Short Communication

Desensitization of Kitten Atria to Chronotropic, Inotropic and Adenylyl Cyclase Stimulating Effects of (−)Isoprenaline

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Summary. Desensitization of kitten atria with 30 μM (−)isoprenaline resulted in a 6-fold and 15-fold increase in the EC50's of (−)isoprenaline for its positive chronotropic effects (sinus pacemakers) and positive inotropic effects (left atria), respectively, but only in a 2-fold increase of the EC50 of (−)isoprenaline for adenylyl cyclase stimulation in membrane particles from atria. However, maximum cyclase stimulation by (−)isoprenaline was decreased to 1/2 in membranes from (−)isoprenaline-treated atria, whereas maximum increase in rate of sinus pacemakers and force of left atria were unchanged and reduced by 15%, respectively. The high affinity β-adrenoceptor blocker (−)bupranolol antagonized the adenylyl cyclase stimulation by (−)isoprenaline to similar extent in membranes from (−)isoprenaline and untreated atria, suggesting that the apparent affinity of β-adrenoceptors for ligands is unchanged by desensitization. The evidence is compatible with the concept that desensitization is associated with decreased availability of receptors and with the view that near maximal positive chronotropic effects of catecholamines may be caused by only threshold increases in membrane adenylyl cyclase activity.

Key words: (−)Isoprenaline desensitization — Kitten atria — Adenylyl cyclase — β-adrenoceptors — Sinus pacemaker and left atrial myocardium.

INTRODUCTION

Stimulation of adenylyl cyclase by catecholamines can be decreased by exposure of tissues or cells of various systems to high concentrations of amine (Deguchi and Axelrod, 1973; Franklin and Foster, 1973; Franklin et al., 1975; Mukherjee et al., 1975; Mickey et al., 1975; Hopkins, 1975). In heart, apparent affinities of many ligands for β-adrenoceptors mediating positive chronotropic and inotropic, relaxant and adenylyl cyclase stimulating effects of catecholamines are of the same order of magnitude, suggesting that a single receptor is involved in conveying heterogeneous effects (Kaumann and Birnbaumer, 1973, 1974b; Kaumann, 1974). However, 2 or 3 orders of magnitude greater concentrations of catecholamine are required to stimulate adenylyl cyclase in heart membrane particles (Kaumann and Birnbaumer, 1974a, b) than to cause positive chronotropic and inotropic effects (Kaumann, 1972). This dissociation suggests that stimulation of the adenylyl cyclase is either unrelated to the positive chronotropic and inotropic, and relaxant effects of catecholamines, or that small increases of cAMP modify substantially heart functions. The present experiments were carried out to study the relationship between the enhanced (−)isoprenaline-mediated production of cAMP by membrane bound adenylyl cyclase and the effects of (−)isoprenaline on sinus pacemakers and left atrial myocardium by comparing the desensitizations to each of these effects of the catecholamine.

METHODS

Eight kittens of either sex, coming from 2 litters of 4 each, weighing 300 g (not weaned) were used. To reduce endogenous noradrenaline which may occupy β-adrenoceptors of membrane particles and act additively with exogenous catecholamine (Kaumann and Birnbaumer, 1974b, p. 7878), the kittens were pretreated with 2.5 mg/kg reserpine i.p. 18 h before sacrificing. The kittens were anesthetized with chloroform; their hearts were washed free of blood with salt solution and rapidly removed. The physiological salt solution contained: (mM) Na+ 140, K+ 5, Ca2+ 2.25, Mg2+ 1, Cl− 98.5, SO42− 1, HCO3− 29, HPO42−, glucose 10, acetate 20, EDTA (ethylene-diaminetetraacetic acid, disodium salt) 0.04. Water was redistilled.
Fig. 1A and B. Desensitization at 32.5 °C of a sinus pacemaker and atrial membranes to the chronotropic and adenylyl cyclase stimulating effects of (-)isoprenaline. (A) Spontaneously beating kitten atrium. Closed and open circles, concentration-effect curves for (-)isoprenaline before and 4 h after a 3-h exposure to 30 μM (-)-isoprenaline. (B) Membrane particles of atria were incubated for (-)isoprenaline without (black squares, open circles) and with (-)isoprenaline (30 μM), as described above. The reaction was stopped by addition of a solution containing 10 mM cAMP, 40 mM ATP and 1% sodium dodecyl sulphate, followed by immediate boiling for 3.5 min. Membranes indicated by circles (17 μg protein/assay) and squares (15 μg protein/assay) were from the atrium shown in (A) and from an electrically stimulated control atrium (not exposed to (-)-isoprenaline), respectively.

RESULTS AND DISCUSSION

Exposure of atria to 30 μM (-)-isoprenaline caused desensitization (Fig. 1, Table 1) to the amine with the following characteristics: The EC50's (−log M) of (-)-isoprenaline increased from (mean ± S.E.M.) 9.17 ± 0.21 and 8.83 ± 0.20 to 8.39 ± 0.19 and 7.67 ± 0.21 in right (n = 6) and left (n = 4) atria, respectively. Maximum rates and force (mean ± S.E.M.) achieved with (-)-isoprenaline were 190 ± 4 and 188 ± 3 beats/min in right atria and 2.8 ± 0.2 and 2.3 ± 0.2 g tension in left atria, before and 4 h after the 3-h incubation with 30 μM (-)-isoprenaline. Equilibrium chronotropic effects of 30 μM (-)-isoprenaline were somewhat smaller than maximum effects, and similar in desensitized and desensitized atria (Fig. 1).

In membrane particles from the (-)-isoprenaline-treated atria, maximal stimulation of adenylyl cyclase activity was only 1/2 of that of electrically stimulated atria (Fig. 1, Table 1). The EC50 (Fig. 1, Table 1) for cyclase stimulation by (-)-isoprenaline increased somewhat from 88 nM to 167 nM. To see whether this small increase in EC50 was due to a decrease in apparent affinity of the β-adrenoceptors for their ligands, the potency of the high affinity, pure β-adrenoceptor blocker 1-(6'-chloro-3'-methylphenoxy)-tert-(butylaminopropane-2-ol) [(−)KL255, (−)butpranolol] was investigated (Kaumann, 1972, Kaumann and...