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CARDIAC ADENYLATE CYCLASE—II

STRUCTURE–ACTIVITY RELATIONSHIPS FOR THE ACTIVATION OF RAT VENTRICLE ADENYLATE CYCLASE BY β-ADRENOCEPTOR AGONISTS

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Abstract—The ability of phenylethylamine derivatives to stimulate adenylate cyclase activity of a sarcolemma fraction of rat ventricular muscle has been studied. The structure–activity relationships for maximal activation of adenylate cyclase activity showed that a phenylethylamine derivative required two adjacent hydroxyl groups at the 3 and 4 positions of the phenyl ring, a hydroxyl group on the asymmetric β-carbon of the ethylamine side chain and an isopropyl group on the amine. The configuration of the hydroxyl group on the β-carbon was critical since the *laevo* isomer of this amine (isoprenaline) was highly active whilst the *dextro* isomer was almost inactive. The activation of adenylate cyclase activity by (−)-isoprenaline was blocked by (−)-propranolol but not (+)-propranolol or phentolamine. These structure–activity relationships are identical with those found for the actions of β-agonists and antagonists on cardiac muscle and therefore strengthen the hypothesis that the β-adreno-receptor is a component of adenylate cyclase in cardiac muscle. The order of potency of catecholamine derivatives to stimulate adenylate cyclase activity in intact cubes of rat ventricles was similar to that found in homogenates of ventricles, although the relative sensitivity of catecholamines in cubes was approximately 50 times higher. It was concluded that homogenization and preparation of sarcolemma membranes alters the sensitivity of adenylate cyclase to activation by catecholamines.

The enzyme adenylate cyclase catalyses the formation of adenosine 3′5′-monophosphate (cyclic AMP) from ATP in the presence of Mg^{2+}. In liver, fat cells and kidney, adenylate cyclase is a membrane-bound enzyme found almost exclusively in the plasma membrane (see [1] for a review) and recently we have demonstrated a sarcolemmal localization of adenylate cyclase in cardiac muscle [2, 3].

In homogenates of cardiac muscle, the rate of formation of cyclic AMP is markedly increased by the addition of a β-adrenoceptor agonist and this stimulapion can be blocked on the addition of a β-adrenoceptor antagonist [4, 5]. In isolated perfused hearts, the tissue levels of cyclic AMP are elevated within 1–3 sec of β-agonist application which is shortly before the inotropic and metabolic response of the heart to the agonist [6–8]. Again, the prior administration of a β-antagonist blocks the elevation of cyclic AMP and also the characteristic responses of the heart to the β-agonist. Thus it has been proposed that the cardiac β-receptor is a component of adenylate cyclase and that cyclic AMP is the intracellular mediator of the β-agonist-induced changes in cardiac contractility [7, 9], and metabolic processes such as glycogenolysis [7, 9, 10].

Mayer [4] demonstrated that the order of potency of catecholamines in stimulating adenylate cyclase in dog heart homogenates was the same as that for stimulation of cardiac contractility, i.e. isoprenaline > adrenaline > noradrenaline. However, there has been no detailed study of the structure–activity relationships for compounds which stimulate cardiac adenylate cyclase. A study was therefore undertaken of the ability of structural analogues of isoprenaline (the classical β-adreno-agonist) to stimulate adenylate cyclase activity in rat ventricular muscle. The structure–activity relationships determined could then be compared with the known structural requirements for stimulation of cardiac β-receptors [11–14].

A subcellular fraction isolated from homogenates of rat ventricle by differential centrifugation and characterised as being rich in both plasma membrane and catecholamine-sensitive adenylate cyclase, was used as the source of enzyme for this structure–activity analysis. The preparation and characterization of this subcellular fraction (P_4) is detailed in a preceding paper [3].

EXPERIMENTAL

Adenylate cyclase activity in a membrane fraction from rat heart homogenate

A particulate fraction (P_4) prepared as described previously [3] was resuspended in 4 ml of ice-cold sucrose (0·25 M) containing dithiothreitol (2 mM) and Tris–HCl (10 mM, pH 7·2) and used as a source of catecholamine-sensitive adenylate cyclase. Adenylate cyclase activity was determined using the standard incubation mixture of Drummond and Duncan [15] except that (a) the final incubation volume was 50 μl, (b) phosphatidylinositol (4 μg) was included in the incubation mixture [16], (c) the amount of pyruvate kinase included was 1·54 I.U., (d) the particulate fraction contained 40–60 μg of protein and (e) amines

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were added in a volume of 5 μl. [^{3}H]cyclic AMP was isolated as described by Krishna and Birnbaumer [17]. Adenylate cyclase activity was determined within 20 min of preparation of the subcellular fraction P_4. At least three concentrations of each amine were tested for their ability to stimulate adenylate cyclase activity. Results were expressed as a percentage of basal adenylate cyclase activity and significant stimulation was determined using the Students' t-test with $P < 0.05$. In cases where four concentrations of an amine produced significant stimulation of enzyme activity, the approximate concentration of the amine producing 50% of the maximal activation of adenylate cyclase activity (EC_{50}) was estimated graphically by eye.

The protein content of the particulate fraction was determined by the method of Campbell and Sargent [18].

Adenylate cyclase activity in intact heart cubes

Male Wistar rats (250–350 g) were injected with pentobarbitone (100 mg/kg, i.p.) and heparin (1000 I.U.) and when anaesthetized, the hearts were removed. Three hearts were used in each experiment. Blood was washed out of the hearts by retrograde perfusion through the aorta with 20 ml modified Tyrode solution containing NaCl 130 mM, KCl 5·6 mM, $CaCl_2$ 2·16 mM, $NaHCO_3$ 25 mM, glucose 11·1 mM, sucrose 13·1 mM, NaH_2PO_4 9·1 mM, $MgSO_4$ 1·4 mM and previously gassed with 95% O_2:5% CO_2. Atria were removed and the ventricles chopped into 0·5-mm cubes as described by Lee *et al.* [19]. The ATP pool in the cubes was prelabelled with [2-^{3}H]adenine and the effects of catecholamines on the conversion of newly-synthesized [^{3}H]ATP to [^{3}H]cyclic AMP was determined [19, 20].

Reagents

Cyclic AMP (monosodium salt), bovine serum albumin (fatty acid free) and ATP (Tris salt) were purchased from Sigma. Dithiothreitol, 2-phosphoenolpyruvate (trisodium salt) and pyruvate kinase were obtained from Calbiochem. [2-^{3}H-5']Adenosine triphosphate and [2-^{3}H]adenine were purchased from The Radiochemical Centre, Amersham and phosphatidylinositol (ex yeast) was obtained from Koch-Light Laboratories, U.K. All other reagents were of the highest purity available.

Amines. Compounds and suppliers were as follows: (−)-isoprenaline bitartrate, (±)-noradrenaline HCl, (±)-adrenaline HCl, (−)-adrenaline bitartrate (Sigma); (+)-isoprenaline bitartrate, (±)-isoprenaline HCl, (±)-*N*-ethyl-noradrenaline HCl, (±)-*N*-ter-butyl-noradrenaline methane sulphonate, *N*-isopropyl-dopamine, (±)-1-(3-hydroxyphenyl)-2-isopropyl-aminoethanol sulphate, *N*-isopropylphenylethylamine HCl, (±)-*N*-isopropylphenylethanolamine HCl, (+)-noradrenaline bitartrate, (+)-adrenaline bitartrate (*Sterling-Winthrop Research Institute, U.S.A.); 3,4-dihydroxy-alpha-(isopropylamino)-acetophenone HCl (Aldrich Chemical Co., U.S.A.); (±)-(3-hydroxy-methyl, 4-hydroxyphenyl)-2-ter-butylaminoethanol (*Allen and Hanburys, U.K.); (±)-(3,5-dihydroxy-phenyl)-2-isopropylaminoethanol HCl, (±)-(3-meth-oxy,4-hydroxyphenyl)-2-isopropylaminoethanol HCl (*Boehringer Ingelheim, Germany); (±)-1-(4-hydroxy-phenyl)-2-isopropylaminoethanol HCl (*Alcon Laboratories, Texas); (−)-noradrenaline bitartrate, (−)-phenylephrine HCl (Koch–Light, U.K.); phento-lamine methane sulphonate (*CIBA, Switzerland); (+)-propranolol HCl and (−)-propranolol HCl (*I.C.I., U.K.).

* Asterisks indicate organizations which generously donated compounds and we gratefully acknowledge these gifts.

RESULTS

Adenylate cyclase activity in a sarcolemma fraction from heart homogenates

Basal adenylate cyclase activity of membrane fraction P_4, determined using substrate concentrations of 0·4 mM ATP and 15 mM $MgSO_4$, was 21·4 pmoles cyclic AMP formed/min/mg protein (S.E.M. = 2·1, n = 33) at 37°, and was expressed as 100·0% (±10·2%).

(i) *Activation of adenylate cyclase by isoprenaline and its derivatives.* Since the previous study [3] showed that (±)-isoprenaline stimulated the basal adenylate cyclase activity of P_4 in the presence of phosphatidylinositol, the following systematic study of the ability of structural derivatives of isoprenaline to activate adenylate cyclase activity was made. Compounds have been grouped to illustrate the effects of structural alterations on the parent isoprenaline molecule.

(a) Effect of *N*-substitutions (Fig. 1). The primary amine, (±)-noradrenaline, stimulated adenylate cyc-

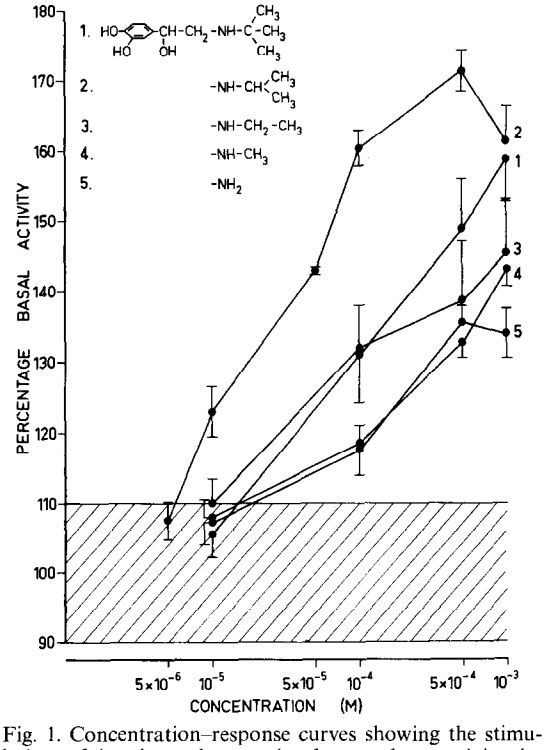


Fig. 1. Concentration–response curves showing the stimulation of basal rat heart adenylate cyclase activity by various *N*-substituted catecholamines. Each point represents the mean ± S.E.M. of 4–6 determinations and the hatched area represents the mean ± S.E.M. of the basal enzyme activity.

lase activity, and this indicates that an alkyl substituent of the nitrogen is not an absolute requirement for activation of the enzyme. However, increasing monoalkyl substituents through methyl, ethyl to isopropyl led to progressively greater and more potent activation of adenylate cyclase. Maximal stimulation (72% at 500 μM) was achieved with an isopropyl group since the addition of a tertiary butyl group resulted in decreased activation. (See Table 1 for EC_{50} values). The racemic mixture of these amines was used since not all amines were available as the resolved optical isomer. Theoretical considerations [21] have shown that monosubstitution of noradrenaline with methyl, ethyl and isopropyl groups changes neither the conformation of the side chain relative to the catechol ring nor the positive charge around the nitrogen. Thus the increase in adenylate cyclase stimulation in this series is suggestive of a favourable interaction of the alkyl group with an isopropyl-like moiety on the binding site of the enzyme by long-range dispersion binding. Since these studies suggested that optimal activation was achieved with the isopropyl derivative (isoprenaline), subsequent studies were made with isoprenaline derivatives.

(b) Effect of β-carbon substitutions (Fig. 2). Since the β-carbon is asymmetric, isoprenaline has two isomeric forms: (−)- and (+)-isoprenaline. The (−) isomer produced a 90% activation of adenylate cyclase with an EC_{50} of 10 μM (Table 1a). The (+) isomer significantly ($P < 0.05$, t-test) stimulated adenylate cyclase only at a concentration of 1 mM and the racemic mixture (±) maximally activated the enzyme by 72% with an EC_{50} of 40 μM (Table 1a). The response with the racemic mixture suggests that (+)-isoprenaline slightly antagonizes the effects of (−)-isoprenaline but this may not be significant. The effect of removing the hydroxyl group on the β-carbon was tested using N-isopropyl dopamine and this amine only significantly stimulated adenylate cyclase activity at a concentration of 1 mM (Table 2b). Substitution of the hydroxyl group with a carbonyl group was tested with 3,4-dihydroxy-alpha-(isopropylamino)-acetophenone and this compound failed to activate adenylate cyclase at all concentrations employed (Table 2b). This suggests that in the asymmetric *laevo* isoprenaline molecule, three of the four groups (the amine, the aromatic ring and the alcoholic hydroxyl group) linked to the asymmetric carbon are needed for optimal interaction with the enzyme. With *dextro* isoprenaline, N-isopropyl dopamine and the carbonyl derivative, only a two-point interaction is possible.

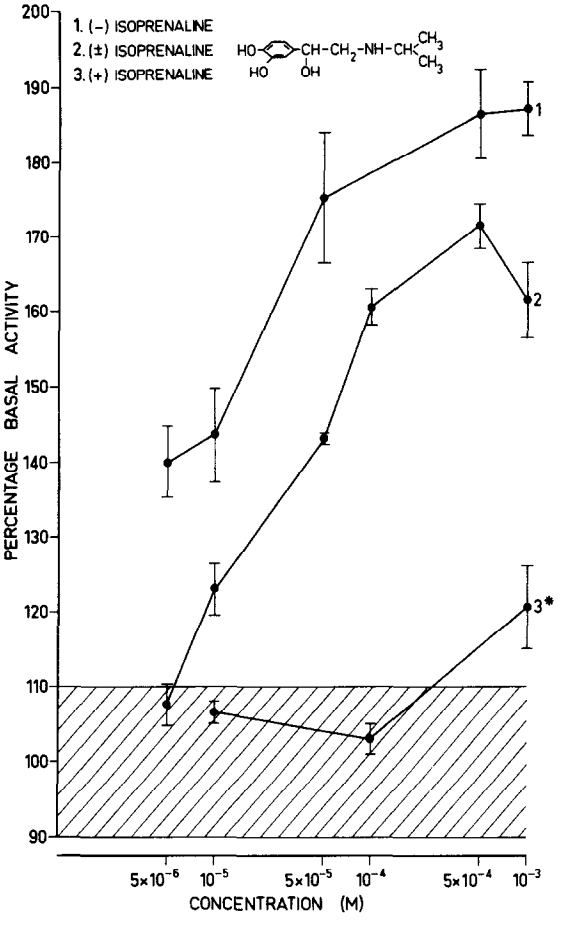


Fig. 2. Concentration–response curves showing the stimulation of basal adenylate cyclase activity by *laevo*, *dextro* and the racemic mixture of isoprenaline isomers. An asterisk indicates significant stimulation ($P < 0.05$) at that concentration. For other details, see Fig. 1 legend.

Table 1. EC_{50} values for phenylethylamine derivatives which activated adenylate cyclase in (a) a membrane fraction prepared from rat ventricle homogenates and (b) intact heart cubes

	Compound	EC_{50} (μM)
(a)	(−)-Isoprenaline	10
	(±)-Isoprenaline	40
	(±)-Noradrenaline	100
(b)	(−)-Isoprenaline	0·2
	(−)-Adrenaline	2
	(−)-Noradrenaline	20

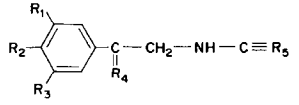
Values have been estimated graphically by eye for compounds whose responses reached maximum at the concentrations employed.

(c) Effects of phenyl substitutions. Substitution of the phenolic hydroxyl group at the 3-position of the catechol ring (Table 2c) abolished the stimulant activity of (±)-isoprenaline and (±)-N-ter-butyl noradrenaline. Thus (3-methoxy, 4-hydroxy-phenyl)-2-isopropylaminoethanol and (3-hydroxymethyl, 4-hydroxy-phenyl)-2-ter-butylaminoethanol (Salbutamol®) were inactive even at 1 mM.

It was shown that a phenolic hydroxyl group in 4-position only, in the 3- and 5-positions (Orciprenaline®) or in the 3-position only, markedly reduce the ability of isoprenaline to stimulate adenylate cyclase (Table 2d). Thus 1-(3-hydroxyphenyl)-2-isopropylaminoethanol does not stimulate the enzyme even at 1 mM but Orciprenaline® [(3,5-dihydroxyphenyl)-2-isopropylaminoethanol] and 1-(4-hydroxyphenyl)-2-isopropylaminoethanol do produce some significant activity at this concentration.

Elimination of the phenolic hydroxyl groups in the presence and absence of the β-hydroxyl substituent,

Table 2. The effects of (a) (±)-isoprenaline and structural derivatives of this compound possessing (b) β-carbon substitutions, (c) phenyl substitutions and (d) altered phenolic hydroxyl groupings, on the adenylate cyclase activity of a partially purified sarcolemmal preparation, P_4, isolated from rat myocardium



		R_1	R_2	R_3	R_4	R_5	% Basal adenylate cyclase activity at concentrations shown: 5×10^{-6} M	10^{-5} M	5×10^{-5} M	10^{-4} M	5×10^{-4} M	10^{-3} M
(a)	Parent compound (±)-isoprenaline	H	OH	OH	HOH	$H(CH_3)_2$	108 ± 2·9(4)	123 ± 3·6(13)*	143 ± 0·5(4)*	160 ± 2·4(12)*	172 ± 3·1(10)*	161 ± 5·1(13)*
(b)	β-Carbon substitutions	H	OH	OH	H_2	$H(CH_3)_2$	—	102 ± 4·6(6)	—	106 ± 2·6(6)		116 ± 4·8(6)*
		H	OH	OH	O	$H(CH_3)_2$	—	99 ± 6·0(6)		94 ± 6·3(6)	—	100 ± 8·2(6)
(c)	Phenyl substitutions											
	(i) On (±)-isoprenaline	H	OH	OCH_3	HOH	$H(CH_3)_2$	—	99 ± 8·1(6)	—	106 ± 6·8(6)		111 ± 9·4(6)
	(ii) On (±)-*N*-tert-butyl noradrenaline	H	OH	OH	HOH	$(CH_3)_3$	—	106 ± 3·3(6)	—	131 ± 6·8(7)*	149 ± 7·0(7)*	159 ± 5·6(4)*
		H	OH	CH_2OH	HOH	$(CH_3)_3$		95 ± 3·1(6)		102 ± 5·6(6)	106 ± 2·6(3)	107 + 6·3(6)
(d)	Displacement or removal of phenolic-OH groups	H	OH	H	HOH	$H(CH_3)_2$	—	100 ± 2·2(2)	—	111 ± 0·4(3)	113 ± 2·6(3)	121 ± 2·8(3)*
		OH	H	OH	HOH	$H(CH_3)_2$		107 ± 1·9(6)	—	111 ± 2·2(6)		118 ± 1·6(6)*
		H	H	OH	HOH	$H(CH_3)_2$		109 ± 5·1(3)	—	109 ± 2·3(3)	103 ± 1·8(3)	107 ± 3·3(3)
		H	H	H	HOH	$H(CH_3)_2$	—	106 ± 6·2(3)	—	112 ± 1·1(3)	—	106 ± 3·6(3)
		H	H	H	H_2	$H(CH_3)_2$	—	103 ± 2·0(3)	—	101 ± 1·3(3)	—	99 ± 5·4(3)

Results are expressed as a percentage of the basal adenylate cyclase activity (100%, S.E.M. = 10·2%, n = 33) ± S.E.M., and the number of observations are represented in brackets. An asterisk indicates significant ($P < 0.05$, *t*-test) activation of adenylate cyclase by compounds at the concentrations shown.

Table 3. Effects of (−)-isoprenaline (10 μM and 100 μM) alone, and in the presence of (−)-propranolol (1 μM), (+)-propranolol (1 μM) or phentolamine (1 μM), on the adenylate cyclase activity of a partially purified sarcolemmal preparation P_4

			(−)-Propranolol, 1 μM	(+)-Propranolol, 1 μM	Phentolamine, 1 μM
(−)-Isoprenaline	10 μM	144 ± 5·9(3)	123 ± 3·6(2)	149 ± 2·4(3)	137 ± 6·9(3)
	100 μM	202 ± 4·1(3)	154 ± 7·3(3)	189 ± 12·0(3)	187 ± 18·8(3)

Results are expressed as % basal adenylate cyclase activity (100%, S.E.M. = 10·6%, n = 3) ± S.E.M., for the number of observations in brackets. See text for further details.

as in *N*-isopropyl-phenylethanolamine and *N*-isopropyl-phenylethylamine (Table 2d) completely destroyed adenylate cyclase stimulating ability.

(ii) *Actions of other adreno-agonists and antagonists on adenylate cyclase activity.* In one experiment, (−)-isoprenaline (10 and 100 μM) activated adenylate cyclase activity by 44% and 102% respectively (Table 3). (−)-Propranolol (1 μM) partially but significantly blocked this activation. However, (+)-propranolol (1 μM) and the α-adreno-receptor antagonist phentolamine (1 μM) failed to affect the response to (−)-isoprenaline. Furthermore, the α-adreno-receptor agonist, (−)-phenylephrine at three concentrations (10,100 and 1000 μM) failed to activate adenylate cyclase.

Adenylate cyclase activity in intact cardiac cells

The rate of formation of [^{3}H]cyclic AMP from [^{3}H]ATP pools (pre-labelled using [^{3}H]adenine) in cubes of rat ventricles was studied in the presence of catecholamines. (−)-Isoprenaline maximally activated adenylate cyclase in intact cells by 350% (see Table 1b for EC_{50} values). (−)-Adrenaline and (−)-noradrenaline produced a similar maximal activation but at higher concentrations. The *dextro* isomers of all three catecholamines had markedly less ability to stimulate the formation of cyclic AMP than the *laevo* isomers.

DISCUSSION

If the hypothesis that the cardiac β-adrenoceptor is a regulatory sub-unit of the enzyme adenylate cyclase [9] is correct, then the structural requirements for catecholamines to activate cardiac adenylate cyclase should be the same as those for activation of β-adrenoceptors in intact cardiac muscle. The results of a structure–activity analysis for the activation of cardiac adenylate cyclase by isoprenaline and its derivatives, are presented in this communication. Furthermore, we have made some deductions about the configuration of the catecholamine receptor site of adenylate cyclase on the assumption that the effective amines exhibit a degree of complementarity with this receptor site. The source of rat cardiac adenylate cyclase used was fraction P_4 whose isolation and characterization was described in a preceding paper [3].

The structural requirements for activation of cardiac adenylate cyclase by phenylethylamines were found to be distinct. Optimal stimulation was achieved with two adjacent phenolic groups at the 3 and 4 positions of the phenyl ring, a hydroxyl group on the asymmetric β-carbon giving the *laevo* isomer and an isopropyl group on the nitrogen, that is (−)-isoprenaline.

Isoprenaline is a positively charged molecule at physiological pH but it was not possible to test if the positively charged nitrogen was essential for activation of the enzyme. Although the carbon isostere of isoprenaline has been synthesized and shown to have beta-adreno activity in cardiac muscle [22], our attempts to synthesize this compound failed. However, it is of interest that the carbon isostere has limited solubility in water [22], and in view of its pharmacological activity, the role of the nitrogen may simply be to confer water solubility on isoprenaline.

A number of comprehensive studies of the actions of phenylethylamine derivatives on pharmacological preparations of cardiac muscle have been made [14, 23]. However, these studies have only defined the structure–activity relationships for stimulation of cardiac muscle by phenylethylamines and not for the activation of cardiac beta-adrenoceptors. More recently Furchgott [13, 24] has demonstrated that it is possible to define the structure–activity relationships for activation of adrenoceptors by careful experimental design. He demonstrated that the minimal

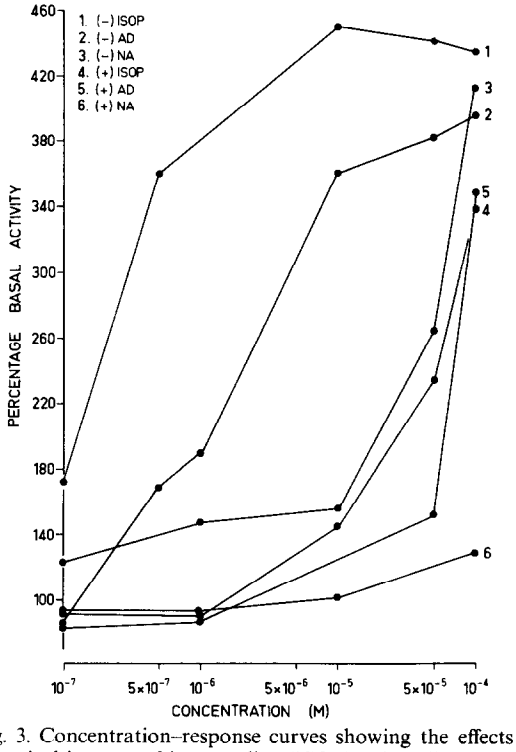


Fig. 3. Concentration–response curves showing the effects of optical isomers of isoprenaline (ISOP), adrenaline (AD) and noradrenaline (NA) on the conversion of [^{3}H]ATP to [^{3}H]cyclic AMP in intact cubes of rat ventricle. Each point represents the mean of two determinations.

structural requirement of phenylethylamine derivatives was one phenolic hydroxyl group and a benzylic hydroxyl group. Furthermore these derivatives were only partial agonists (i.e. the maximal increase in force of contraction was approximately 10% of the maximal increases in force produced by full agonists such as isoprenaline). Full agonist action required a catechol nucleus and the order of potency of catecholamines for activating the cardiac β-adrenoceptor was (−)-isoprenaline > (−)-adrenaline > (−)-noradrenaline. Interestingly, substitution of the phenolic group of the 3-position of isoprenaline with an hydroxymethyl group (Salbutamol®), or replacement of the catechol group with a resorcinol group (Orciprenaline®) resulted in a partial agonist response [13,25]. Salbutamol® and Orciprenaline® produce a full agonist response on bronchial and vascular smooth muscle and this was used as some of the evidence for the subdivision of the β-adrenoceptors into β_1 (cardiac muscle) and β_2 (bronchial and vascular smooth muscle) [25]. In the present study, Salbutamol® and Orciprenaline® (β_2-agonists) produced only marginal stimulation of cardiac adenylate cyclase (10–20%) at the highest concentration studied.

These structure–activity relationships for phenylethylamine derivatives on cardiac muscle are remarkably similar to those determined in the present study for activation of cardiac adenylate cyclase. However, β-adrenoceptors are not only defined according to the order of potency of catecholamines which stimulate the receptor but also according to the types of compounds which produce blockade of the response to the agonist [26]. In the present study, stimulation of adenylate cyclase activity in the particulate fraction of cardiac muscle by (−)-isoprenaline was blocked by (−)-propranolol but not by the same concentration of (+)-propranolol. In intact cardiac muscle preparations, (−)-propranolol is a competitive β-antagonist and is at least one hundred times more active than (+)-propranolol [27]. Recently, a more detailed kinetic study of the interaction of a number of β-adreno-antagonists and their stereoisomers on a feline myocardial preparation of adenylate cyclase has demonstrated [28] that the affinity of these compounds for the enzyme was almost identical to the affinity determined in intact cardiac muscle. Thus these findings are consistent with the hypothesis that the cardiac β-adrenoceptor is a component of adenylate cyclase.

Whilst the present study was in progress, Mayer [4] published results showing that the order of potency of isoprenaline, adrenaline and noradrenaline in stimulating adenylate cyclase activity in a particulate fraction from dog heart homogenates was similar to the order of potency of these amines on intact cardiac muscle. However, he pointed out that the concentrations of amines needed to produce a 50% maximal activation of adenylate cyclase was 275–660 times greater than the concentration producing 50% of maximal contraction of cardiac muscle *in vitro*. In the present study with rat hearts, a similar discrepancy occurs. Mayer [4] suggested that one explanation for this discrepancy could be that the absolute, but not the relative sensitivity of adenylate cyclase to activation by catecholamines was markedly reduced upon homogenization of cardiac muscle. We have tested this possibility using intact cubes of rat ventricular muscle with the intracellular ATP pools pre-labelled using [^{3}H]adenine. Upon addition of catecholamines to the cubes, there was an increase in the formation of [^{3}H]cyclic AMP in the tissue and the concentration of (−)-isoprenaline giving a 50% maximal rise in cyclic AMP was 0·2 μM. This is 50 times less than the concentration producing a 50% maximal activation of adenylate cyclase activity in homogenates. Levey [29] has suggested that the process of homogenization of cardiac muscle could alter the lipid–enzyme relationship in the cell wall resulting in a reduced sensitivity of the enzyme to hormonal stimulation since the addition of phosphatidylinositol to a particulate preparation of cardiac muscle lowered the concentration of noradrenaline producing half-maximal activation. However, it should be noted that in the present study, phosphatidylinositol was added to the particulate preparation of adenylate cyclase and there was still a discrepancy between the cubes and the fraction prepared by homogenization. Thus other factors such as alterations in the ionic environment of the receptor upon homogenization could be responsible. Although homogenization does appear to alter the sensitivity of adenylate cyclase to activation by catecholamines, there still remains a discrepancy between the concentration producing a 50% maximal rise in cyclic AMP in cubes of cardiac muscle, and that producing 50% of maximal contraction of intact cardiac muscle. The most likely explanation is that the concentration of isoprenaline producing a half-maximum pharmacological response may require only a slight activation of adenylate cyclase and rise in cyclic AMP. Thus the concept of 'spare receptors' [23,30] could apply in this situation.

In conclusion, it appears that cardiac adenylate cyclase and the β-adreno-receptor in intact cardiac muscle require the same structural configuration of agonists and antagonists in order for interaction to occur. These results strongly support the hypothesis that the β-adrenoceptor in the heart is a regulatory component of the enzyme, adenylate cyclase. The recent demonstrations that intracellular iontophoresis of cyclic AMP into Purkinje fibres [31] and sinoatrial nodal cells [32] mimics the chronotropic actions of β-adreno agonists and that elevations of intracellular levels of cyclic AMP produce a positive inotropic effect through activation of the slow Ca^{2+} influx channels in ventricular cells [33], with a subsequent net increase in cellular Ca^{2+} levels, further strengthen the hypothesis [9] that cyclic AMP mediates the mechanical responses of the heart to catecholamines.

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