diffusion velocity inversely to molecular weight, since the molecular weights of salbutamol and isoprenaline are similar. Since there was probably no difference between preparations in concentration of the drugs at the receptor, and if the responses observed in the two preparations were both reflections of solely direct interaction of the drug and receptor, then a possible explanation comes from alterations of agonist affinity and efficacy in an Ariëns-Stephenson sense (see Ariëns, 1954 and Stephenson, 1956). A parallel shift of the dose-response curve reflects a change in affinity of the drug for the receptor. Comparison of the dose ratios for the two preparations showed that, relative to isoprenaline, it took a greater concentration of salbutamol to elicit a response in the in vitro papillary muscle than in the in vivo denervated myocardium, thus supporting the proposal of a variation in affinity. Further, the difference in slopes of the dose-response curves for salbutamol, between the in vivo denervated heart preparation and the in

<u>vitro</u> papillary muscle preparation indicated that an alteration in the efficacy of the drug molecule for the receptor site had occurred also. The <u>in</u> <u>vitro</u> papillary muscle experimentation was performed at 25°C. Alterations of the temperature of the organ bath have been shown in the frog (Kumos et al., 1973) to affect the receptor-type. The adrenergic receptors of the frog heart apparently underwent transformation from β- to α-adrenergic receptors with a change in temperature from 22°C to 17°C and reverted back to β-adrenergic receptors when the temperature was again raised. The significance of this observation when applied to mammalian physiology is not yet clear (see Nickerson, 1973). Although this hypothesis provided an interesting explanation of the results, it is doubtful that it occurred. Nayler (1971) tested her isolated papillary muscles at 35°C and observed a very similar response pattern to that reported in this study.

Another mechanism which may have come into play at the level of the drug-receptor interaction within the black box, is indirect; the release of noradrenaline from the nerve endings. This indirect action is especially prevalent in compounds which differ from the catecholamine structure by the absence of one of the two phenolic hydroxyl groups (Muscholl, 1966). Salbutamol also differs from the catecholamine structure in the aromatic ring, with a methanol group substituting for the <u>m</u>-phenolic hydroxyl group. The tendency for salbutamol to act as an indirect sympathomimetic has not been tested, but salbutamol

would not be expected to act in this manner. The reason for this is that salbutamol is capable of forming the same electrostatic chelation to a positive charge on the receptor area as a catecholamine, since the methanol substituent contains a negatively charged hydroxyl group. Further, it was unlikely that any difference existed in the ability of either the in vivo denervated myocardium or the in vitro papillary muscle to release noradrenaline from the nerve endings, since the classical preparation used to determine a drug's indirect effect is the isolated atrium of the reserpinised guinea pig (Muscholl, 1966).

Examination of steps beyond the drug-receptor interaction in the black box becomes more difficult because of the lack of direct experimental observations. It cannot be said for certain that the drug has elicited its response solely through direct action on the myocardial adrenergic receptors. Peripheral vasodilatation was observed to be greater during salbutamol infusion than isoprenaline infusion in the in vivo dog. Assuming coronary vasodilatation to parallel that in the periphery coronary blood flow would be expected to increase since attempts were made to maintain constant aortic pressure. Nayler (1971) has shown a marked decrease in resistance to flow through the coronary circulation with salbutamol following K^+ arrest of the heart, supporting the concept of increased flow. The increased blood flow will result in a higher delivery rate of O_2 to the working muscle. Myocardial contractility has been shown to vary with oxygen supply (see Dempsey and Cooper, 1972). Some investigators (Fisher et al., 1969 and McRaven et al.,1971)

found no variation in contractility with O_2 concentration near physiological levels. However, Bacaner et al., 1971 reported increased contractility of the isolated perfused dog heart to O_2 delivery rates higher than physiologically normal. Attempts to determine if vasodilatation, independent of direct myocardial stimulation, resulted in increased myocardial contractility in the in vivo perfused dog heart (McRaven et al., 1971) proved to be negative. This may have been because coronary blood flow was maintained at a constant level by the perfusion pump, thereby not allowing increased O_2 delivery to occur. Since no measurements of arterio-venous O_2 differences nor of coronary flow rates were made in the present study, it is impossible to confirm the hypothesis of increased contractility being due to an increased supply of O_2 to the myocardium.

The answer to the question concerning the different responses observed in the two preparations, the in vivo denervated dog myocardium and the in vitro dog papillary muscle, has not been made. The components of the system within the physiological black box have been considered individually. The answer probably lies at some intermediate point, a combination of direct and indirect mechanisms interating to produce the final response differences.

This study attempted to structually characterize the β-adrenergic receptors involved in the myocardial chronotropic and inotropic responses of the dog by testing structure-activity relationships of the β-agonists isoprenaline and salbutamol in the in vivo denervated dog myocardium. Salbutamol differs

structurally from isoprenaline primarily at the m-position
of the phenyl ring by the substitution at this point of a
methanol group for the hydroxyl group. Any differences in
the relative dose-response ratios of the inotropic and chrono-
tropic responses would, therefore, be a reflection of a
difference in the structure of the receptor sites at a position
complementary to the phenyl ring of the agonist molecule. No
definite statement regarding receptor structure can be made in
light of the results from the three portions of this study.
Although the indication from both the in vivo denervated myo-
cardium of the dog and the in vitro isolated guinea pig atrium
is that there is no difference in the structure of the β -
adrenergic receptors involved in the myocardial inotropic and
chronotropic responses, the data obtained from the in vitro
isolated dog papillary muscle preparation questions the nature
of the inotropic response measured in the in vivo denervated
myocardium. The data also points to the species variation in
the efficacy of salbutamol for the adrenergic receptors media-
ting the inotropic response in the in vitro isolated dog
papillary muscle and the in vitro isolated guinea pig atrium.
The discrepancy between the two dog ventricular muscle prepa-
rations, the in vivo denervated myocardium and the in vitro
papillary muscle was analysed in terms of a physiological black
box problem. No definitive answer could be found, however,
to explain why the in vitro papillary muscle was so unresponsive
to salbutamol compared with the in vivo denervated myocardium.
Salbutamol does, within the limits of a normal, physiological

maximum rise in heart rate, produce an effect on the _in vivo_ denervated dog myocardium that is similar to that produced by isoprenaline with regard to both heart rate and myocardial contractile strength.

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