β -Adrenoceptor blocking effects of a selective β_2 -agonist, mabuterol, on the isolated, blood-perfused right atrium of the dog

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1 Effects of ($\pm$)-1-(4-amino-3-chloro-5-trifluoromethyl-phenyl)-2-tert.-butylamino-ethanol hydrochloride (mabuterol) on pacemaker activity and atrial contractility were investigated in the isolated and blood-perfused right atrium of the dog.

2 Mabuterol, injected into the sinus node artery of the isolated atrium, dose-dependently increased atrial rate and contractile force at doses of 0.01–10 nmol but the responses to over 10 nmol of mabuterol gradually decreased and mabuterol at higher doses induced biphasic cardiac responses, i.e., negative followed by positive cardiac responses.

3 The maximal increases in atrial rate and contractile force induced by mabuterol were 41.4% and 12.9%, respectively, of the maximal chronotropic and inotropic effects of isoprenaline.

4 Positive chronotropic and inotropic responses to mabuterol were dose-dependently inhibited by a selective β_2 -adrenoceptor antagonist, ICI 118,551. These responses were only slightly attenuated by atenolol.

5 Mabuterol (1–300 nmol) dose-dependently inhibited both dobutamine- and procaterol-induced positive chronotropic and inotropic responses.

6 These results indicate that mabuterol causes weak positive chronotropic and inotropic effects on the perfused canine right atrium by activating β_2 -adrenoceptors, and that higher concentrations non-selectively block both β_1 - and β_2 -adrenoceptors.

Introduction

Mabuterol, (($\pm$)-1-(4-amino-3-chloro-5-trifluoromethyl-phenyl)-2-tert.-butylamino-ethanol hydrochloride), is a newly synthesized orally active and long-acting β_2 -adrenoceptor agonist and bronchodilator. Mabuterol exhibits marked bronchodilator activity accompanied by only slight effects on the cardiovascular system in guinea-pigs and dogs (Murai *et al.*, 1984). Osada *et al.* (1984) found that i.v. administration of mabuterol dose-dependently decreased blood pressure and increased heart rate, but at higher doses, mabuterol decreased heart rate in rats, cats and dogs. They also reported that a high dose of mabuterol possessed β_1 -adrenoceptor blocking activity in guinea-pig isolated atrium.

Lands *et al.* (1967a,b) first subclassified β -adrenoceptors into β_1 - and β_2 subtypes with absol-

ute organ-specific distribution such as β_1 -receptors in the heart and β_2 -receptors in the lung. Recently, it has been shown that both receptor subtypes exert a physiological role in cat, dog and human hearts by use of pharmacological (O'Donnell & Wanstall, 1980; Zerkowski *et al.*, 1986; Friedman *et al.*, 1987), biochemical (Brodde *et al.*, 1984; Gille *et al.*, 1985; Kaumann & Lemoine, 1987) and receptor-binding studies (Hedberg *et al.*, 1980; Manalan *et al.*, 1981; Stiles *et al.*, 1983). However, only β_1 -receptors mediate positive chronotropic and inotropic responses in rat and guinea-pig hearts (Wilson & Lincoln, 1984; Molenaar & Summers, 1987). Recently, we have reported the existence of positive chronotropic and inotropic responses mediated by β_2 -adrenoceptors in addition to β_1 -adrenoceptors in the isolated, blood-perfused right atrial and left ventricular preparations of the dog (Akahane *et al.*,

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1988; 1989). Therefore, this study was carried out to examine the selectivity of mabuterol for β -receptor subtypes. We also studied whether mabuterol selectively blocked β_1 -adrenoceptors in the isolated, blood-perfused right atrium of the dog.

Methods

Preparation of the isolated, blood-perfused atrium of the dog

Thirty six donor dogs, weighing 8–25 kg, were anaesthetized with sodium pentobarbitone, 30 mg kg⁻¹ i.v. Sodium heparin (500 USP units kg⁻¹, i.v.) was administered at the beginning of the perfusion of the isolated atrial preparation and 200 USP units kg⁻¹ were given each hour thereafter. Isolated right atrial preparations were obtained from another 36 mongrel dogs weighing 7–15 kg. Each dog was anaesthetized with sodium pentobarbitone 30 mg kg⁻¹ i.v. Sodium heparin (200 USP units kg⁻¹, i.v.) was given, and the right atrium was then excised and immersed in cold Tyrode solution. The wet weight of the atrial preparations varied between 8–14 g. The sinus node artery was cannulated via the right coronary artery and perfused with blood conducted from the carotid artery of the support dog by the aid of a peristaltic pump (Harvard Apparatus Model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mmHg. The rate of blood flow to the isolated atrium was 3–9 ml min⁻¹.

The ventricular margin of the atrium was anchored to a stainless steel bar and was placed in a cup-shaped glass container which was kept at a constant temperature of 37°C. The superior part of the atrium was connected to a force-displacement transducer (Nihon Kohden AP 620G) by a silk thread. The atrium was usually stretched to a resting tension of 2 g. The isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden WT 685T). A pair of electrodes, each with an inter-electrode distance of 1.5 mm, was brought into contact with the epicardial surface of the isolated atrium in order to record the atrial electrogram. The atrial rate was derived from the atrial electrogram with a cardiometer (Nihon Kohden AT 600G). The details of this preparation have been described previously (Chiba *et al.*, 1975a,b).

Experimental protocols

Chronotropic and inotropic responses to mabuterol ($n = 8$) and isoprenaline ($n = 6$) were first determined

in the isolated, blood-perfused right atrial preparation. Positive chronotropic and inotropic responses to either mabuterol, to a selective β_1 -agonist, dobutamine (Williams & Bishop, 1981) or to a selective β_2 -agonist, procaterol (Yabuuchi *et al.*, 1977) were obtained and the effects of a selective β_1 -antagonist, atenolol or a selective β_2 -antagonist, ICI 118,551 (Bilski *et al.*, 1983) were studied. Finally, effects of pretreatment with mabuterol on positive chronotropic and inotropic responses to dobutamine, procaterol, 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor), Bay K 8644 (a dihydropyridine calcium agonist) and CaCl₂ were examined in order to determine whether mabuterol possesses β_1 - and/or β_2 -receptor antagonistic activities.

Drugs

The drugs used in this study were ($\pm$)-1-(4-amino-3-chloro-5-trifluoromethyl-phenyl)-2-tert-butyl-amino-ethanol hydrochloride (mabuterol, generously donated by Kaken Pharmaceutical Co. Ltd., Tokyo, Japan), (-)-isoprenaline hydrochloride (Nikken Kagaku, Tokyo, Japan), dobutamine hydrochloride (Shionogi Corp., Osaka, Japan), procaterol hydrochloride (Otsuka Pharmaceutical Corp., Tokushima, Japan), atropine sulphate (Wako Pure Chemical, Osaka, Japan), atenolol (Sigma Chemical Corp., St. Louis, MO, U.S.A.), ICI 118,551 (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol generously donated by Imperial Chemical Industries, Macclesfield, England), 3-isobutyl-1-methylxanthine (IBMX, Aldrich Chemical, Milwaukee, WIS, U.S.A.) and Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-fluoromethylphenyl)-pyridine-5-carboxylate, generously donated by Dr F. Seuter, Bayer AG, Wuppertal-Elberfeld, F.R.G.).

Bay K 8644 was dissolved in ethanol and the other drugs were dissolved in physiological saline before the start of the experiment. Drugs were injected into the sinus node artery through a rubber tube with a microsyringe (Terumo Co.). The amount of drug solution injected was 0.01–0.03 ml over a period of 4 s.

The degree of chronotropic or inotropic effect was expressed as maximum percentage change of the basal atrial rate or developed tension. To examine any β -receptor antagonistic activity of mabuterol, the response to each agonist was assessed 1–1.5 min after pretreatment with mabuterol. Inhibition by an antagonist was expressed as percentage inhibition of the control value which was obtained just before treatment with an antagonist. Data were expressed as the mean $\pm$ s.e.mean and analysed by analysis of variance for multiple comparisons of data and Student's *t* test for unpaired data. A probability of

Figure 1 Changes in atrial rate and contractile force in response to 0.1, 1, 10, 100 and 1000 nmol mabuterol injected into the sinus node artery of an isolated, blood-perfused atrium of the dog.

less than 0.05 was considered as statistically significant.

Results

Effects of mabuterol on pacemaker activity and atrial contractility in the isolated, blood-perfused atrium of the dog

Figure 1 shows representative responses on atrial rate and atrial contractility induced by mabuterol at doses of 0.1–1000 nmol in an isolated, blood-perfused right atrium of the dog. When mabuterol was injected at doses of 0.1–10 nmol into the preparation, dose-dependent positive chronotropic and inotropic responses were evoked. However, at higher doses, chronotropic and inotropic responses became biphasic, i.e., negative followed by positive responses and the positive effects gradually decreased. At a dose of 1000 nmol, a biphasic chronotropic response and only a negative inotropic response was observed. Dose-response curves for atrial rate and atrial developed tension expressed as maximum percentage changes of the resting level in response to mabuterol ($n = 8$) and isoprenaline ($n = 6$) in isolated and blood-perfused right atria are shown in Figure 2. Mabuterol increased atrial rate and atrial tension dose-dependently over the range 0.01–10 nmol, and these positive chronotropic and inotropic responses gradually decreased at higher doses. Chronotropic (over 100 nmol) and inotropic responses (over 3 nmol) became biphasic and at doses of 300 and 1000 nmol, only a negative inotropic response was evoked. On the other hand, isoprenaline at doses of 0.001–0.3 nmol, dose-dependently increased atrial rate and atrial tension. The maximal positive chronotropic and inotropic responses to 10 nmol of mabuterol were $31.4 \pm 2.8\%$ and $32.4 \pm 2.6\%$, respectively, and these responses

Figure 2 Mean dose-response curves for percentage changes in the maximum positive or negative chronotropic (a) and inotropic (b) responses to mabuterol (●, $n = 8$) and isoprenaline (○, $n = 6$) in isolated, blood-perfused atria of the dog. Vertical lines show s.e.mean. The basal atrial rate and contractile force in atrial preparations were 106.6 ± 4.4 (mean $\pm$ s.e.mean) beats min^{-1} and 1.6 ± 0.2 g, respectively.

were 41.4% and 12.9%, respectively, of the maximal positive chronotropic and inotropic responses induced by 0.3 nmol isoprenaline (Figure 2). The mabuterol-induced negative cardiac responses at higher doses were not inhibited by atropine ($n = 3$).

Effects of atenolol on positive chronotropic and inotropic responses to mabuterol, dobutamine and procaterol

A selective β_1 -agonist, dobutamine and a selective β_2 -agonist, procaterol were used for comparison. Mabuterol (1 nmol, $n = 8$), dobutamine (1 nmol, $n = 8$) and procaterol (1 nmol, $n = 7$) increased atrial rate by $13.8 \pm 2.4\%$, $18.2 \pm 6.1\%$ and $21.5 \pm 5.3\%$, respectively and atrial contractility by $29.2 \pm 6.3\%$, $75.9 \pm 11.8\%$ and $41.0 \pm 7.5\%$, respectively. In our previous study, dobutamine (0.1–10 nmol) and procaterol (0.03–30 nmol) dose-dependently increased atrial rate and contractile force in the isolated right

Figure 3 Effects of atenolol on increases in atrial rate (a) and atrial tension (b) in responses to mabuterol (●, $n = 8$), dobutamine (▲, $n = 8$) and procaterol (○, $n = 7$). The dose of each agonist was 1 nmol. Data are expressed as a percentage inhibition of the control value (Control) which was obtained just before treatment with atenolol. Points show mean values with s.e.mean.

atrium of the dog (Akahane *et al.*, 1988; 1989). It has been reported that procaterol possesses β_1 -agonist activities at high concentrations (Johansson & Persson, 1983; O'Donnell & Wanstall, 1985; Moleenaar & Summers, 1987). Thus, the dose of each β -agonist which evoked about a 15–20% increase in atrial rate was selected for the control. Atenolol, a selective β_1 -antagonist, at doses of 0.1–10 nmol dose-dependently inhibited dobutamine-induced positive chronotropic and inotropic responses. Atenolol also reduced the positive responses to mabuterol and procaterol. However, the inhibition by atenolol of the mabuterol- or procaterol-induced responses was much less than the inhibition of the dobutamine-induced responses. Summarized data are shown in Figure 3.

Effects of ICI 118,551 on the responses to β -agonists

Positive chronotropic and inotropic responses to mabuterol were dose-dependently inhibited by ICI

Figure 4 Effects of ICI 118,551 on increases in atrial rate (a) and atrial tension (b) in responses to mabuterol (●, $n = 8$), dobutamine (▲, $n = 7$) and procaterol (○, $n = 7$). The dose of each agonist was 1 nmol. Data are expressed as a percentage inhibition of the control value (Control) which was obtained just before treatment with ICI 118,551. Points show mean values with s.e.mean.

118,551 (0.01–10 nmol). Increases in atrial rate and atrial tension evoked by procaterol were more effectively inhibited by ICI 118,551 than those of mabuterol, and the difference in two inhibition curves for atrial rate and atrial tension were statistically significant by an analysis of variance ($P < 0.01$, respectively). Dobutamine-induced positive chronotropic and inotropic responses were only slightly attenuated by ICI 118,551 at doses of 0.01–10 nmol. The summarized data are shown in Figure 4.

Blocking effects of mabuterol on dobutamine-, procaterol-, IBMX-, Bay K 8644- and CaCl_2 -induced positive cardiac responses

Pretreatment with mabuterol at doses of 3 and 30 nmol reduced to positive chronotropic and inotropic responses to both dobutamine and procaterol (Figure 5). Effects of mabuterol on increases in atrial rate and atrial tension evoked by 1 nmol dobutamine or 1 nmol procaterol in 6 right atrial preparations of the dog are shown in Figure 6. Both dobutamine- and procaterol-induced positive chrono- and inotro-

Figure 5 Effects of pretreatment with mabuterol (3 and 30 nmol) on changes in atrial rate and contractile force in response to 1 nmol dobutamine (a) and 1 nmol procaterol (b) in an isolated, blood-perfused atrium of the dog.

pic responses were dose-dependently inhibited by mabuterol at doses of 1–300 nmol. Mabuterol at doses of 100–300 nmol completely suppressed dobutamine- and procaterol-induced responses. Mabuterol inhibited more effectively procaterol-induced atrial rate ($P < 0.05$) and atrial tension ($P < 0.01$) than dobutamine-induced responses. Fifty percent inhibitory doses (IC_{50}) of mabuterol for atrial rate and atrial tension to the effects of procaterol were 1.1 ± 0.4 nmol and 2.9 ± 1.3 nmol, respectively, and to the effects of dobutamine, 5.9 ± 2.0 nmol and 7.2 ± 2.0 nmol, respectively.

In order to examine whether mabuterol selectively inhibited β -adrenoceptors, effects of pretreatment with mabuterol on positive chronotropic and inotropic responses induced by a phosphodiesterase inhibitor, IBMX, a dihydropyridine calcium agonist, Bay K 8644, and $CaCl_2$ were studied in 5–9 isolated right atrial preparations of the dog. Since a direct injection of $CaCl_2$ into the preparation induced a much smaller chronotropic response than inotropic one (Furukawa *et al.*, 1988), the dose of a non- β -agonist which increased the atrial tension to almost the same extent (50–100%) as β -agonists with an obvious chronotropic response was selected, i.e., IBMX 135 nmol, Bay K 8644 0.8 nmol and $CaCl_2$ 3 μ mol, respectively. The positive chronotropic and inotropic responses to IBMX, Bay K 8644 or $CaCl_2$ were not

Figure 6 Effects of mabuterol (1–300 nmol) on the mean percentage changes in chronotropic (a) and inotropic responses (b) to 1 nmol of dobutamine (▲, $n = 6$) and 1 nmol of procaterol (○, $n = 6$) in canine isolated, blood-perfused atrial preparations. Data are presented as a percentage inhibition of the control value (Control) which was obtained just before treatment with mabuterol. Vertical lines represent s.e.mean.

modified by pretreatment with 100 nmol mabuterol which completely suppressed the positive cardiac responses evoked by dobutamine or procaterol. Summarized data are shown in Table 1.

Discussion

Carlsson *et al.* (1972) reported that both β_1 - and β_2 -adrenoceptors mediate chronotropic responses in the cat heart. Carlsson *et al.* (1977) also showed that the increases in heart rate and ventricular contractility were mediated in part by β_2 -adrenoceptors and suggested that the different responses might be responsible for differences in the relative distribution of β_1 - and β_2 -adrenoceptors in the sinus node and in the myocardium. Brodde *et al.* (1982) determined the amount of β_2 -adrenoceptors in atria and ventricle of the rabbit using radioligand binding. As in animal hearts, the coexistence of β_1 - and β_2 -adrenoceptors has been reported in the receptor binding assay of human right atrium and left ventricle (Stiles *et al.*,

Table 1 Effect of pretreatment with mabuterol (100 nmol) on positive chronotropic and inotropic responses induced by several cardiotonics in the canine isolated, blood-perfused atrium

Compound	n	Control		Mabuterol	
		AR (%)	CF (%)	AR (%)	CF (%)
Dobutamine (1 nmol)	9	13.0 ± 3.2	113.3 ± 12.8	0.6 ± 0.2**	9.7 ± 2.9**
Procaterol (1 nmol)	7	18.9 ± 6.0	58.6 ± 15.0	0.1 ± 0.1*	2.9 ± 2.4**
IBMX (135 nmol)	5	23.3 ± 1.9	52.7 ± 6.5	30.3 ± 7.8	95.4 ± 19.5
Bay K 8644 (0.8 nmol)	7	9.5 ± 2.7	59.4 ± 13.8	7.8 ± 2.9	58.5 ± 12.9
CaCl ₂ (3 µmol)	5	2.2 ± 1.0	94.4 ± 10.7	2.2 ± 0.3	68.5 ± 7.6

Each value indicates the mean ± s.e.mean.

AR, atrial rate; CF, contractile force; IBMX, 3-isobutyl-1-methylxanthine.

Significantly different from each control value at **P* < 0.05 and ***P* < 0.01.

1983). Stimulation of β_2 -adrenoceptors increases heart rate and cardiac contractility in human heart (Arnold *et al.*, 1985; Gille *et al.*, 1985; Kaumann & Lemoine, 1987), although β_1 -receptor-mediated responses are predominant. Previous findings have indicated that β_2 -selective drugs affected chronotropy more than inotropy (Carlsson *et al.*, 1977; Stiles *et al.*, 1983; Friedman *et al.*, 1987). Our present study clearly shows that mabuterol is a selective β_2 -adrenoceptor agonist; it increased heart rate more markedly than atrial force (41.4% in atrial rate and 12.9% in atrial tension of the maximal responses to isoprenaline), though maximal positive chronotropic and inotropic responses induced by 10 nmol mabuterol were much smaller than those of isoprenaline. We have reported that atrial contractile force increases in parallel with the pacing rate when an isolated, blood-perfused right atrium of the dog was paced at a rate of 2–4 Hz (a positive frequency-force relationship, Chiba, 1976; Furukawa *et al.*, 1986). Since the mabuterol-induced positive inotropic response was greater than that induced by the increase in the pacing rate and almost equalled in extent the mabuterol-induced positive chronotropic response, mabuterol itself increased the atrial tension independently of the mechanism of a positive frequency-force relationship. Osada *et al.* (1984) reported that mabuterol increases the atrial tension of the isolated, electrically driven left atrium of guinea-pig. The mabuterol- and procaterol-induced positive chronotropic and inotropic responses were inhibited more by a β_2 -selective antagonist, ICI 118,551, than by a selective β_1 -antagonist, atenolol. In contrast, the positive chronotropic and inotropic responses induced by dobutamine were inhibited by atenolol much more than by ICI 118,551. These results suggest that mabuterol exhibits positive chro-

notropic and inotropic responses mainly through β_2 -adrenoceptors in the isolated, blood-perfused dog atrium.

Murai *et al.* (1984) found that mabuterol has a high selectivity for bronchial smooth muscle and has little effect on cardiac muscle in conscious guinea-pigs. Although β_2 -adrenoceptors have been identified by radioligand binding studies in the guinea-pig, rat and rabbit atria (Minneman *et al.*, 1979a,b; Hedberg *et al.*, 1980; Brodde *et al.*, 1982; Molenaar *et al.*, 1987), only β_1 -, and not β_2 -adrenoceptors, mediate positive chronotropic and inotropic responses in these species (O'Donnell & Wanstall, 1979; 1980; Wilson & Lincoln, 1984; Molenaar & Summers, 1987). Results obtained from these species might, therefore, tend to underestimate the chronotropic and inotropic responses to selective β_2 -agonists in man. Manalan *et al.* (1981) reported that the β_1/β_2 adrenoceptor ratio was approximately 85%/15% for dog ventricular myocardium. In the anaesthetized dog, propranolol inhibits the increase in heart rate induced by exercise more markedly than atenolol, suggesting that sinoatrial β_2 -adrenoceptors might be stimulated by circulating catecholamines (Friedman *et al.*, 1987). We have also shown the existence of β_2 -adrenoceptor-mediated positive chronotropic and inotropic responses in the isolated, blood-perfused canine right atrium and left ventricle (Akahane *et al.*, 1988; 1989). Thus, dogs may be more suitable than guinea-pigs, rats or rabbits for predicting chronotropic and inotropic effects of β -adrenoceptor-agonist compounds in man.

In the dog isolated atrium, mabuterol, at high doses, induced negative cardiac responses. The negative cardiac responses were not mediated by muscarinic cholinergic receptors because the negative cardiac responses were not modified by atropine.

Osada *et al.* (1984) found that more than $0.1 \mu\text{g ml}^{-1}$ mabuterol inhibits the positive inotropic effect of isoprenaline and decreases the maximum driving frequency in guinea-pig isolated left atria, finally suggesting a β_1 -blocking activity and a quinidine-like activity of mabuterol at higher doses. In our experiments, mabuterol suppressed not only dobutamine- but also procaterol-induced positive chronotropic and inotropic responses, whereas IBMX-, Bay K 8644- and CaCl_2 -induced responses were not inhibited by mabuterol. These results indicate that mabuterol possesses both β_1 - and β_2 -adrenoceptor blocking activities at higher doses. Fifty percent inhibitory doses of mabuterol on the positive chronotropic and inotropic responses to procaterol were smaller than those to dobutamine. Osada *et al.* (1984) showed that the concentration of mabuterol that induces a 50% relaxation of the tracheal smooth muscle of the guinea-pig is one-two hundredth of the concentration that inhibits the

β_1 -receptor-mediated positive inotropic response to isoprenaline by 50%. However, in the present study, mabuterol inhibited both β_1 - and β_2 -receptor-mediated responses and was rather more effective in inhibiting β_2 -receptor-mediated responses.

In conclusion, mabuterol is a selective β_2 -agonist which evokes small positive chronotropic and much weaker positive inotropic responses, and mabuterol also possesses a non-selective β -antagonistic activity in isolated and blood-perfused canine right atrium. Caution may be required in the use of this drug when the dosage of mabuterol is very high or its excretion is disturbed in asthmatic patients.

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