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Lack of relationship between myocardial cyclic AMP concentrations and inotropic effects of sympathomimetic amines

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Summary

1. Sympathomimetic amines which increase the contractility of the isolated heart were tested for effects on cyclic AMP concentrations in rabbit heart slices and on adenyl cyclase activity in rabbit heart homogenate.

2. Noradrenaline, as expected, stimulated adenyl cyclase activity and increased cyclic AMP concentrations, but dopamine and phenylephrine were ineffective.

3. This result does not support the concept that cyclic AMP plays an essential role in the inotropic effect of sympathomimetic amines.

Introduction

The catecholamines, noradrenaline, adrenaline and isoprenaline, stimulate the activity of myocardial adenyl cyclase in broken cell preparations (Murad, Chi, Rall & Sutherland, 1962), slices (LaRaja & Reddy, 1969), perfused isolated hearts (Cheung & Williamson, 1965 ; Robison, Butcher, Øye, Morgan & Sutherland, 1965), and *in vivo* (Namm & Mayer, 1968). The cyclic AMP concentration is increased just before or simultaneously with the increase in contraction amplitude in perfused hearts. The question is whether or not the metabolic response to catecholamines in heart muscle is required for the inotropic effect of these agents.

Dopamine and phenylephrine were chosen to test the concept that cyclic AMP plays an essential role in the effect of sympathomimetic amines on cardiac contractility. They differ from noradrenaline and adrenaline in the absence of a single hydroxyl group. The inotropic effect of dopamine has been demonstrated in the isolated rabbit atrium (Lee & Yoo, 1964) and the anaesthetized dog (McDonald & Goldberg, 1963), and that of phenylephrine in the isolated rabbit atrium (Lee & Yoo, 1964) and the isolated perfused dog heart (Kabela, Jalife, Peon, Cros & Mendez, 1969).

Methods

Contractility

New Zealand white rabbits were killed by a blow on the neck and strips of left atrium suspended at 31° C in 100 ml of a solution containing 137 mM NaCl, 2·68 mM KCl, 1·8 mM $CaCl_2$, 0·362 mM NaH_2PO_4, 11·9 mM $NaHCO_3$, 5·55 mM glucose, and 0·01 mM EDTA, aerated with 5% CO_2 in oxygen. The muscle strips were stimulated at 1 Hz with 1 ms square-wave pulses and a voltage 20% greater than

threshold. Contractions were recorded with a Grass transducer. The amines were added cumulatively. One concentration-effect curve was obtained from each preparation.

Cyclic AMP concentrations

New Zealand white rabbits were killed as before and ventricle slices (120–200 mg), prepared with a Stadie–Riggs tissue slicer, suspended at 37° C in 2 ml of the previously described solution.

After preincubation for 45 min with 2 μCi of ^{14}C-adenine (spec. act. 52–58 mCi/mmol) in a metabolic shaker, the medium was replaced by fresh medium and the amines added 5 min later in a volume of 0·02 ml. In the first type of experiment the slices were incubated for 15, 30, 45, or 60 seconds. In the second type they were incubated for 5 min in a medium containing 6·7 mM theophylline, 0·11 mM $MgSO_4$, and 50% of the concentration of $CaCl_2$ (0·9 mM) in addition to the previously listed components.

After incubation the slices were placed in 1 ml of water containing 50 μg carrier cyclic AMP and 0·1 μCi ^{3}H-cyclic AMP, boiled for 8 min, homogenized with a tissue grinder, and the homogenate was centrifuged for 10 min at 1,500 *g*. The cyclic AMP in the supernatant was purified by column chromatography on Dowex 50 and negative adsorption on nascent $BaSO_4$ and $Zn(OH)_2$ (Krishna, Weiss & Brodie, 1968), followed by paper chromatography in ethanol/boric acid (Streeto & Reddy, 1967).

The radioactivity was eluted with water and counted in a Picker 330 liquid scintillation counter using standard methods for double isotope counting with external standardization. The counting data were processed by a PDP 8L computer (Digital Equipment Corp.) which was programmed to compute d.p.m. for both isotopes. All ^{14}C counts were corrected to 100% on the basis of the tritium recovered. Recovery ranged from 26 to 38%.

Protein was determined by the biuret method (Kabat & Mayer, 1961).

Adenyl cyclase activity

New Zealand white rabbits were killed as before and 2 g of heart tissue were homogenized for 25 s in a Sorvall Omnimixer in 8 ml of 2 mM Tris-HCl, pH 7·4, and 1 mM $MgSO_4$. The homogenate was centrifuged at 2,000 *g*, the supernatant discarded, the pellet resuspended in 10 ml of the same solution, the suspension centrifuged at 2,000 *g*, and the supernatant discarded.

The pellet (100 mg wet weight) was incubated for 3 min at 37° C in 2 ml of a solution containing 40 mM Tris-HCl, pH 7·4, 3·5 mM $MgSO_4$, 6·7 mM theophylline, 10 mM phosphoenolpyruvic acid, 100 μg pyruvate kinase, and 2 mM ^{14}C-ATP (spec. act. 0·248 mCi/mmol). The reaction mixture was boiled for 3 min immediately after adding 0·1 μCi ^{3}H-cyclic AMP; the suspension was centrifuged at 2,000 *g*, and the cyclic AMP purified by the method of Krishna *et al.* (1968). All ^{14}C counts were corrected to 100% on the basis of the tritium recovered. Recovery ranged from 46 to 69%.

Drugs and chemicals

These included: (−)-phenylephrine HCl and dopamine HCl (K & K Labs.), (−)-noradrenaline bitartrate (Winthrop), phosphoenolpyruvic acid and pyruvate

kinase (Sigma), ^{14}C-adenine (spec. act. 52–58 mCi/mmol), 3′,5′-cyclic AMP, ^{3}H-3′,5′-cyclic AMP (spec. act. 16·3 C/mmol), 5′-ATP and ^{14}C-5′-ATP (spec. act. 45–53 mCi/mmol, Schwarz BioResearch), and AG 50W-X4 (200–400 mesh, Bio-Rad Labs.).

Results

Contractility

Dopamine, phenylephrine and noradrenaline had an inotropic effect of similar magnitude on the isolated rabbit left atrium (Fig. 1). Dopamine was 9 times and phenylephrine 3 times less potent than noradrenaline.

Cyclic AMP concentrations

Unlike noradrenaline, dopamine and phenylephrine did not increase the concentration of cyclic AMP in rabbit heart slices (Figs. 2 and 3). The greatest effect of noradrenaline on the concentration of cyclic AMP was an approximately 3-fold increase in the absence of theophylline (1 mM noradrenaline present for 30 s, Fig. 2) and an approximately 6-fold increase in the presence of theophylline (0·1 mM noradrenaline, Fig. 3).

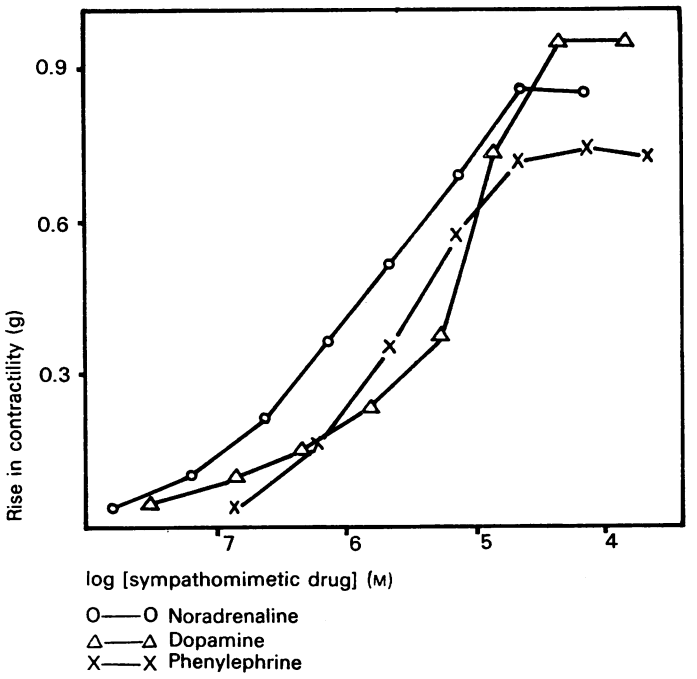

FIG. 1. Effects of noradrenaline, dopamine and phenylephrine on the contractility of rabbit left atrium. Means of four–eight experiments.

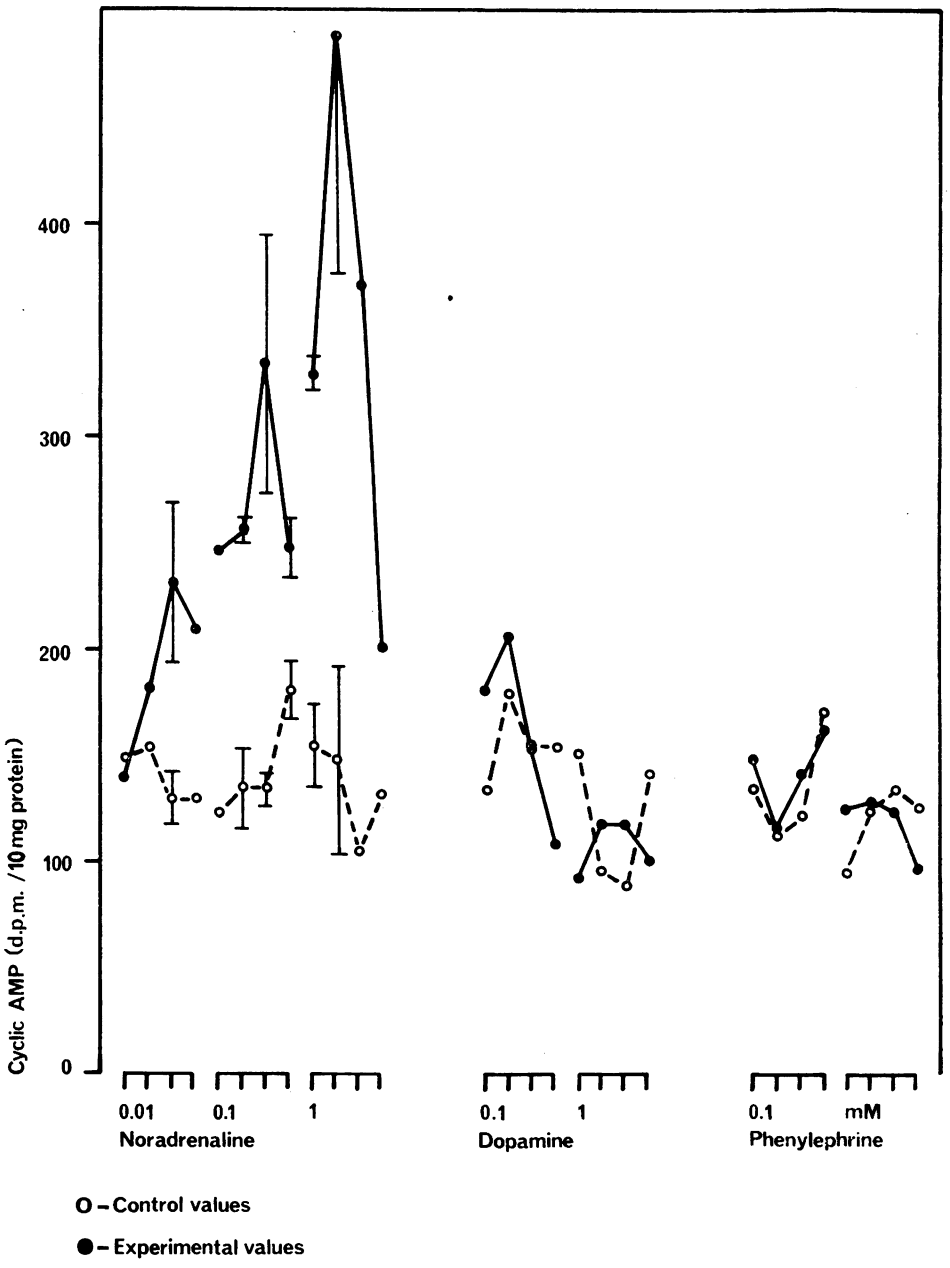

FIG. 2. Effects of noradrenaline, dopamine and phenylephrine on cyclic AMP concentrations in rabbit heart slices, incubated for 15, 30, 45 and 60 seconds. Means of three–five experiments. The vertical bars represent the standard error of the means; they are shown only when the difference between control and experimental values was significant ($P<0.05$).

Adenyl cyclase activity

In rabbit heart homogenate noradrenaline significantly increased the rate of formation of cyclic AMP; dopamine and phenylephrine were ineffective (Fig. 4). Noradrenaline (1 mM) caused an approximately 3-fold increase in the amount of cyclic AMP.

Discussion

There is evidence that the primary sites of action of dopamine and phenylephrine on the myocardium differ from the site of action of noradrenaline. In the rabbit atrium reserpine pretreatment reduced the inotropic effect of dopamine, indicating that part of the effect of dopamine is exerted through an action on adrenergic nerve fibres (Lee & Yoo, 1964). In the cat heart, van Rossum (1966) found special dopamine receptors. β-Adrenoceptor blocking drugs inhibited the inotropic effect of dopamine on the dog heart (McDonald & Goldberg, 1963), on the rabbit atrium (Lee & Yoo, 1964), and on the guinea-pig atrium (Furchgott, 1970).

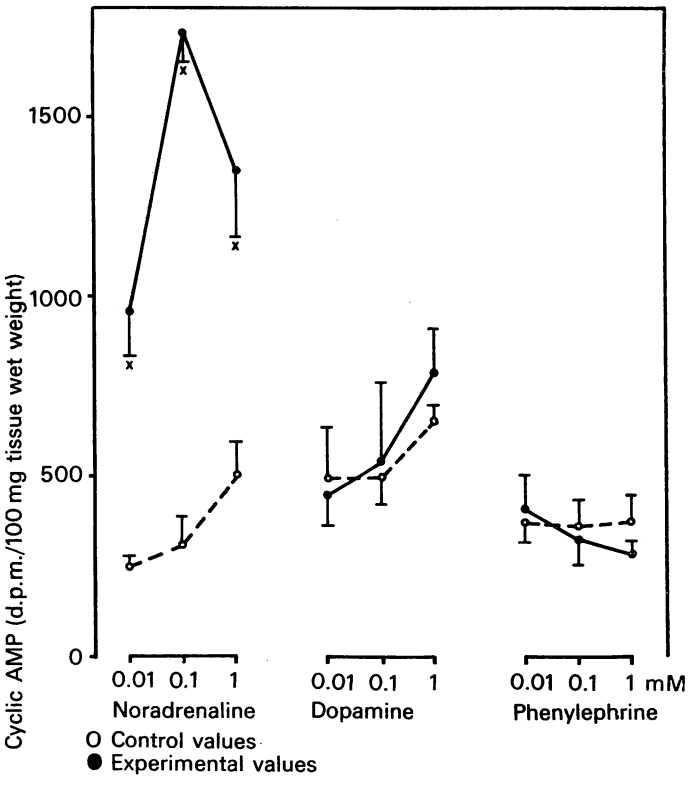

FIG. 3. Effects of noradrenaline, dopamine and phenylephrine on cyclic AMP concentrations in rabbit heart slices, incubated for 5 min in the presence of theophylline. Means of three experiments. The vertical bars represent the standard error of the means; the asterisks indicate a significant difference between control and experimental values ($P<0.05$).

The inotropic effect of phenylephrine on the rabbit atrium was not altered by pretreatment with reserpine (Lee & Yoo, 1964). Studies with α-adrenoceptor blocking drugs gave evidence that phenylephrine acts on α-adrenoceptors in the isolated rat ventricle (Wenzel & Su, 1966) and rabbit left atrium (Benfey & Varma, 1967). β-Adrenoceptor blocking drugs inhibited the inotropic effect of phenylephrine on the rabbit right atrium (Lee & Yoo, 1964 ; Leong & Benfey, 1968) and on the isolated perfused dog heart (Kabela *et al.*, 1969).

Dopamine and phenylephrine differ from noradrenaline and adrenaline in the absence of a single hydroxyl group. The catecholamine, dopamine, lacks the alcoholic group of noradrenaline ; the phenolamine, phenylephrine, lacks the *p*-phenolic group of adrenaline.

In this study dopamine and phenylephrine had inotropic activity but no biochemical activity. This result could indicate that cyclic AMP does not play an essential role in the effect of sympathomimetic amines on cardiac contractility.

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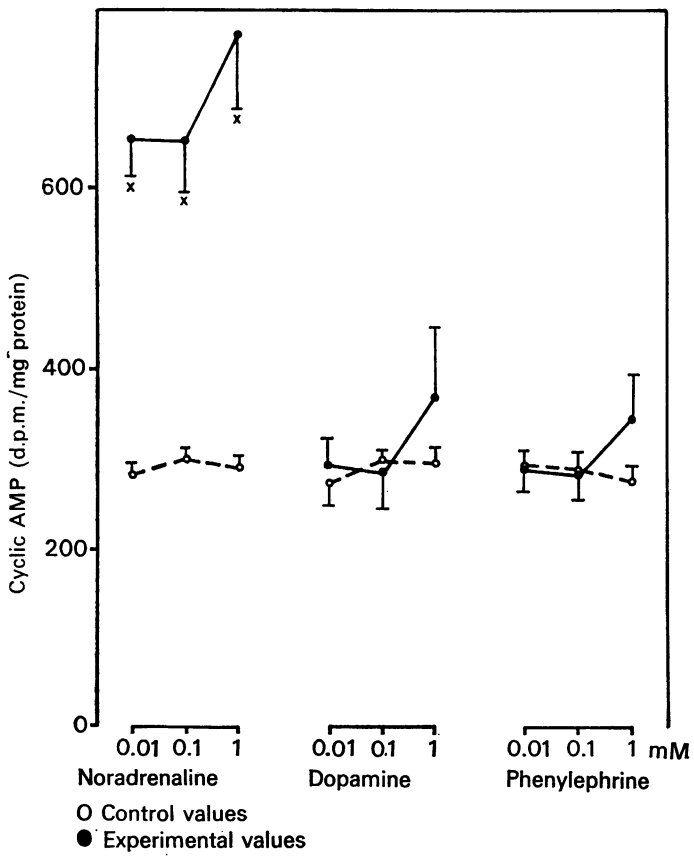

FIG. 4. Effects of noradrenaline, dopamine and phenylephrine on adenyl cyclase activity in rabbit heart homogenate. Means of three experiments. The vertical bars represent the standard error of the means; the asterisks show a significant difference between control and experimental values ($P<0{\cdot}05$).

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