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Reduction in isoprenaline-induced cyclic AMP formation in guinea-pig heart after exposure to isoprenaline or salbutamol

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Occasionally it has been observed that prolonged treatment of a tissue (*in vitro* or *in vivo*) with a sympathomimetic amine results in a decrease in its responsiveness to subsequent applications of that, or in some cases another sympathomimetic amine [1–8]. In rat pineal gland [6], guinea-pig macrophages [7], and human embryonic fibroblasts [8] a desensitization in catecholamine-induced cyclic AMP accumulation was observed. Conversely, an increase in the formation of cyclic AMP was observed following denervation of rat pineal gland [9].

However, conflicting reports exist on the occurrence of a change in responsiveness to catecholamines following prolonged exposure to adrenergic agonists in cardiac muscle. Atkinson and Rand [2] found that during maintained infusion of adrenaline or isoprenaline in the anaesthetised cat there was a reduction in the rise in heart rate in response to subsequent doses of either amine. Similar observations were made in man and dog by Conolly *et al.* [4]. In contrast, Kingsley *et al.* [10] found that in man such a reduction only occurred because the initial heart rate was already elevated; in addition the dose of isoprenaline required to obtain maximal chronotropic effects was unchanged. Similarly, McDivitt *et al.* [11] found no evidence of resistance to isoprenaline in anaesthetised dogs. Recently it has been reported that maintained exposure of guinea-pig atria to salbutamol, soterenol, MJ7999-1, orciprenaline or terbutaline results in a competitive antagonism of the positive chronotropic actions of *l*-isoprenaline [1, 12]. In the present experiments it was attempted, therefore, to demonstrate whether a change occurs in the accumulation of catecholamine-induced cyclic AMP in cardiac muscle after exposure to isoprenaline or salbutamol.

METHODS

Guinea-pigs of either sex, weighing between 250 and 400 g were killed by a blow to the head. Each heart was collected and perfused at a rate of 6 ml/min via an aortic cannula with McEwen's solution [13] maintained at 37° and saturated with 5% CO_2 in O_2. In some experiments *d,l*-isoprenaline or *d,l*-salbutamol was added to the McEwen's solution using a motor-driven syringe. Isoprenaline was administered as single injections either alone or during the continued perfusion with isoprenaline or salbutamol at a point close to the aortic cannula in volumes of 0·5 ml over a period of 5 sec. Tissue was collected for cyclic AMP assay 15 sec after the injection of a single dose of isoprenaline or in other cases, after varying periods of perfusion. The ventricle was cut from the heart, shaken to remove excess fluid and quickly frozen in a brass clamp chilled in liquid N_2. The tissue was then weighed and ground to a fine powder under liquid N_2 and extracted with 5% trichloracetic acid. In order to determine the recovery of the nucleotide, [^{3}H]cyclic AMP (25 nCi, 0·8 pmole, Radiochemical Centre, Amersham) was added to the extract. The extract was then centrifuged at a temperature of 4° for 15 min and a force of 10,000 *g*. Subsequent procedures for the analysis of cyclic AMP were in accord with the method of Gilman [14]. In addition, an aliquot of the solution for analysis was assayed for recovery of [^{3}H]cyclic AMP. A Packard Tri-Carb Liquid Scintillation Spectrometer was employed for estimations of radioactivity.

RESULTS AND DISCUSSION

After hearts had been perfused with McEwen's solution for 10 min, cyclic AMP levels were found to be 0·23 ± 0·03 nmoles/g wet wt. Continual perfusion of hearts with 10^{-4}M salbutamol increased cyclic AMP levels by 240 per cent when hearts were assayed at 20 sec, 1 and 10 min after commencement of perfusion with the drug (Table 1). However, after perfusion for 45 min with salbutamol, levels of cyclic AMP were similar to those in control hearts. The loss of agonist activity which occurred after perfusion with salbutamol for 45 min may be explained in terms of a change in receptor availability. This may have been due to either a slowly-developing auto-inhibi-

Table 1. Concentrations of cyclic AMP in perfused guinea-pig hearts*

	Cyclic AMP (nmole/g)	
Time of perfusion (min)	0·3–10	45
Control	0·23 ± 0·03 (6)	
Salbutamol (10^{-4}M)	0·55 ± 0·02 (6)†	0·23 ± 0·05 (4)
Isoprenaline (10^{-8}M)	0·28 ± 0·02 (4)	0·37 ± 0·07 (4)†

* Values are expressed as means ± S.E.; the number of observations in each case is shown in parenthesis.
† $P \leqslant 0.05$ relative to control hearts.

tion by salbutamol or a change in receptor orientation as has been suggested for the β-adrenergic receptor of aortic muscle by Fleisch and Titus [3].

Single injections of isoprenaline in hearts perfused for 45 min with McEwen's solution produced a dose-dependent increase in the levels of cyclic AMP when tissues were frozen 15 sec after completion of the injection (Fig. 1). Doses of 1·5 nmoles of isoprenaline appeared to produce a maximum increase in cyclic AMP formation since doses of 5 nmoles produced no further increase in the formation of the cyclic nucleotide. A shift to the right in the log dose–response curve to isoprenaline without reduction in the maximal response was observed when hearts were perfused with 10^{-5}M salbutamol for 45 min prior to injection of isoprenaline (Fig. 1). The results suggest that salbutamol competitively inhibits the activation of the β-adrenoceptor by isoprenaline rather than causing a change in receptor availability by inactivation due to change in receptor orientation. However the possibility cannot be dismissed that receptor availability is in excess of that required to achieve maximum adenyl cyclase activation, i.e. maximal cyclic AMP formation may be achieved in the presence of incomplete receptor availability.

When hearts were perfused with 10^{-8}M *d,l*-isoprenaline for 45 min, a small rise in cyclic AMP levels occurred (Table 1). This concentration of isoprenaline also caused a shift to the right of the log dose–response curve to single injections of isoprenaline (Fig. 1). As with salbutamol, the maximum level of cyclic AMP formed in response to isoprenaline was unchanged. These results contrast with those of Malta and Raper [12] who were unable to demonstrate a decrease in the chronotropic action of *l*-isoprenaline after maintained perfusion with the drug. In the present experiments it is possible that it was the *d*-form of the drug which decreased the responsiveness to later applications of isoprenaline. In support of this conclusion Bilezekian and Aurbach [15] observed that *d*-isoprenaline inhibited the activation of adenyl cyclase by *l*-isoprenaline in turkey erythrocytes. Similarly, in the experiments of Atkinson and Rand [2] and of Conolly *et al.* [4] which demonstrated a tolerance to isoprenaline the racemic mixture was employed. Such a difference in the behaviour of the enantiomers of salbutamol may also have occurred.

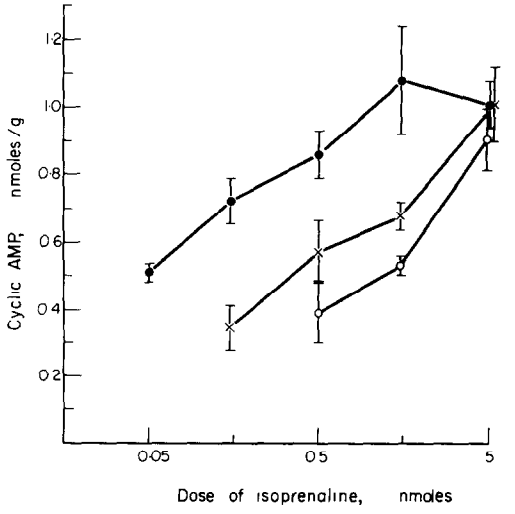

Fig. 1. Cyclic AMP levels in perfused guinea-pig hearts 15 sec after a dose of isoprenaline. Prior to injection hearts were perfused for 45 min with McEwen's solution (●), McEwen's solution containing 10^{-5}M salbutamol (○), or McEwen's solution containing 10^{-8}M isoprenaline (×). Each point is the mean ± S.E. of four observations.

The results suggest that prolonged exposure of the guinea-pig myocardium to either a partial or a full agonist of the β_1-adrenoceptor leads to a decrease in the activity of the receptor with respect to its ability to generate cyclic AMP. The studies of Raper and Malta [1] suggest that, at least in the case of salbutamol, this is coupled with a decrease in the chronotropic action of β-receptor agonists. It would appear, therefore, that a reduction in substrate availability is coupled with a decrease in the work demands on the heart. It has been claimed that different receptors are involved in catecholamine-induced cyclic AMP formation and rise in heart rate [16–17]. However, this claim has not been fully supported by the studies of McNeill *et al.* [18–19] and Caron and Lefkowitz [20]. Should the receptors for the two events not be identical, then a difference might be observed in the magnitude of the changes in activity. On the basis of the present experiments 10^{-5}M salbutamol produced approximately a ten-fold shift in the isoprenaline dose–response curve whereas this concentration of salbutamol produced a dose ratio of approximately 2 in experiments where chronotropic actions of isoprenaline were assayed [1].

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N-methylation of 1-methyltryptamines by indolethylamine *N*-methyltransferase

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Our finding that the methyltetrahydrofolate-mediated *N*-methyltransferase did not methylate the amino-nitrogen [1] led us to suggest a possible methylation on the indole ring nitrogen to give 1-methyl derivatives. As a part of our continuing studies on the kinetics of indolethylamine *N*-methyltransferase (INMT), we have used two 1-methyl derivatives of tryptamine, 1-methyltryptamine (1-MeT) and 1-methyl-*N*-methyltryptamine (1-MeNMT), as substrates in order to study the relationship between the structure of the substrate and the activity of the enzyme. The effects of *N*,*N*-dimethyltryptamine (DMT), bufotenin (Aldrich Chemical Co., Milwaukee, Wis.), 1-methyl-*N*,*N*-dimethyltryptamine (1-MeDMT) and *S*-adenosylhomocysteine (SAH) (Sigma Chemical Co., St. Louis, Mo.) on the activity of the enzyme were also examined. The results of our experiments are reported in this communication.

INMT was obtained from rabbit lung [2]. Fifty milligrams of lyophilized human serum was also used as a source of enzyme. The assay medium, which contained 0·5 m-mole *S*-adenosyl[methyl-^{14}C]methionine (SAM, Amersham–Searle Corp., Arlington Heights, Ill., sp. act. 51 mCi/m-mole), 0·5 μmole non-labeled SAM (Sigma Chemical Co., St. Louis, Mo.), 100 μg enzyme, amine [*N*-methylserotonin (NMS), *N*-methyltryptamine (NMT) (Aldrich Chemical Co., Milwaukee, Wis.), 1-MeT or 1-MeNMT] in various amounts and 50 μmoles potassium buffer (pH 8) in a total volume of 0·5 ml, was incubated at 37° for 60 min. The methylated products were extracted with ethyl acetate at pH 10, and the radioactivity was measured [3].

To identify the methylated products, two-dimensional t.l.c. of the ethyl acetate extract and of reference standards was carried out on Silica gel G [4]. The products were visualized by spraying the plates with methanolic sulfuric acid. Those fluorescent spots isographic with the standards were scraped, eluted with methanol and the radioactivity in the eluate was counted [3].

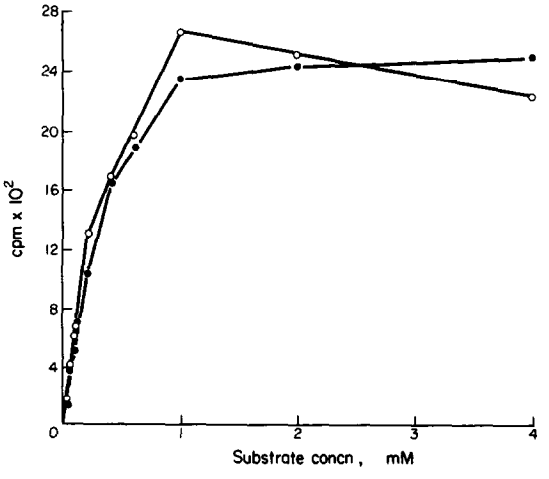

Fig. 1. Effect of substrate concentration on the *N*-methylation of 1-methyltryptamine (1-MeT, solid circles) and 1-methyl-*N*-methyltryptamine (1-MeNMT, open circles) by indolethylamine *N*-methyltransferase from rabbit lung.

G.l.c.–m.s. analyses and quantitation of the reaction products were carried out on a Varian CH 7 mass spectrometer interfaced with a Varian model 2740 gas chromatograph. The g.l.c. conditions were: 6-ft column, 3% OV-225 on Gas Chrom Q at 180° isothermal, helium flow-

Table 1. G.l.c. and g.l.c.–m.s. data of the methylation products of 1-methyltryptamine (1-MeT) and 1-methyl-*N*-methyltryptamine (1-MeNMT) with indolethylamine *N*-methyltransferase from rabbit lung

Compound	R_T* (min)	G.l.c.–m.s.†		
1-MeNMT				
Standard	6·66	188‡ (3)	144 (100)	145 (90)
Product of 1-MeT	6·66	188‡ (3)	144 (100)	145 (90)
1-MeDMT§				
Standard	5·53	202‡ (4)	144 (13)	58 (100)
Product of 1-MeNMT	5·53	202‡ (4)	144 (13)	58 (100)

* G.l.c. column conditions: 3% OV-225 on Gas Chrom Q, 6-ft column, 180° isothermal, He 30 ml/min.
† *m/e* (relative abundance).
‡ Molecular ion.
§ 1-Methyl-*N*,*N*-dimethyltryptamine.