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EFFECT OF ACETYLCHOLINE ON CHANGES IN CONTRACTILITY, HEART RATE AND PHOSPHORYLASE ACTIVITY PRODUCED BY ISOPRENALINE, SALBUTAMOL AND AMINOPHYLLINE IN THE PERFUSED GUINEA-PIG HEART

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1 Isolated perfused hearts of guinea-pigs were given graded doses of isoprenaline, salbutamol and aminophylline, both before and during acetylcholine infusion.

2 The three agonists produced increases in contractile force, heart rate and ventricular glycogen phosphorylase activity.

3 Acetylcholine, in the concentration used, had no effect on any of the measured variables but did antagonize the effects of the three agonists on contractility and phosphorylase activity. The positive chronotropic responses were unaltered by acetylcholine infusion.

4 The ratio of the dose required for a standard heart rate response to the dose producing a standard contractile force response was different for each agonist.

5 The selective antagonism of the contractile response to isoprenaline, salbutamol and aminophylline suggest that different mechanisms are involved in the initiation of positive inotropic and chronotropic responses.

Introduction

Cardiac β-adrenoceptor stimulation produces increases in both heart rate and contractile force. It has been proposed that stimulation of cardiac β-adrenoceptors by catecholamines leads to enhanced cyclic adenosine 3′,5′-monophosphate (cyclic AMP) production which acts as a 'second messenger' to initiate the positive inotropic and chronotropic responses (Robison, Butcher, Øye, Morgan & Sutherland, 1965; Kukovetz & Pöch, 1972). However, a low concentration of acetylcholine that does not affect ventricular contractility, rate, phosphorylase activity, or cyclic AMP formation has been shown to antagonize the initial adrenaline-induced increases in contractility, phosphorylase activity, and cyclic AMP formation without affecting the positive chronotropic effect (Chamales, Williams, Pauk & Ellis, 1971). These results have led to the proposal that cardiac inotropic and chronotropic responses may be mediated by different mechanisms. The purpose of the present investigation was to test this hypothesis by studying the cardiac responses to isoprenaline, aminophylline and salbutamol, both alone and in combination with acetylcholine. It has been suggested that isoprenaline and aminophylline produce their cardiac effects through acceleration of synthesis of cyclic AMP and inhibition of cyclic AMP degradation, respectively (Robison, Butcher & Sutherland, 1971). Salbutamol is considered to act selectively on β_2-adrenoceptors (Cullum, Farmer, Jack & Levy, 1969), but produces effects on the heart qualitatively similar to those of isoprenaline, a less selective β-adrenoceptor agonist. Acetylcholine has been shown to reduce the elevated cyclic AMP concentrations seen after addition of adrenaline or isoprenaline to particulate preparations from heart tissue (Lee, Kuo & Greengard, 1971; Kuo, Lee, Reyes, Walton, Donnell & Greengard, 1972) or after catecholamine administration to guinea-pig isolated hearts (Chamales *et al.*, 1971). It has been proposed that this effect at the cyclic AMP level may be the basis for the observed acetylcholine-adrenaline antagonism (LaRaia & Sonnenblick, 1971).

The present study examines the interactions between drugs thought to act by increasing cyclic AMP accumulation, and acetylcholine which acts by decreasing cyclic AMP concentration, to determine whether cyclic AMP accumulation is

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directly related to the production of both heart rate and contractile force responses.

Methods

Isolated perfused hearts

Guinea-pigs of either sex exceeding 400 g in weight were injected with heparin, and their hearts quickly removed under pentobarbitone anaesthesia. Hearts were perfused at 27° C by the Langendorff technique using Krebs-Henseleit bicarbonate solution containing 0.2% glucose and equilibrated with 95% O_2 and 5% CO_2 (Vincent & Ellis, 1963). All hearts were perfused at a pressure of 40 cm of water and were allowed to stabilize for 30 min before any drug infusion. Contractile force was recorded on a Grass polygraph via a Grass FT-03 force displacement transducer attached to the apex of the ventricles. Heart rate was determined from the contractile force recording. The calibration of the recording system before each experiment was performed as described by Blukoo-Allotey, Vincent & Ellis (1969). Diastolic tension was adjusted to 10 g before drug administration was begun. All hearts used for phosphorylase activity determinations received only one injection. Hearts receiving multiple drug injections were allowed recovery periods sufficient to reestablish pre-injection heart rate values.

Acetylcholine iodide, stored as a 10 mg/ml stock solution at 4° C, and isoprenaline hydrochloride, aminophylline, and salbutamol, prepared daily as stock solutions, were further diluted with 0.9% w/v NaCl solution (saline) immediately before administration. Acetylcholine was administered by infusion delivered into the aortic cannula by a syringe pump at a rate of 0.1 ml/minute. Isoprenaline, aminophylline, or salbutamol was administered into the aortic cannula by bolus injection in volumes of 0.2 ml or less.

Increasing concentrations of isoprenaline, aminophylline or salbutamol were alternately injected alone and 2 min after starting an infusion of 0.018 μg/min of acetylcholine. Acetylcholine infusions were allowed to continue 5 min after each drug injection and then were turned off for at least 10 min before the next drug injection.

Hearts used for phosphorylase activity determinations were frozen 30 s or 1 min after drug injection by clamping between modified Wollenberger tongs (Wollenberger, Ristau & Schoffa, 1960) precooled in liquid nitrogen. Tissues were stored in liquid nitrogen until biochemical assays were performed. Phosphorylase activity was determined in the hearts used for physiological studies.

Biochemical methods

Tissue samples were excised from the apex region of the ventricle and weighed in a refrigerated room (−20° C). Phosphorylase activity was determined in both the presence and absence of adenosine 5′-monophosphate by the enzymatic method of Hardman *et al.* (1965). Phosphorylase activity is expressed as percent phosphorylase *a*

$$\left(\frac{\text{phosphorylase } a \text{ activity}}{\text{total phosphorylase activity}}\right) \times 100$$

Drugs and chemicals

Acetylcholine iodide and aminophylline were purchased from Sigma Chemical Co. Salbutamol was obtained from the Schering Corp., U.S.A. and (−)-isoprenaline hydrochloride from the Sterling-Winthrop Research Institute, U.S.A.

Phosphoglucomutase, glucose-6-phosphate dehydrogenase, and nicotine-adenosine dinucleotide phosphate used in the phosphorylase assay were purchased from Calbiochem, U.S.A. All enzymes were of grade A purity.

Statistical methods

Contractile force and heart rate effects of agonists alone and in combination with acetylcholine were determined in the same hearts, and were statistically compared using Student's *t* test for paired samples. Phosphorylase activities were compared by the *t* test for grouped data (Runyon & Haber, 1967). A *P* value of 0.05 was chosen as the level of significance.

Results

Isoprenaline in doses of 0.001 to 0.05 μg produced dose-related increases in contractile force and heart rate. Figure 1 shows the responses of guinea-pig isolated hearts measured 1 min after isoprenaline injection. Acetylcholine (0.018 μg/min) infused alone had no effect on ventricular contractility or heart rate (Figure 2). However, the positive inotropic response to all doses below 0.01 μg of isoprenaline was antagonized by infusion of acetylcholine. In contrast, the heart rate response to isoprenaline was no different in the presence or in the absence of acetylcholine. Time-response curves are presented to indicate that acetylcholine effects shown in dose-response curves are not simply a phenomenon of sampling time (Figure 2).

Salbutamol at doses of 0.25 to 1 μg produced a positive chronotropic effect without a con-

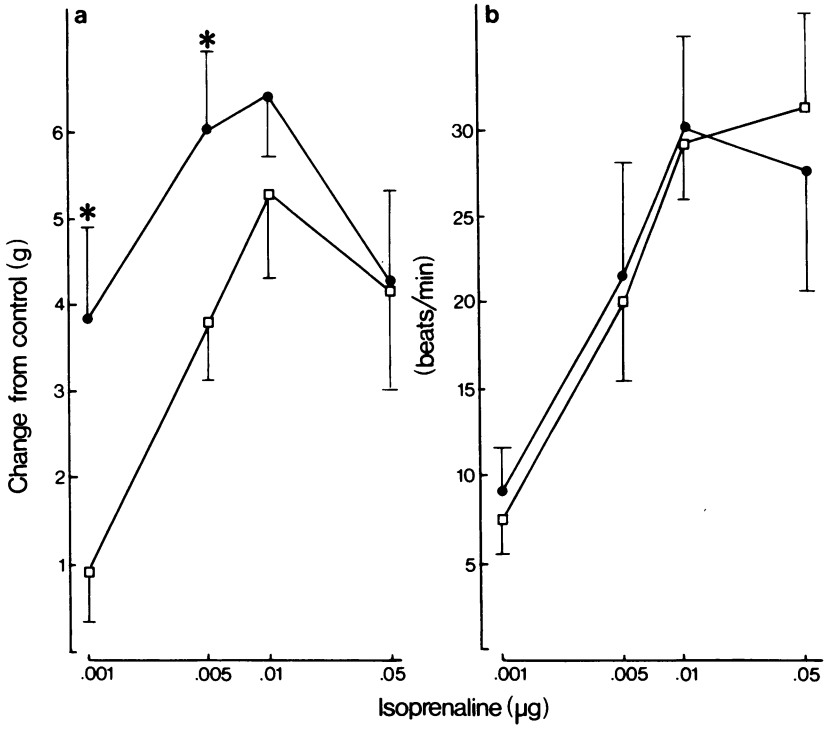

Figure 1 Contractile force (a) and heart rate (b) responses of isolated spontaneously beating heart of guinea-pig to injections of isoprenaline. Responses were measured 1 min after isoprenaline injection both before (•) and during infusion of acetylcholine, 0.018 μg/minute (□). Each point represents the mean of 4 or more experiments. In this and the following 5 figures, vertical bars represent s.e. mean and * denotes responses to agonist alone which differ significantly from the response of the same dose of agonist during acetylcholine infusion.

comitant positive inotropic effect, and 0.5 μg of salbutamol actually caused a slight, but significant decrease in contractile force. Higher doses produced increases in contractile force as well as heart rate. Acetylcholine antagonized the positive inotropic response to all except the highest dose of salbutamol used (Figures 3, 4). During acetylcholine infusion, all doses of salbutamol below 10 μg caused statistically significant decreases below control contractile force. Again, heart rate responses were unaffected by acetylcholine infusion.

Aminophylline produced dose-related increases in both contractile force and heart rate (Figure 5). Acetylcholine infusion prevented the positive inotropic response to all except the highest dose of aminophylline used. The contractile force in response to all doses of aminophylline below 3 mg was not significantly different from pre-injection control values or from the response to acetylcholine infusion alone (Figures 2, 5 & 6).

Control heart rates before administration of any dose of agonist alone and before the same dose of agonist with acetylcholine did not differ significantly. Only in the cases of the highest dose of both isoprenaline and aminophylline were control contractile force values for agonist alone significantly different from control values before agonist plus acetylcholine. However, in neither of these instances was agonist effect on contractility altered by acetylcholine.

Phosphorylase activity was determined on a separate series of guinea-pig isolated hearts frozen 60 s after drug injection. Doses of 0.005 and 0.01 μg of isoprenaline increased phosphorylase activity within 1 min after injection (Figure 7). Acetylcholine infusion antagonized the phosphorylase response to both doses of isoprenaline. The enhanced phosphorylase activity produced by salbutamol (10 μg) or by aminophylline (1 mg) was also significantly decreased by concomitant acetylcholine infusion.

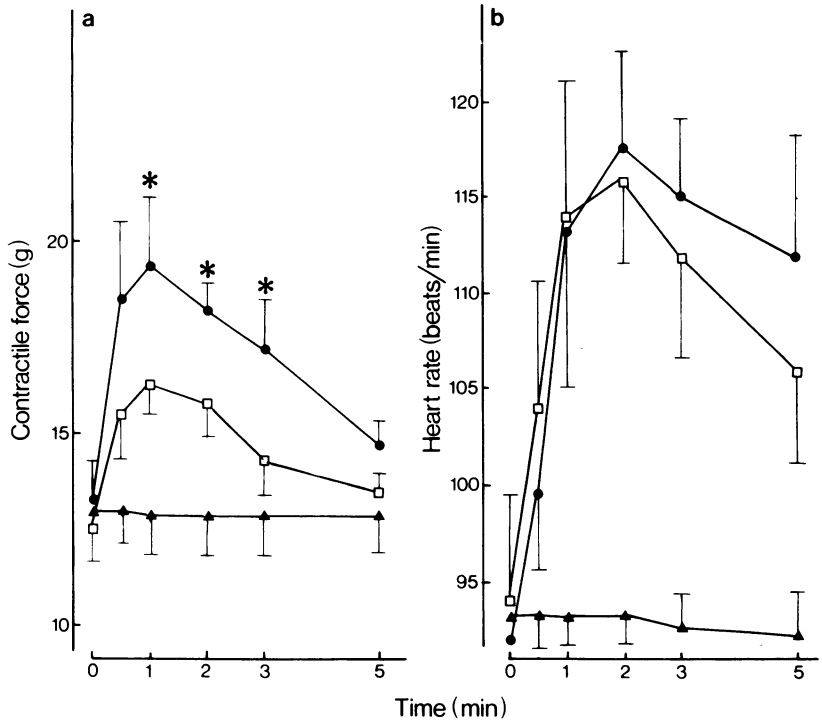

Figure 2 Time course of effects of contractile force (a) and heart rate (b) produced by isoprenaline injection (0.005 μg) in the absence (●) and the presence of acetylcholine (ACh) 0.018 μg/minute (□). (▲) shows effect of acetylcholine alone. Each point represents the mean of 4 experiments.

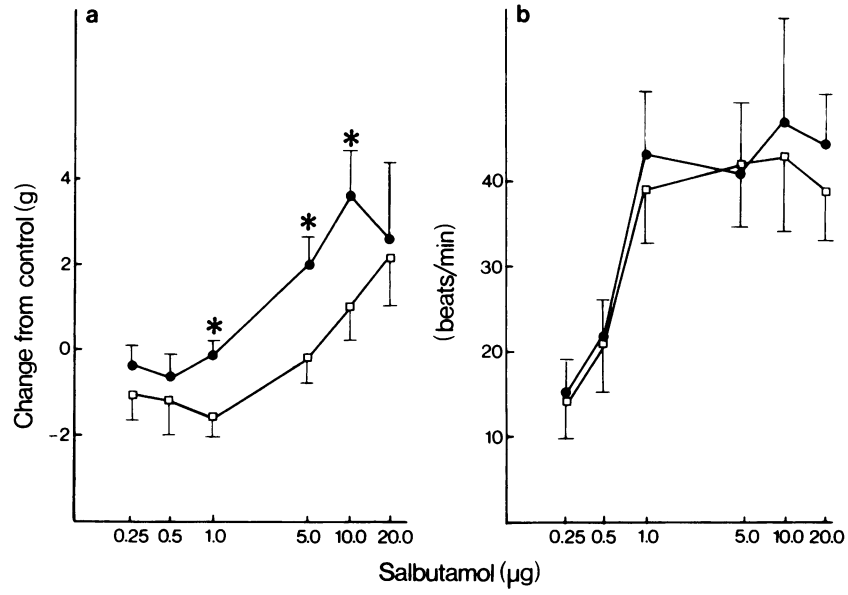

Figure 3 Effects of salbutamol alone (●) or during acetylcholine (ACh) infusion (0.018 μg/min) (□) on contractile force (a) and heart rate (b) in isolated perfused hearts of guinea-pigs. Responses were measured 1 min after salbutamol injection. Each point represents the mean of 4 or more experiments.

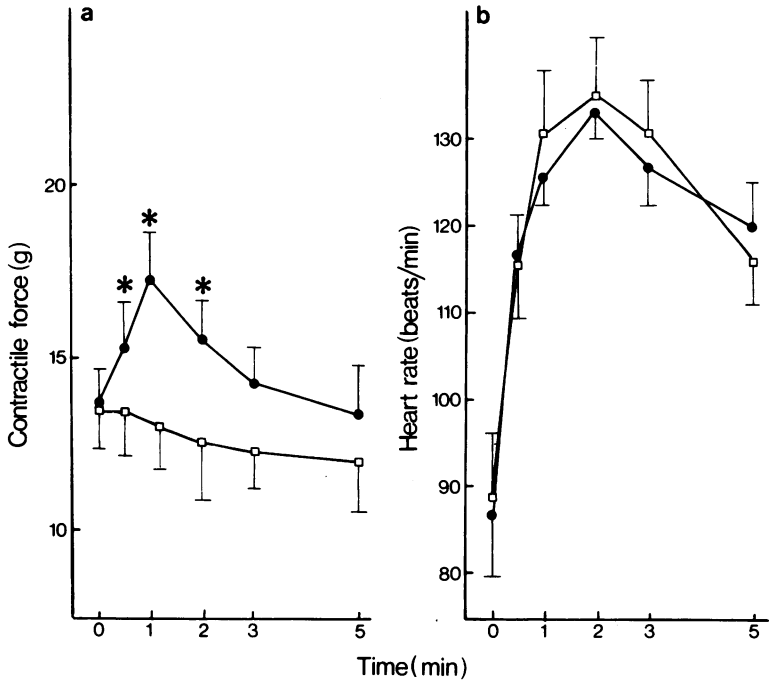

Figure 4 Time course of the effect of salbutamol (5 μg) on contractile force (a) and heart rate (b) both before (•) and during acetylcholine infusion (0.018 μg/min) (□). Each point represents the mean of 5 experiments.

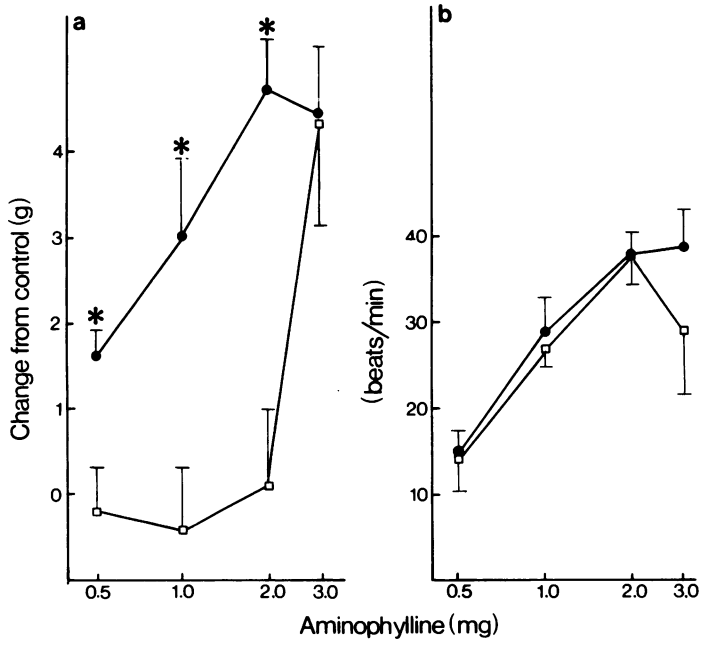

Figure 5 Effect of aminophylline injected before (•) and during acetylcholine infusion (0.018 μg/min) (□) in the guinea-pig isolated heart. Contractile force (a) and heart rate (b) responses were measured 2 min after aminophylline injection. Each point represents the mean of 4 or more experiments.

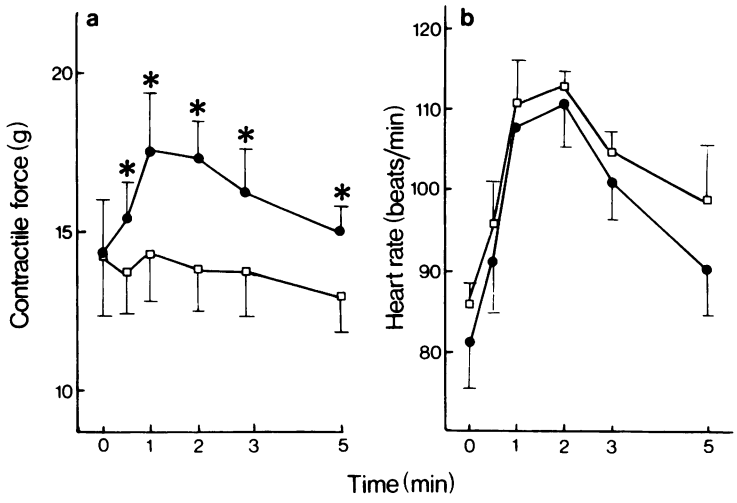

Figure 6 Time course of effect of aminophylline (1 mg) on contractile force (a) and heart rate (b) in the absence (•) and presence of acetylcholine (0.018 μg/minute) (□). Each point represents the mean of 5 experiments.

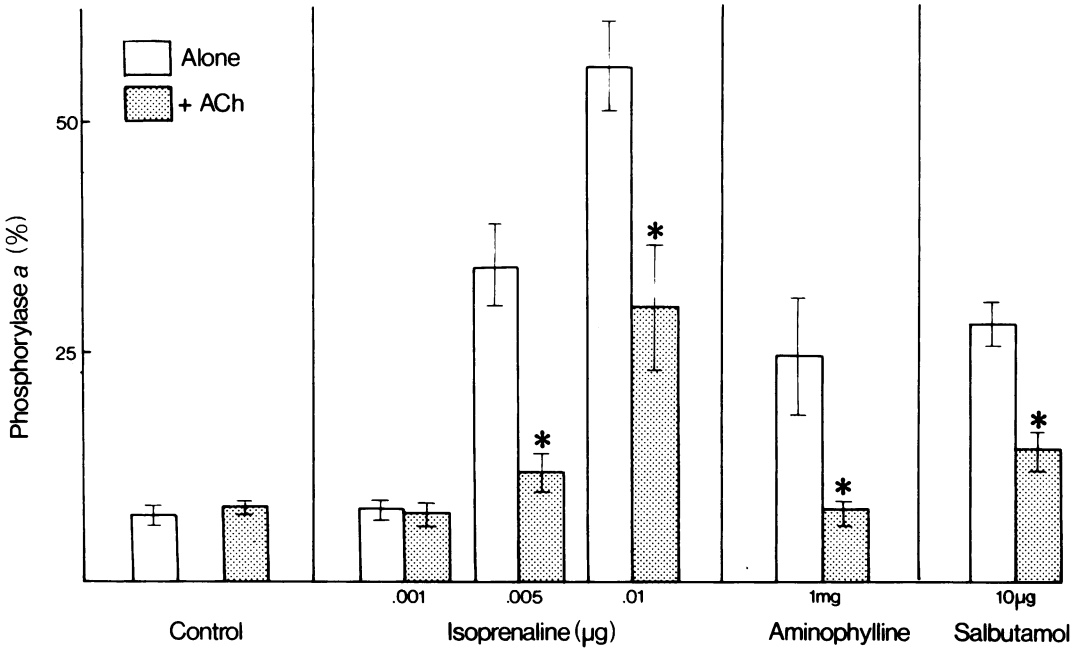

Figure 7 Effect of acetylcholine (ACh) on the activation of glycogen phosphorylase induced by isoprenaline, aminophylline and salbutamol. Results are expressed as % phosphorylase *a*

$$\left(\frac{\text{phosphorylase } a \text{ activity}}{\text{total phosphorylase activity}}\right) \times 100$$

since total phosphorylase activity was not changed from control by any treatment. Vertical bars indicate s.e. mean. * phosphorylase activity in the presence of acetylcholine which differs significantly from enzyme activity induced by the corresponding dose of isoprenaline, aminophylline or salbutamol. Each column represents the mean of 3-8 experiments.

Discussion

It has been proposed that enhancement of cardiac cyclic AMP concentration mediates the inotropic effect of β-adrenoceptor agonists and of phosphodiesterase inhibitors (Robison *et al.*, 1971). Acetylcholine has been shown to antagonize the increase in cyclic AMP produced by adrenaline or isoprenaline in various preparations (Lee *et al.*, 1971; Chamales *et al.*, 1971; Kuo *et al.*, 1971). It has further been proposed that an effect on cyclic AMP production may mediate the antagonism by acetylcholine of the cardiac effects of catecholamines (LaRaia & Sonnenblick, 1971).

The present study was performed in order to test the hypothesis that agents which antagonize the enhancement of cyclic AMP production will also antagonize the cardiac effects of catecholamines and phosphodiesterase inhibitors. Isoprenaline stimulates cardiac adenyl cyclase (Lee *et al.*, 1971) and salbutamol produces phosphorylase activation (Figure 7), presumably through cyclic AMP production. Both these agents stimulate cardiac rate and force, but salbutamol causes a positive inotropic response only with doses that produce a maximal increase in heart rate, whereas isoprenaline causes a positive inotropic response in doses which produce less than 15% increase in heart rate. From the present experiments a ratio between the dose causing a standard change in heart rate and the dose causing a standard change in contractile force was calculated for each agonist. These ratios are shown in Table 1. The large differences among the ratios for the three agonists suggest that the rate and contractility responses are not mediated by a single intermediate.

The concentration of acetylcholine used in these experiments had no measurable effect on either rate or contractile force, but did antagonize the effect of all three agonists on contractility. It has previously been shown that only concentrations of acetylcholine which produce a bradycardia can antagonize the chronotropic effect of adrenaline (Chamales *et al.*, 1971).

The negative inotropic effect seen when salbutamol was administered with acetylcholine, or when 0.5 μg of salbutamol was administered alone appears to have been a function of the different inotropic and chronotropic potencies of this agonist. These decreases in contractile force occurred primarily as increases in end-diastolic resting tension, rather than decreases in systolic tension, and could have resulted from increases in rate without changes in time to peak tension. This response to 0.5 μg of salbutamol could reflect the interaction of a sub-threshold inotropic dose with an effective chronotropic dose, and with acetylcholine could result from antagonism of inotropic, but not chronotropic effects.

In the present experiments the positive inotropic responses and phosphorylase activation induced by isoprenaline, aminophylline and salbutamol were antagonized in parallel by acetylcholine. A role for cyclic AMP in the production of these responses cannot be ruled out on the basis of the present data. However, the differences among the potency ratios for the three agonists, and the lack of effect of acetylcholine on the chronotropic response suggest that heart rate changes are not mediated solely by cyclic AMP. Although cyclic AMP participation in the heart rate response cannot be eliminated on the basis of these experiments, the data indicate that other factors must be involved.

Table 1 Ratio between dose required to produce 25% increase in heart rate (HR ED_{25}) and dose which produces 25% increase in contractile force (CF ED_{25})

Agonist	$\frac{HR\ ED_{25}}{CF\ ED_{25}}$
Isoprenaline	4.808
Salbutamol	0.039
Aminophylline	0.636

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