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β_2-Adrenoceptors in guinea-pig atria

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The occurrence of β_2-adrenoceptors in the isolated, spontaneously beating right atrium and the electrically driven left atrium of the guinea-pig was studied. Isoprenaline was used as reference compound and procaterol as selective β_2-agonist. Cumulative concentration response (C/R) curves were obtained with the agonists. The C/R curve of procaterol was biphasic in both preparations. Compared with isoprenaline, procaterol was a partial agonist, with a mean maximum response of $0{\cdot}78 \pm 0{\cdot}04$ in the right atrium and $0{\cdot}29 \pm 0{\cdot}05$ in the left atrium. The β_2-selective antagonist ICI 118,551, 10^{-7} mol litre^{-1}, caused a small but significant shift of the C/R curve of isoprenaline to a higher concentration range in both preparations. The same concentration of the β_2-blocker changed the shape of the C/R curve of procaterol from biphasic to monophasic by blocking the responses to low concentrations of procaterol. Practolol, a β_1-selective antagonist, 10^{-6} mol litre^{-1}, gave a highly significant shift of the C/R curve of isoprenaline to a higher concentration range in both preparations but had no effect on the responses to low concentrations of procaterol. The effect of practolol on the responses to high concentrations of procaterol is discussed. We conclude that the guinea-pig atria may contain β_2-adrenoceptors mediating positive chronotropic and inotropic effects.

According to Lands et al (1967a, b), the β-adrenoceptors can be divided into two subgroups: the β_1- and β_2-adrenoceptors; and the response of an organ or tissue to β-agonists is mediated by one of the two subgroups.

In studies on cat isolated hearts, where non-selective, β_1- and β_2-selective drugs were used, Carlsson (1972) obtained results that were inconsistent with Land's theory. In a later study, Carlsson et al (1972) found that the cat heart contains both β_1- and β_2-adrenoceptors and that both kinds of adrenoceptors mediate the same kind of response. This theory of Carlsson and co-workers has also been applicable to other organs (Furchgott et al 1975; O'Donnell & Wanstall 1981a).

In more recent studies O'Donnell & Wanstall (1979, 1981b) and Zaagsma et al (1979) found no functional support for the existence of β_2-adrenoceptors in the guinea-pig heart. This heart may thus differ from the cat heart by containing only β_1-adrenoceptors. However, in binding studies, Hedberg et al (1980), analysing the kinetics of the inhibition of (^{125}I) iodohydroxybenzylpindolol (IHYP) by β_1- and β_2-selective drugs in the right atrium of cats and guinea-pigs, found β_1- and β_2-adrenoceptors in the atria of both species. In our study we have used the highly selective β_2-agonist procaterol (Yoshizaki et al 1976; Yabuuchi 1977) to re-examine whether or not there is functional evidence for the presence of β_2-adrenoceptors mediating rate and force responses in the right and the left atrium of guinea-pig.

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MATERIALS AND METHODS

Male guinea-pigs (Dunkin Hartley from Sahlin's, Malmö, Sweden); 400–600 g, were pretreated with reserpine (5 mg kg^{-1}) 15–20 h before the experiment to avoid possible interference by endogenous catecholamines. They were then killed by cervical dislocation, the heart removed, and the ventricular tissue cut away as far as possible. Separate preparations of the right and the left atrium were mounted in a jacketed, temperature-controlled (37 °C), 25-ml organ bath containing Krebs solution of the following composition (mmol litre^{-1}): NaCl, 118; KCl, 4·7; $CaCl_2 \times 2H_2O$, 2·5; $MgSO_4 \times 7H_2O$, 1·16; $NaHCO_3$, 24·9; KH_2PO_4, 1·15; and D-glucose, 11·1. The Krebs solution was gassed with 95% O_2 and 5% CO_2.

The initial tension was adjusted to 5·9 mN and the contractions were recorded with a Grass force displacement transducer, FTO3C, connected to a Grass polygraph, model 7 D. The right atrium was beating spontaneously, and its frequency was recorded with a Grass tachograph triggered by the contraction amplitude. The left atrium was electric-

ally driven by square pulses from a Grass stimulator, model S4GR (frequency: 3 Hz, pulse duration: 1 ms, voltage: 2 × threshold value). After they were mounted, the preparations were left 30–60 min to equilibrate. Concentration response (C/R) curves of the non-selective agonist isoprenaline and the β_2-selective agonist procaterol were obtained cumulatively. The doses were increased logarithmically with each subsequent dose added to the bath when the effect of the preceding concentration of the compound had reached equilibration. This was obtained within 3 min. Any extraneuronal uptake was blocked by the addition to the bath of metanephrine to a final concentration of 10^{-5} mol litre^{-1}. Because it was difficult to obtain two identical C/R curves of procaterol in consecutive replications, a cumulative C/R curve for isoprenaline was always obtained first as a reference. After washout and 30 min rest, the β_2-selective antagonist ICI 118,551, 10^{-7} mol litre^{-1}, or the β_1-selective antagonist practolol, 10^{-6} mol litre^{-1}, was added to the bath. Thirty min later a C/R curve of isoprenaline or procaterol was run. The controls were given 60 min rest before the second run. During the first 30 min the bathing medium was changed every 10 min in both tests and controls. The effect of the agonists as a percentage of maximum isoprenaline response, obtained in the initial isoprenaline run, was plotted against log concentration of the agonist. The negative logarithm of the concentration producing a 50% effect, pD_2, was obtained by interpolation. In the biphasic procaterol curves, a pD_2 value was calculated from each component of the C/R curve.

Compounds and solutions

(−)-Isoprenaline HCl (Sigma), procaterol HCl (through Warner-Lambert Company), ICI 118,551 (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) HCl (ICI), practolol (Eraldin, ICI), metanephrine HCl (Calbiochem), and reserpine (Serpasil, Ciba).

Statistics

Mean values are given together with the standard error of the mean (s.e.). Comparisons were made using the Student's *t*-test.

RESULTS

Right atrium

Fig. 1A and B show the effects of isoprenaline and procaterol on the rate of the right guinea-pig atrium. Both compounds caused a concentration-dependent increase in rate. The mean resting rate was 184 ± 5 beats min^{-1} and the mean maximum increase in rate for isoprenaline was 146 ± 5 beats min^{-1}. The pD_2 value of isoprenaline was 8·74 ± 0·03 (n = 13). ICI 118,551 in the concentration 10^{-7} mol litre^{-1} gave a small but significant parallel shift of the C/R curve of isoprenaline to a higher concentration range (pD_2 8·66 ± 0·06, n = 10, $0{\cdot}05 > P > 0{\cdot}02$). Practolol in the concentration 10^{-6} mol litre^{-1} caused a highly significant shift of the C/R curve of isoprenaline to a higher concentration range (pD_2 8·16 ± 0·04, n = 7, $P < 0{\cdot}001$) (Fig. 1A).

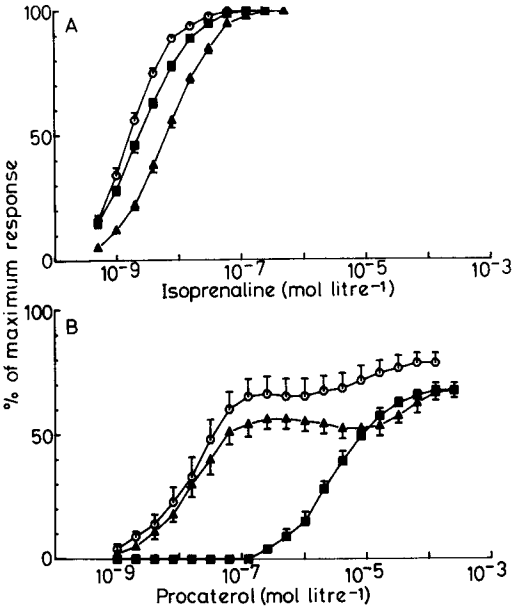

FIG. 1A. Selective blockade of the isoprenaline-induced chronotropic effect on guinea-pig right atrium. Control (○), n = 13; 10^{-6} mol litre^{-1} practolol (▲), n = 7; and 10^{-7} mol litre^{-1} ICI 118,551 (■), n = 10. Bars indicate s.e. Each response is expressed as a percentage of the maximum response obtained with isoprenaline. B. Selective blockade of the procaterol-induced chronotropic effect on guinea-pig right atrium. Control (○), n = 6; 10^{-6} mol litre^{-1} practolol (▲), n = 7; and 10^{-7} mol litre^{-1} ICI 118,551 (■) n = 5. For further explanation, see Fig. 1A.

The C/R curve of procaterol was biphasic (Fig. 1B). Compared with isoprenaline, procaterol was a partial agonist, with a mean maximum response of 0·78 ± 0·04. For the first phase of the C/R curve, the pD_2 value of procaterol was 7·79 ± 0·09 and for the second phase 5·05 ± 0·08 (n = 6). Pretreatment of the preparation with the β_2-blocking agent ICI 118,551 in the concentration 10^{-7} mol litre^{-1} changed the shape of the C/R curve of procaterol from biphasic to monophasic by blocking the responses to low concentrations of procaterol (Fig. 1B). The β_2-blocker also caused a small depression

of the mean maximum response. Practolol, 10^{-6} mol litre^{-1}, did not affect the responses to low concentrations of procaterol, but caused a small depression of the mean maximum response of the C/R curve corresponding to that caused by ICI 118,551 (Fig. 1B).

Left atrium

The effects of isoprenaline and procaterol on the force of the electrically driven left atrium are shown in Fig. 2A and 2B. Isoprenaline caused a concentration dependent increase in force. The mean maximum increase was 5·0 ± 0·6 mN and the pD_2 value 8·29 ± 0·05 (n = 14).

The antagonists shifted the C/R curve of isoprenaline in a way similar to that in the right atrium (pD_2 8·15 ± 0·07, n = 10, $P = 0·02$ after β_2-blockade and 7·66 ± 0·07, n = 8, $P < 0·001$ after β_1-blockade) (Fig. 2A).

The effect of procaterol in this preparation was weak with a mean maximum effect of 0·29 ± 0·04 (n = 7) times that of isoprenaline. The C/R curve of procaterol was biphasic in this preparation too (Fig. 2B), and the two components of the C/R curve appear in the same concentration ranges as for the rate in the right atrium. ICI 118,551, 10^{-7} mol litre^{-1}, changed the shape of the C/R curve of procaterol from biphasic to monophasic by blocking the responses of low procaterol concentrations and caused an increase in the mean maximum response (Fig. 2B).

Practolol in the concentration 10^{-6} mol litre^{-1} did not influence the responses of procaterol in its low concentration range.

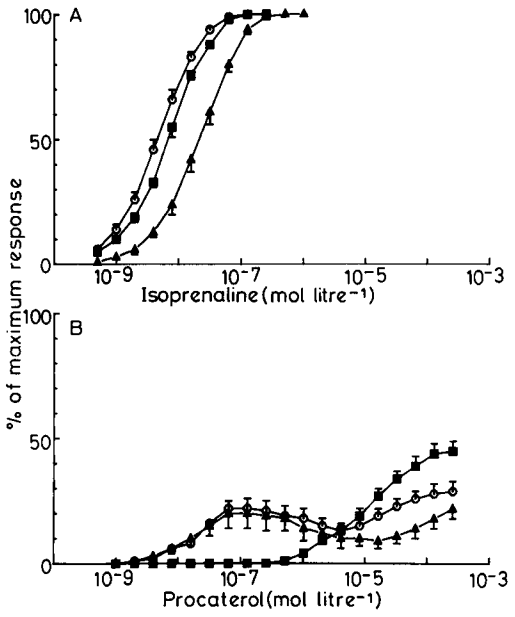

FIG. 2A. Selective blockade of the isoprenaline-induced inotropic effect on guinea-pig left atrium. Control (○), n = 14; 10^{-6} mol litre^{-1} practolol (▲), n = 8; and 10^{-7} mol litre^{-1} ICI 118,551 (■), n = 10. For further explanation, see Fig. 1A. B. Selective blockade of the procaterol-induced inotropic effect on guinea-pig left atrium. Control (○), n = 7; 10^{-6} mol litre^{-1} practolol (▲), n = 8; and 10^{-7} mol litre^{-1} ICI 118,551 (■), n = 5. For further explanation, see Fig. 1A.

DISCUSSION

The use of selective agonists alone or in combination with selective antagonists is a common technique for the characterization of adrenoceptors. Recently, two new β_2-selective compounds were introduced. One is procaterol, a β_2-selective agonist (Yoshizaki et al 1976; Yabuuchi 1977), the other is ICI 118,551, a potent and specific β_2-antagonist (Bilski et al 1980; O'Donnell & Wanstall 1980).

Procaterol induced a biphasic C/R curve for both the chronotropic and the inotropic response. A biphasic C/R curve for procaterol was also obtained by Hedberg & Mattsson (1981) in the cat papillary muscle. They also studied the inhibition of the binding of IHYP by procaterol in the cat ventricular myocardium. This study revealed a biphasic receptor-binding curve in the interaction with procaterol, indicating that the β-adrenoceptors in the cat ventricular myocardium are not homogeneous. These authors suggested that the two components of the C/R curve were mediated by β_2- and β_1-adrenoceptors, respectively, because the effect of procaterol in low concentrations on the papillary muscle was selectively blocked by a β_2-blocker and in high concentrations by a β_1-blocker.

The response of procaterol obtained by us on the right and the left guinea-pig atrium and its interaction with ICI 118,551 and practolol are qualitatively the same as those reported by Hedberg & Mattsson on the cat papillary muscle. In our study, ICI 118,551 changed the C/R curve of procaterol from biphasic to monophasic in both preparations by blocking the responses to low procaterol concentrations. These results and the fact that practolol had no blocking effect in this concentration range indicate that this component of the C/R curve is mediated by β_2-adrenoceptors. The weaker response of the left atrium to procaterol compared with that of the right one may indicate a higher density of β_2-adrenoceptors in the sinus node than in the left atrium. Practolol, like ICI 118,551, caused a small

depression of the C/R curve of procaterol in the right atrium (Fig. 1B) at high procaterol concentrations. This makes it difficult to decide whether or not there is a real shift of this part of the C/R curve towards a higher concentration range. In the left atrium there may be a real shift after practolol treatment, as the C/R curve of procaterol is still rising in the highest concentrations of procaterol studied (Fig. 2B). ICI 118,551 changed the C/R curve of procaterol in the left atrium from biphasic to monophasic and also caused an increase in the mean maximum response compared with that of the control. However, if the increases in force, obtained with procaterol in the low and high concentration ranges (corresponding to the two phases) of the C/R curve, are added together, there is no difference in the mean maximum response between the controls and the ICI 118,551 treated preparations.

In binding studies using IHYP and β_1- and β_2-selective drugs, Hedberg et al (1980) studied the occurrence and relative density of β_1- and β_2-adrenoceptors in the right atria of cat and guinea-pig atria and found that the atria of both species contained both kinds of β-adrenoceptors. About 25% of all the β-adrenoceptors in each species were of the β_2-type. The density of the β_1- and β_2-adrenoceptors was about the same in both species. Because the occurrence of β_2-adrenoceptors in the cat heart is readily demonstrated in effect studies (Carlsson et al 1972; O'Donnell & Wanstall 1979), the failure to find any functional evidence of these adrenoceptors in the guinea-pig heart led to the assumption that the guinea-pig right atrium lacks β_2-adrenoceptors mediating a positive chronotropic effect. According to O'Donnell & Wanstall (1981b) the β_2-adrenoceptors, demonstrated in the binding studies, may mediate other effects, e.g. vasodilator response and glycogenolysis.

We have in this study obtained functional evidence of the occurrence of β_2-adrenoceptors in the guinea-pig atria mediating positive chronotropic and inotropic effects. It appears, however, that more selective agonists are needed to demonstrate the β_2-adrenoceptors in the guinea-pig right atrium than used by others in earlier studies. Presumably, in the guinea-pig right atrium the fraction of the existing β_2-adrenoceptors, which is associated with the positive chronotropic response, is smaller than in the cat right atrium.

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