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DISSOCIATION OF CARDIAC INOTROPIC AND ADENYLATE CYCLASE ACTIVATING ADRENOCEPTORS

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1 At higher temperatures, near the physiological range for mammals and nonhibernating frogs, the adrenoceptors for both inotropic responses to adrenaline and noradrenaline and for cyclic 3′,5′-adenosine monophosphate (cyclic AMP) production in rat and frog isolated heart preparations, had typical β characteristics. Phenoxybenzamine potentiated the inotropic response and the accumulation of cyclic AMP; conversely, propranolol inhibited the two responses.

2 When the ambient temperature was reduced, the adrenoceptors mediating cyclic AMP production changed very little; they were blocked as effectively as at the higher temperature by propranolol and were not blocked by phenoxybenzamine. However, the adrenoceptors mediating the inotropic response were markedly changed by the decrease in temperature; phenoxybenzamine now inhibited this response and the inhibitory activity of propranolol was reduced about tenfold.

3 These results indicate that the adrenoceptors that mediate cardiac inotropic responses at physiological temperatures are distinct from those that mediate the production of cyclic AMP, and that the activation of adenylate cyclase and the accumulation of cyclic AMP are probably not intermediate steps in cardiac inotropic responses to catecholamines.

Introduction

Our objective was to test the hypothesis that cyclic 3′,5′-adenosine monophosphate (cyclic AMP) plays an obligatory role in the effect of catecholamines on cardiac contractility. There is considerable evidence that the inotropic effect of catecholamines is mediated by intracellular cyclic AMP. Following administration of catecholamines, myocardial cyclic AMP levels rise before, or simultaneously with, the rise in contractility; catecholamines have the same order of potency in stimulating adenylate cyclase and in increasing contractility of the heart; β-adrenoceptor blocking drugs inhibit the effects of catecholamines on contractility and on cyclic AMP levels (Robison, Butcher & Sutherland, 1971).

Our study is based on the observation of Kunos & Szentivanyi (1968) amd Kunos, Yong & Nickerson (1973) that the blocking characteristics of inotropic adrenoceptors change with temperature. At low temperatures, α-adrenoceptor blocking drugs inhibit the inotropic effect of catecholamines and β-adrenoceptor blocking drugs decrease in potency. We now report that the blocking characteristics of adrenoceptors mediating cyclic AMP production do not change with temperature.

Methods

Contractility

The hearts of frogs (*Rana pipiens*), kept at 5°C for several weeks before use, were isolated and attached to a J-shaped cannula filled with a solution containing (mM): NaCl 111; KCl 1.88; $CaCl_2$ 1.08; $NaHCO_3$ 2.38 and NaH_2PO_4 0.083. After a 60 min period during which the solution was frequently changed, single doses of adrenaline were added, the maximal effect on contractility determined, and the drug washed out before a change in heart rate occurred.

Male Sprague-Dawley rats of 180-300 g weight were anaesthetized with ether, the hearts quickly removed and the left atria suspended in a solution containing (mM): NaCl 115.3; KCl 4.6; $CaCl_2$ 1.8; $MgSO_4$ 1.1; $NaHCO_3$ 22.1; KH_2PO_4 1.1 and glucose 11.1. The solution was aerated with 5% CO_2 in oxygen. The atria were driven by a Grass stimulator (model SD5) at 1 Hz with 3 ms square-wave pulses at a voltage slightly above threshold (3-6 V). Isometric contractions were recorded with a force-displacement transducer (Grass FT 83C) and a polygraph (Grass model 5). Noradrenaline was added cumulatively.

Exposure to phenoxybenzamine was for

40 min, the solution being changed every 10 min, and the preparation was then washed repeatedly for 1-2 h before adrenaline or noradrenaline was added. Exposure to propranolol was for 10 min and the drug remained in the bath.

The results are expressed as the dose-ratio. The dose-ratio is the ratio of the concentrations of the agonist causing 50% of the maximal effect after and before exposure to the antagonist or potentiating drug. As in most experiments at low temperatures phenoxybenzamine depressed the maximum of the effect of the agonist by more than 50%, the concentration of agonist causing 20% of the maximal effect was used for the calculation of the dose-ratio in these experiments.

Cyclic AMP accumulation

Ventricles of frogs (*Rana pipiens*) were cut in half and suspended in the solution used for the contractility experiments. Ventricle slices from male Sprague-Dawley rats anaesthetized with ether were prepared with a Stadie-Riggs tissue slicer and suspended in a solution containing (mM): NaCl 137; $CaCl_2$ 1.8; KCl 2.68; $NaHCO_3$ 11.9; NaH_2PO_4 0.362; glucose 5.55; and disodium edetate 0.01 which was aerated with 5% CO_2 in oxygen.

After preincubation for two periods of 30 min with 2 μCi [^{14}C]-adenine in a metabolic shaker, the medium was replaced four times with fresh medium containing 6.7 mM theophylline, and adrenaline or noradrenaline was then added for 15 minutes. Exposure to phenoxybenzamine or propranolol was for 60 minutes. Phenoxybenzamine was removed from the bath before the addition of the catecholamines and propranolol remained in the bath.

After incubation with the catecholamines the slices were placed in 1 ml 6% TCA containing 50 μg carrier cyclic AMP and 0.1 μCi [^{3}H]-cyclic AMP, homogenized with a tissue grinder, and the homogenate centrifuged for 10 min at 2,000 *g*. The supernatant was shaken once with benzene and twice with ether, the organic phase discarded and the aqueous phase purified by column chromatography on Dowex 50 and negative adsorption on nascent $BaSO_4$ (Krishna, Weiss & Brodie, 1968). Radioactivity was counted in a Picker 330 liquid scintillation counter using standard methods of 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/min for both isotopes. All ^{14}C counts were corrected to 100% on the basis of the tritium recovered. Mean recovery was 40%.

Protein was determined by the biuret method (Kabat & Mayer, 1961). For each incubation two half frog ventricles (mean protein content, 11 mg) or one rat ventricle slice (mean protein content, 18 mg) were used.

The mean basal levels of cyclic AMP (d/min per mg protein) were as follows: frog, 14°C, 10.6; 24°C, 12.3; rat, 14°C, 17.1; 37°C, 20.5. Phenoxybenzamine or propranolol did not significantly change these values.

The validity of the prelabelling method for the study of changes in myocardial cyclic AMP levels has been confirmed. Lee, Kuo & Greengard (1971) compared the prelabelling method in rat heart slices with the protein kinase method, which measures total cyclic AMP, and concluded that the ATP newly synthesized from the radioactive adenine was in equilibrium with the existing pool used for the production of cyclic AMP. Brooker (1971) prelabelled the isolated frog ventricle with [^{14}C]-adenosine and found the specific activity of cyclic [^{14}C]-AMP and [^{14}C]-ATP to be similar.

Drugs and chemicals

These included (−)-noradrenaline bitartrate and (−)-adrenaline bitartrate (Winthrop), phenoxybenzamine hydrochloride (Smith, Kline & French), propranolol hydrochloride (Inderal, Ayerst, McKenna & Harrison), cyclic 3′,5′-AMP, [^{3}H]-3′,5′-cyclic AMP (sp. act. 16.3 Ci/mmol) and [^{14}C]-adenine (sp. act. 52-58 mCi/mmol; Schwarz BioResearch), and AG 50W-X4 (200-400 mesh; Bio-Rad Labs.).

Results

Contractility

At low temperatures (14° and 17°C), both the α-adrenoceptor blocking drug phenoxybenzamine and the β-adrenoceptor blocking drug, propranolol, inhibited the inotropic effect of adrenaline on the frog isolated ventricle and of noradrenaline on the rat isolated atrium (Figures 1 and 2).

At higher temperatures (24° and 31°C), phenoxybenzamine potentiated the inotropic effect of the catecholamines, and the inhibitory potency of propranolol was approximately ten times greater than at low temperatures (Figures 1 and 2).

Cyclic AMP accumulation

The effects on cyclic AMP accumulation of adrenaline in frog isolated ventricles and of noradrenaline in rat heart slices are shown in

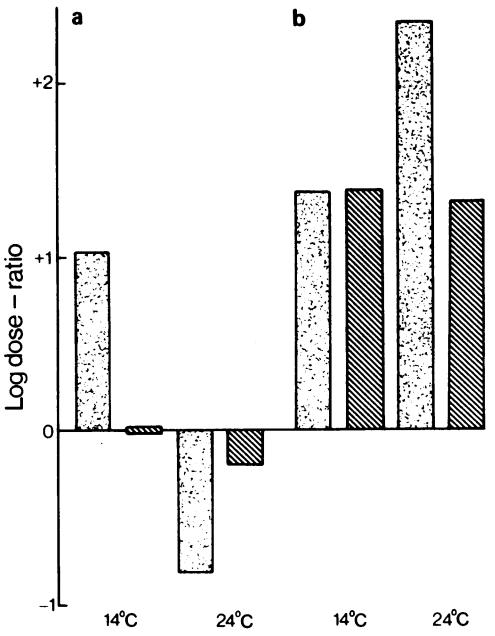

Fig. 1 Effects of (a) phenoxybenzamine (7.4 μM) and (b) propranolol (0.34 μM) on the dose-ratio of adrenaline in frog ventricles. Stippled columns, inotropic responses; hatched columns, cyclic AMP accumulation. A positive dose-ratio indicates inhibition and a negative dose-ratio indicates potentiation of responses to adrenaline.

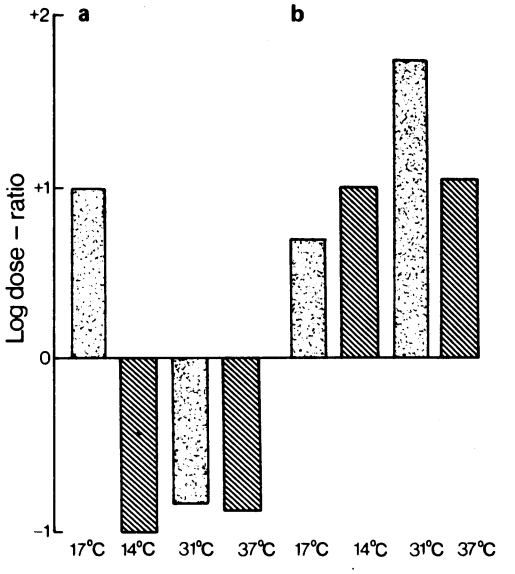

Fig. 2 Effects of (a) phenoxybenzamine (7.4 μM) and (b) propranolol (0.34 μM) on the dose-ratio of noradrenaline in rat atria and rat heart slices. Stippled columns, inotropic responses; hatched columns, cyclic AMP accumulation. A positive dose-ratio indicates inhibition and a negative dose-ratio indicates potentiation of responses to noradrenaline.

Figures 3 and 4. At a low temperature (14°C), phenoxybenzamine did not inhibit the effect of the catecholamines on cyclic AMP accumulation, even when the concentration of the drug was increased tenfold (74 μM).

At higher temperatures (24° and 37°C), the effects of phenoxybenzamine or propranolol on cyclic AMP accumulation produced by adrenaline and noradrenaline were not unlike those at low temperature (Figures 1 and 2).

Discussion

The results described here confirm previous observations on the effects of an α-adrenoceptor antagonist, phenoxybenzamine, and a β-adrenoceptor antagonist, propranolol, on inotropic responses to catecholamines. At temperatures approximating to the physiological range for mammals (31°C in the present study) or for non-hibernating frogs (24°C) phenoxybenzamine potentiated inotropic responses (Furchgott, 1959; Benfey & Greeff, 1961; Kunos *et al.*, 1973; and others). At the same temperatures, propranolol very effectively blocked inotropic responses. As previously observed (Kunos & Szentivanyi, 1968; Buckley & Jordan, 1970; Kunos *et al.*, 1973), the α-adrenoceptor antagonist blocked responses in both the frog and rat preparations at lower temperatures, and propranolol was about ten times less potent.

In contrast to the change in the blocking characteristics of inotropic adrenoceptors with temperature, the stimulation of cyclic AMP production by adrenaline or noradrenaline remained an entirely β-adrenoceptor response. Phenoxybenzamine produced no block of cyclic AMP accumulation at either temperature and propranolol was equally effective at the lower temperature.

It was previously shown that the inotropic effect of phenylphrine is blocked by α-adrenoceptor antagonists in the driven rat ventricle (Wenzel & Su, 1966), rabbit atrium (Benfey & Varma, 1967; Benfey, 1973) and guinea-pig atrium (Govier, 1968). It was also shown that phenylephrine does not stimulate cyclic AMP accumulation in rabbit heart slices or broken cell preparations (Benfey, 1971) or guinea-pig broken

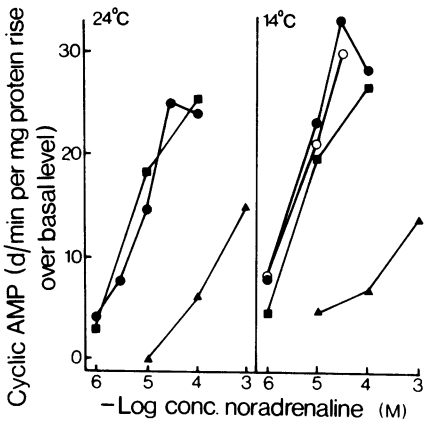

Fig. 3 Effects of adrenaline on cyclic AMP accumulation in frog ventricles in the absence (●) and presence of propranolol, 0.34 μM (▲); phenoxybenzamine, 7.4 μM (■); and phenoxybenzamine, 74 μM (○). Means of 6-10 incubations.

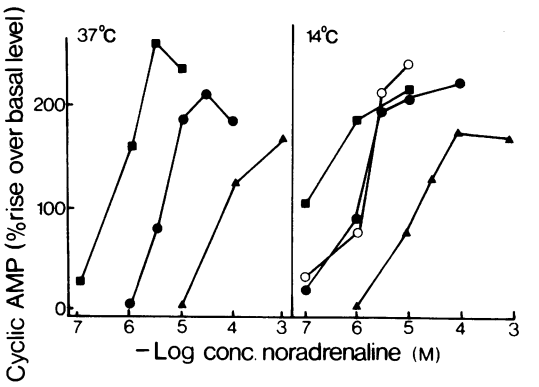

Fig. 4 Effects of noradrenaline on cyclic AMP accumulation in rat heart slices in the absence (●) and presence of propranolol, 0.34 μM (▲); phenoxybenzamine, 7.4 μM (■); and phenoxybenzamine 74 μM (○). Means of 6-12 incubations.

cell preparations (McNeill, Davis & Muschek, 1972). These observations indicate that cyclic AMP is not an obligatory intermediate in inotropic responses to sympathomimetic amines. Combined with the results of other experimental procedures as reviewed by Sobel & Mayer (1973), they strongly suggest that the many reported correlations between myocardial cyclic AMP production and inotropic responses to sympathomimetic amines, may reflect parallel processes rather than a cause and effect relationship. However, this evidence appears not to have seriously shaken faith in the obligatory role of cyclic AMP as a 'second messenger' in inotropic responses to sympathomimetic amines, perhaps because the dissociation has not been fully related to the β-adrenoceptors that normally dominate responses of the myocardium.

In the present study at the higher, more usual, experimental temperatures, the receptors mediating both cyclic AMP accumulation and increase in force of contraction appeared to be typical β-adrenoceptors. There was no significant α-adrenoceptor component of either response. However, when the temperature was lowered, only the receptors mediating the inotropic response assumed α-adrenoceptor characteristics, i.e., phenoxybenzamine inhibited the response and propranolol became much less effective. There was no significant change in the characteristics of the adrenoceptors mediating cyclic AMP accumulation. Thus, it appears that at temperatures approaching the physiological range for mammals and nonhibernating amphibians, the myocardium has at least two populations of β-adrenoceptors independently involved in the increased force of contraction and in the production of cyclic AMP in response to sympathomimetic amines.

It appears that the present observations on cardiac β-adrenoceptors may be correlated with previous reports indicating an absence of stimulation of cyclic AMP production during α-adrenoceptor stimulation of the myocardium, on the basis of evidence that the α- and β-adrenoceptors are simply different configurations of the same entity (Kunos *et. al.*, 1973). The results make it unnecessary to assume that one configuration (β) but not the other is coupled to the contractile response through cyclic AMP.

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