Selective and full β_1 -adrenoceptor agonist action of a catechol derivative of denopamine (T-0509) in the guinea-pig cardiac muscle and trachea: comparison with denopamine, xamoterol and isoprenaline

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1 The pharmacological actions of T-0509, a 3-hydroxy derivative of denopamine, were studied in various guinea-pig tissues; these effects were compared with those of isoprenaline, denopamine and xamoterol.

2 The intrinsic activities of the positive inotropic actions of T-0509, denopamine and xamoterol compared with isoprenaline (= 100%) in the papillary muscle were 99%, 83% and 28%, respectively, while their relative potencies (EC_{50} agonist EC_{50} isoprenaline) were 0.23, 33 and 1.4, respectively. The intrinsic activities of T-0509, denopamine and xamoterol as positive chronotropic agents in the right atria were 98%, 69% and 48%, respectively, and their equipotent concentrations (isoprenaline = 1) were 0.24, 50 and 4, respectively.

3 The positive chronotropic actions of T-0509 and denopamine were antagonized by bisoprolol (3×10^{-8} M), but not by ICI 118,551 (3×10^{-8} M).

4 The intrinsic activity of T-0509 in histamine-contracted tracheae was similar to that of isoprenaline, but its equipotent concentration was 38; the effects of both agents were antagonized by ICI 118,551 (3×10^{-8} M), but not by bisoprolol (3×10^{-8} M). Denopamine and xamoterol did not show any agonist activity on guinea-pig trachea.

5 Denopamine and xamoterol antagonized the positive chronotropic (pA_2 , denopamine: 6.98, xamoterol: 7.75) and tracheal relaxant (pA_2 , denopamine: 5.39, xamoterol: 6.25) effects of isoprenaline.

6 Isoprenaline, T-0509 and denopamine, but not xamoterol, contracted the guinea-pig aorta in a decreasing order in the presence of propranolol (10^{-6} M).

7 Based on the above studies, T-0509 appears to be a highly selective β_1 -adrenoceptor agonist with full agonist properties, while denopamine and xamoterol appear to be selective, but partial β_1 -adrenoceptor agonists.

Keywords: T-0509; denopamine derivative; selective β_1 -adrenoceptor with full agonist action; cardiac muscle; trachea; aorta

Introduction

In mammalian hearts, β -adrenoceptors are predominantly of the β_1 -subtype (Stiles *et al.*, 1984). β_1 -Adrenoceptor stimulation is known to cause positive inotropic action through activation of adenylate cyclase (Lemoine *et al.*, 1989a,c). The orally-active cardiotonic agents, denopamine and xamoterol, are highly selective β_1 -adrenoceptor agonists with partial agonist properties (Nagao *et al.*, 1984; Ikeo *et al.*, 1985; Narita *et al.*, 1986; Yokoyama *et al.*, 1988; Lemoine *et al.*, 1989a; Gurden *et al.*, 1989) and have been introduced recently for the treatment of patients with congestive heart failure (Kino *et al.*, 1983; Ikeda *et al.*, 1987; Furlong & Brogden, 1988). Denopamine causes positive inotropic effects with small increases in heart rate and in myocardial oxygen consumption in both dogs (Ikeo & Nagao, 1985) and man (Thormann *et al.*, 1985).

Partial β_1 -adrenoceptor agonists such as denopamine, xamoterol, prenalterol, ICI 89,963 and ICI 119,033 have marked positive inotropic action and only limited stimulatory action on adenylate cyclase (Franklin & Twose, 1979; Hedberg & Mattson, 1981; Main, 1982; Bing *et al.*, 1984; Inamasu *et al.*, 1987; Lemoine *et al.*, 1989a). These agonists

have been thought to be less prone to cause arrhythmogenic side-effects and β -adrenoceptor desensitization than full agonists (Pouleur *et al.*, 1984; Chemnitz & Bing, 1985; Yabana *et al.*, 1986; Benovic *et al.*, 1988; Narita *et al.*, 1988). However, it is not known whether either β_1/β_2 -adrenoceptor selectivity or partial agonist activity is responsible for the favourable properties of partial β_1 -adrenoceptor agonists in the heart. Therefore, the advent of a highly selective β_1 -adrenoceptor agonist having full agonist activity aids in the understanding of the favourable properties of partial β_1 -adrenoceptor agonists.

T-0509 is a catechol derivative of denopamine (Figure 1), and has been reported to produce full β_1 -adrenoceptor agonist activity with a small increase in adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels in the dog isolated ventricular muscle (Kurosawa *et al.*, 1990). However, details of β_1/β_2 -adrenoceptor selectivity and α -adrenoceptor agonist activity of T-0509 have not been reported.

In the present paper, we have studied the pharmacological properties of T-0509 by using guinea-pig cardiac muscle, trachea and aorta as systems with functions of β_1 -, β_2 - and α -adrenoceptors (Buckner & Patil, 1971; Patil *et al.*, 1971; 1972; Malta *et al.*, 1985; Hattori *et al.*, 1987), respectively. We have compared T-0509 with a β -adrenoceptor full agonist, isoprenaline, and the partial agonists, denopamine and xamoterol. Furthermore, to evaluate β_1/β_2 -adrenoceptor selectivity of T-0509, the effects of the highly selective β_1 -

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Figure 1 Chemical structures of denopamine, T-0509, xamoterol and isoprenaline.

adrenoceptor antagonist, bisoprolol (Schliep & Harting, 1984; Klockow *et al.*, 1986) and the highly selective β_2 -adrenoceptor antagonist, ICI 118,551 (O'Donnell & Wanstall, 1980; Bilski *et al.*, 1983), have also been examined. A preliminary account of this work has previously been presented to The Japanese Pharmacological Society (Yabana *et al.*, 1989).

Methods

Male guinea-pigs weighing 210–550 g (4–10 weeks old, Hartley strain, Shizuoka Laboratory Center (SLC), Shizuoka, Japan) were treated with reserpine (5 mg kg⁻¹, i.p.), 16 h before the experiments in order to deplete thenoradrenaline content of the cardiac muscle. Animals were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.) and killed by exsanguination from the carotid artery. The heart, trachea or descending aorta was rapidly removed and placed in Krebs-Henseleit solution (composition mM: Na⁺ 143.3, K⁺ 5.9, Ca²⁺ 2.6, Mg²⁺ 1.2, Cl⁻ 128.3, H₂PO₄⁻ 2.2, HCO₃⁻ 24.9, SO₄²⁻ 1.2 glucose 5.6 and pH 7.4).

The left ventricular papillary muscle and the right atrium from the heart were suspended under a resting tension of 0.3–0.6 g in the Krebs-Henseleit solution at 30°C. The tracheal chain (5 mm in length, four preparations per trachea) and the descending aorta (ring segments of 5 mm in length) were suspended under a resting tension of 1.0–1.5 g in Krebs-Henseleit solution at 37°C. The Krebs-Henseleit solution was aerated with 95% O₂:5% CO₂. The isometric tension was measured with a strain gauge transducer (UL-10GR, Minebea, Tokyo) and recorded on a pen recorder (WR-3701, Graphtec, Tokyo). The left ventricular papillary muscle was driven by a field stimulation of 1.3 Hz frequency and 0.5 ms duration with 1.5 times greater voltage than the threshold. The spontaneous beating rate of the right atria was counted with a heart rate meter (AT-601G, Nihon Kohden, Tokyo). The preparations were equilibrated for 60 min before starting the experiments.

Tracheae were contracted with histamine (10⁻⁵ M), which produced submaximal contraction, and agonists were added on the plateau phase of the histamine contraction. In the cardiac muscle and trachea, a single cumulative concentration-response curve (CCRC) to isoprenaline, T-0509, denopamine or xamoterol was obtained with each preparation. Intrinsic activities of T-0509, denopamine and xamoterol in the cardiac muscle were obtained from the maximum response induced by addition of 10⁻⁴ M isoprenaline at the end of the experiment. Maximum relaxation of the trachea was obtained with papaverine (10⁻⁴ M) at the end of the experiment.

The CCRCs to isoprenaline or T-0509 in the cardiac muscle and trachea were also obtained in the presence of the extraneuronal uptake inhibitor, deoxycorticosterone acetate (DOCA, 2.5 × 10⁻⁵ M) (Henry & Goldie, 1990) in order to evaluate the influence of extraneuronal uptake on agonist potencies in two preparations.

When the β -adrenoceptor antagonistic actions of the drugs in the right atria and trachea were studied, the CCRC to isoprenaline was constructed on each preparation after a 20 min pretreatment period in the presence or absence of the drug (three different concentrations). Each preparation was exposed to only one concentration of the drug.

In other experiments, a single CCRC to T-0509 or denopamine in the right atria and trachea was constructed on each preparation in the presence or absence of the highly selective β_1 -adrenoceptor antagonist, bisoprolol, after a 20 min pretreatment period. The influence of the highly selective β_2 -adrenoceptor antagonist, ICI 118,551, on the positive chronotropic and relaxant actions of T-0509 or denopamine were also investigated.

In the guinea-pig aorta, 1 μ M propranolol was applied in all experiments to inhibit the β -adrenoceptor agonist actions to enable α -adrenoceptor agonist actions to be evaluated. Initially, aortae in the presence of DOCA (2.5 × 10⁻⁵ M) were contracted by adding noradrenaline (10⁻⁵ M). Following a 30 min washout period, the CCRC to the agonist in the absence or presence of 10⁻⁶ M phentolamine was obtained. The responses are expressed as percentage of the noradrenaline (10⁻³ M)-induced maximum response obtained at the end of experiments.

Data analysis

Agonist potencies were expressed in terms of pD₂ values and intrinsic activities. pD₂ is defined as the negative logarithm of EC₅₀, where EC₅₀ is the concentration of the agonist producing half maximum response determined from graphical plots of the CCRC of each preparation. The relative potency of the agonist was represented as EC₅₀ ratio (EC₅₀ for agonist/EC₅₀ for isoprenaline). Intrinsic activities of agonists were represented as percentage of the maximum response to isoprenaline or papaverine.

Antagonist potencies were expressed in terms of pA₂ values. The pA₂ values were estimated from the regression lines obtained in the Schild plot analysis (Arunlakshana & Schild, 1959). If antagonism is competitive, a plot of the logarithm of (concentration ratio – 1) against the negative logarithm of the concentration of antagonist yields a straight line with a slope of unity. The point of intercept of this line with the abscissa scale is the pA₂ value. The concentration-ratio is the ratio of the EC₅₀ for isoprenaline in the presence

and absence of the antagonist. It should be pointed out that in the right atria, in which denopamine increased basal rate, concentration-ratios were not calculated from equiactive isoprenaline concentrations (Stephenson, 1956), but rather from the half-maximal concentration of isoprenaline (Kenakin & Black, 1978).

Experimental values are given as a mean $\pm$ s.e.mean. Results were analysed by unpaired Student's *t* test or Scheffe's test after one way analysis of variance (ANOVA). Probability values less than 0.05 were considered to be significant.

Drugs

The following drugs were used in this study; denopamine hydrochloride [(−)(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl) amino]ethanol]-hydrochloride (Tanabe Seiyaku, Osaka, Japan), T-0509 [(−)(R)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl) amino]ethanol]-hydrochloride (Tanabe Seiyaku, Osaka, Japan), (±)-isoprenaline hydrochloride (Nacalai tesque, Tokyo, Japan), xamoterol (synthesized at Organic Chemistry Research Laboratory of Tanabe Seiyaku), (−)-bisoprolol hemifumarate (E. Merck, Darmstadt, Germany), (±)-ICI 118,551 hydrochloride [(erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol] hydrochloride; synthesized at Organic Chemistry Research Laboratory of Tanabe Seiyaku), propranolol hydrochloride (Nacalai tesque, Tokyo, Japan), reserpine (Apoplon, Daiiti Seiyaku, Tokyo, Japan), deoxycorticosterone acetate (Nacalai tesque, Tokyo, Japan), noradrenaline (Nacalai tesque, Tokyo, Japan) and phentolamine mesylate (Regitin, Ciba-Geigy, Tokyo, Japan). All drugs except reserpine and deoxycorticosterone acetate were dissolved and diluted with Krebs-Henseleit solution containing 0.1 mg ml^{−1} ascorbic acid. Deoxycorticosterone acetate was dissolved in propylene glycol to prepare a 25 mM stock solution. Other chemicals were purchased from commercial sources.

Results

β -Agonist actions in the isolated cardiac muscle

T-0509, denopamine, xamoterol and isoprenaline all exhibited concentration-dependent positive inotropic actions

in the isolated ventricular papillary muscle, as shown in Figure 2a. The pD₂ values determined for these four agonists are summarized in Table 1. The maximum responses to T-0509 (99.1 $\pm$ 0.5%) did not differ significantly from those to isoprenaline (100%), although it was four times more potent than isoprenaline (*P* < 0.01, difference between pD₂s). The slopes of the CCRCs for denopamine and xamoterol were less steep than that for isoprenaline, and maximum responses to denopamine (82.8 $\pm$ 3.7%) and xamoterol (29.3 $\pm$ 5.6%) were significantly lower than that to

Figure 2 Concentration-response curves for the effects of denopamine, T-0509, xamoterol and isoprenaline on contractile force of the left ventricular papillary muscle (a) and spontaneous beating rate of the right atria (b) of the guinea-pig. Initial contractile force and spontaneous beating rate were 396 $\pm$ 66 mg and 150 $\pm$ 5 beats min^{−1}, respectively. The data are expressed as percentages of the maximum response to 10^{−4} M isoprenaline. (○) Isoprenaline (*n* = 9); (●) T-0509 (*n* = 7); (□) denopamine (*n* = 10); (Δ) xamoterol (*n* = 10). The points and vertical bars represent means $\pm$ s.e.mean.

Table 1 Potencies of denopamine, T-0509, isoprenaline and xamoterol in isolated tissue preparations of guinea-pigs

	<i>n</i>	pD ₂ (− log EC ₅₀)	EC ₅₀ agonist/ EC ₅₀ isoprenaline	Intrinsic activity
Papillary muscle (force)				
Isoprenaline	9	8.59 $\pm$ 0.06	1	100
T-0509	7	9.22 $\pm$ 0.15*	0.23	
Denopamine	10	7.08 $\pm$ 0.19**	33	82.8 $\pm$ 3.7*
Xamoterol	10	8.43 $\pm$ 0.07	1.4	28.3 $\pm$ 5.6**
Right atria (rate)				
Isoprenaline	10	8.74 $\pm$ 0.08	1	100
T-0509	8	9.35 $\pm$ 0.11*	0.24	
Denopamine	13	7.07 $\pm$ 0.12**	50	68.7 $\pm$ 3.5* ^a
Xamoterol	8	8.14 $\pm$ 0.24	4	48.3 $\pm$ 7.3** ^b
Trachea (relaxation)				
Isoprenaline	5	9.01 $\pm$ 0.07	1	95.6 $\pm$ 0.5
T-0509	5	7.43 $\pm$ 0.06††	38	95.2 $\pm$ 0.2
Denopamine	9	NC		NC
Xamoterol	6	NC		NC

pD₂ values and intrinsic activities (% of the maximum response to isoprenaline (cardiac muscles) or papaverine (trachea) in the papillary muscle, right atria and trachea were calculated from the experiments of Figures 2a, b and 3, respectively. The relative potencies of agonists were defined as EC₅₀ agonist/EC₅₀ isoprenaline. Values are expressed as mean $\pm$ s.e.mean from *n* preparations. NC: not calculated because the maximum response could not be observed even at highest concentration of 3 $\times$ 10^{−3} M. **P* < 0.05; ***P* < 0.01 as compared with all agonists by one way analysis of variance and Scheffe's test. ††*P* < 0.01 as compared with isoprenaline by unpaired Student's *t* test. ^a*P* < 0.05 as compared with the intrinsic activity of denopamine by unpaired Student's *t* test. ^b*P* < 0.05 as compared with the intrinsic activity of xamoterol by unpaired Student's *t* test.

isoprenaline. The maximum response to denopamine was significantly higher than that to xamoterol, and its pD_2 value was significantly lower.

In isolated right atria, isoprenaline, T-0509, denopamine and xamoterol all caused concentration-dependent positive chronotropic actions (Figure 2b and Table 1). The maximum response to T-0509 ($98.4 \pm 0.5\%$) was again similar to that to isoprenaline, and it was four times more potent than isoprenaline. The maximum responses to denopamine ($68.6 \pm 3.5\%$) and xamoterol ($48.3 \pm 7.3\%$) were lower than that to isoprenaline ($P < 0.01$). The maximum response to denopamine was again higher than that to xamoterol ($P < 0.01$), and its pD_2 was smaller ($P < 0.01$). Furthermore, the maximum positive inotropic response to denopamine was larger than its maximum positive chronotropic response ($P < 0.05$), but for xamoterol, the opposite was apparent ($P < 0.05$).

The potencies of isoprenaline (papillary muscles: $pD_2 = 8.59 \pm 0.08$, $n = 5$; atria: $pD_2 = 8.63 \pm 0.04$, $n = 4$) and T-0509 (papillary muscles: $pD_2 = 9.29 \pm 0.09$, $n = 4$; atria: $pD_2 = 9.22 \pm 0.10$, $n = 4$) in the presence of DOCA (2.5×10^{-5} M) did not differ from those in the absence of DOCA listed in Table 1.

β -Agonist actions in the isolated trachea

T-0509 and isoprenaline demonstrated concentration-dependent relaxant actions in the histamine-contracted trachea (Figure 3). Although T-0509 was less potent than isoprenaline ($P < 0.01$) (see Table 1), the maximum response to T-0509 was comparable to that to isoprenaline. Denopamine and xamoterol caused only about 30% relaxation even at the highest concentration of 3×10^{-3} M. Furthermore, the tracheal relaxant actions of denopamine and xamoterol were not influenced by pretreatment with 10^{-6} M propranolol.

As in the case of the positive inotropic action, the potencies of isoprenaline ($pD_2 = 9.03 \pm 0.09$, $n = 4$) and T-0509 ($pD_2 = 7.45 \pm 0.08$, $n = 4$) in the presence of DOCA (2.5×10^{-5} M) did not differ from those in the absence of DOCA listed in Table 1.

Influences of bisoprolol and ICI 118,551 on responses to T-0509 and denopamine on right atria

The CCRCs for positive chronotropic action of T-0509 or denopamine were shifted to the right in a parallel way in the presence of 3×10^{-8} M bisoprolol, but not 3×10^{-8} M ICI 118,551 (data not shown). The pD_2 s of T-0509 and

Figure 3 Concentration-response curves for the relaxant actions of β -adrenoceptor agonists in the absence ($\circ$, $\bullet$, Δ , $\square$) and the presence ($\blacktriangle$, $\blacksquare$) of 10^{-6} M propranolol on the histamine-induced contraction in the guinea-pig trachea. The data are expressed as percentages of the maximum response to 10^{-4} M papaverine: ($\circ$) isoprenaline ($n = 5$); ($\bullet$) T-0509 ($n = 5$); (Δ) denopamine ($n = 9$); ($\blacktriangle$) denopamine + propranolol ($n = 4$); ($\square$) xamoterol ($n = 6$); and ($\blacksquare$) xamoterol + propranolol ($n = 4$). The points and vertical bars represent means $\pm$ s.e.mean.

denopamine were reduced with bisoprolol from 9.23 ± 0.02 to 8.04 ± 0.11 ($P < 0.01$) and from 7.08 ± 0.08 to 5.99 ± 0.13 , ($P < 0.01$), respectively. Conversely, the CCRC for tracheal relaxant action of T-0509 was shifted to the right in the presence of 3×10^{-8} M ICI 118,551, but not 3×10^{-8} M bisoprolol (data not shown). The pD_2 s of isoprenaline and T-0509 were reduced with ICI 118,551 from 8.03 ± 0.09 to 6.42 ± 0.14 ($P < 0.01$) and from 6.56 ± 0.06 to 5.17 ± 0.05 ($P < 0.01$), respectively.

Influences of denopamine and xamoterol on the response to isoprenaline on right atria

Pretreatment with denopamine itself increased the beating rate of isolated right atria, but caused a parallel rightward shift of the CCRC to isoprenaline after normalization (Figure 4). The Schild plot of the data yielded a linear regression with a slope of 1.06 ± 0.14 , which was not significantly different from unity, and the pA_2 value for denopamine was 6.98 ± 0.2 (Table 2). This pA_2 value was close to its pD_2 value in the right atria. Denopamine also shifted the CCRC of isoprenaline to the right in a parallel fashion in the histamine contracted trachea (data not shown). The Schild plot had a slope of 1.02 ± 0.14 , which was not significantly different from unity, and the pA_2 value for denopamine was 5.39 ± 0.13 (Table 2). Xamoterol also exhibited β -antagonist actions in the right atria ($pA_2 = 7.75 \pm 0.14$, slope = 0.94 ± 0.09) and trachea ($pA_2 = 6.25 \pm 0.11$, slope = 1.06 ± 0.13), respectively (Table 2).

α -Adrenoceptor agonist actions in isolated aorta

In the presence of 10^{-6} M propranolol, isoprenaline, T-0509 and denopamine contracted the aorta, although none of these agonists caused the maximum response even at the highest concentration used (3×10^{-3} M) (Figure 5). T-0509 at concentrations of 3×10^{-5} to 3×10^{-3} M was weaker than isoprenaline ($P < 0.01$). On the other hand, xamoterol (10^{-5}

Figure 4 Influence of denopamine on concentration-response curves for the positive chronotropic action of isoprenaline in the guinea-pig right atria. (a) Concentration-response curves of isoprenaline in the absence and the presence of denopamine expressed as percentages of the maximum response to isoprenaline. (b) Normalized concentration-response curves of isoprenaline. ($\circ$) Control ($n = 15$); ($\bullet$) 10^{-6} M denopamine ($n = 5$); (Δ) 3×10^{-6} M denopamine ($n = 5$); ($\blacktriangle$) 10^{-5} M denopamine ($n = 5$). The points and vertical bars represent means $\pm$ s.e.mean.

Table 2 Antagonistic actions of denopamine and xamoterol in the isolated right atria and trachea of guinea-pig

	n	pA_2	Slope	r
Right atria (rate)				
Denopamine	15	6.98 ± 0.20	1.06 ± 0.14	0.89
Xamoterol	12	7.75 ± 0.14	0.94 ± 0.09	0.94
Trachea (relaxation)				
Denopamine	21	5.39 ± 0.13	1.02 ± 0.14	0.87
Xamoterol	15	6.25 ± 0.11	1.06 ± 0.13	0.91

pA_2 value, slope and r (correlation coefficient of regression line) were obtained from Schild plots according to Arunlakshana & Schild as described in Methods. Values are expressed as the mean $\pm$ s.e.mean from n experiments.

Figure 5 Contractile effects of denopamine, T-0509 and isoprenaline in the guinea-pig aorta pretreated with 10^{-6} M propranolol ($n = 6$). The data are expressed as percentages of the response to 10^{-3} M noradrenaline. (○) Isoprenaline; (●) T-0509; (□) denopamine; (△) xamoterol. The points represent means of 6 experiments and vertical bars show s.e.mean. $**P < 0.01$ as compared with isoprenaline by one way analysis of variance and Scheffe's test.

to 3×10^{-3} M) did not produce any contraction. In the presence of phentolamine (10^{-6} M), isoprenaline, T-0509 and denopamine induced no contractile activity ($n = 4$).

Discussion

The results of the present experiments show that T-0509 is a potent and highly selective β_1 -adrenoceptor agonist with full agonist activity, whereas denopamine and xamoterol are both selective β_1 -adrenoceptor agonists with partial agonist activity. The intrinsic activity of denopamine is higher than that of xamoterol.

The results obtained from the DOCA-treated and non-treated preparations suggest that extraneuronal uptake of catecholamines in the guinea-pig cardiac muscle and trachea (<10 weeks old) is negligible. The extraneuronal uptake inhibitor, hydrocortisone, has been reported not to potentiate either the positive inotropic and chronotropic actions of isoprenaline on cardiac muscle (Goldie, 1975), or its relaxant action on the trachea from young (<12 weeks old) guinea-pigs (Takayanagi *et al.*, 1990). We have obtained similar results with T-0509 and isoprenaline in the present study by using guinea-pigs of 10 weeks old. However, extraneuronal uptake has been reported to play an important role in the β -adrenoceptor action of isoprenaline in the trachea from older (40 and 100 weeks) guinea-pigs (Takayanagi *et al.*, 1990). Therefore, extraneuronal uptake in the guinea-pig trachea appears to be dependent on the age of the guinea-pigs.

Guinea-pig ventricular muscle has been reported to have only β_1 -adrenoceptors (Engel *et al.*, 1980; Hedberg *et al.*, 1980; Freissmuth *et al.*, 1986), and guinea-pig right atria to have predominantly β_1 -adrenoceptors (Hedberg *et al.*, 1980; Johansson & Persson, 1983). Furthermore, β_1 -adrenoceptors

have been reported to couple more efficiently to the positive inotropic and chronotropic responses than β_2 -adrenoceptors (Molenaar & Summers, 1987). In the present paper, T-0509 was demonstrated to be four times more potent as a positive inotropic and chronotropic agent than isoprenaline in the papillary muscle and right atria, and to have similar intrinsic activities to those of isoprenaline. Moreover, T-0509 caused positive chronotropic action through β_1 -adrenoceptors. This finding was consistent with that in the canine ventricular muscle reported by Kurosawa *et al.* (1990) and with that in the guinea-pig right atria reported by Molenaar & Summers (1987).

On the other hand, guinea-pig trachea has been reported to have predominantly β_2 -adrenoceptors (Engel *et al.*, 1980; Carswell & Nahorski, 1983). However, β_1 -adrenoceptor-stimulation has been reported to relax spontaneous tone in the guinea-pig trachea (Zaagsma *et al.*, 1983; Lemoine *et al.*, 1989b). In the present study, T-0509, in contrast to the cardiac muscle, exhibited a relaxant action with a potency 38 times less than isoprenaline through β_2 -adrenoceptors in the histamine-contracted trachea, but with a similar intrinsic activity to isoprenaline. These results indicate that T-0509, like denopamine and xamoterol, did not produce tracheal relaxant action through β_1 -adrenoceptors. Therefore, it is suggested that β -adrenoceptors in the guinea-pig trachea are predominantly of the β_2 -subtype. Thus, T-0509 is considered to be a full agonist at β_2 -adrenoceptors, but to exhibit at least a 150 fold selectivity towards β_1 -adrenoceptors. As an α -adrenoceptor agonist, T-0509 was also less potent than isoprenaline and did not cause maximum responses even at the highest concentration used (3×10^{-3} M). Thus, these results indicate that T-0509 is a highly selective agonist at β_1 -adrenoceptors, having weak β_2 -adrenoceptor agonist and minimal α -adrenoceptor agonist activity. Furthermore, T-0509 exhibits full β -adrenoceptor agonist activity.

Xamoterol exhibited lower intrinsic activity than did denopamine. Xamoterol has been reported either to cause positive inotropic action with low intrinsic activity (Hattori *et al.*, 1987) or not to cause any inotropic activity (Malta *et al.*, 1985). Our results with xamoterol agree with the former, and also with those in the cat papillary muscle reported by Lemoine *et al.* (1989a). Low intrinsic activity of xamoterol seems to result in its acting as an agonist at rest, and as an antagonist during exercise in man (Sato *et al.*, 1987; McCaffrey *et al.*, 1988). On the other hand, denopamine has been reported to produce partial agonist action with high intrinsic activity in the dog ventricular muscle (Yokoyama *et al.*, 1988), but to have full agonist action in the guinea-pig right atria (sinoatrial rate) (Lemoine *et al.*, 1989c) and in the cat papillary muscle and right atria (sinoatrial rate) (Hoshiyama *et al.*, 1985; Lemoine *et al.*, 1989c). Our results have confirmed that denopamine has a high intrinsic activity on β_1 -adrenoceptors, even if it is not a full agonist. Thus, the high intrinsic activity of denopamine seems to be responsible for it acting as an agonist in man (Hori *et al.*, 1991).

Denopamine activated only β_1 -adrenoceptors in the right atria and neither denopamine nor xamoterol showed any tracheal relaxant activity. Xamoterol has been reported to lack agonistic property in the guinea-pig trachea (Hicks *et*

al., 1987), uterus and ileum (Malta *et al.*, 1985) and in the dog femoral artery (Nuttall & Snow, 1982) which are all β_2 -adrenoceptor containing preparations. Moreover, denopamine and xamoterol, when tested for antagonist activity in the right atria and trachea, exhibited different pA_2 values, indicating that both agonists show β_1 - and β_2 -adrenoceptor antagonist actions. The pA_2 values of xamoterol in both tissues were consistent with those reported by Hicks *et al.* (1987) (guinea-pig right atria, $pA_2 = 7.88$; trachea, $pA_2 = 6.57$). These results indicate that denopamine is a partial β_1 -adrenoceptor agonist, and suggest that, like xamoterol, the β_2 -adrenoceptor antagonist action of denopamine contributes to its β_1 -adrenoceptor selectivity. Therefore, we could not confirm the full agonistic action of denopamine in the guinea-pig right atria reported by Lemoine *et al.* (1989c).

The intrinsic activity of xamoterol, like that of another partial agonist prenalterol (Kaumann *et al.*, 1980a,b), has been reported to be slightly higher for positive chronotropy than for positive inotropy (Lemoine *et al.*, 1989b). In contrast, the intrinsic activity of denopamine was reported to be higher in positive inotropy than in positive chronotropy (Hoshiyama *et al.*, 1985; Narita *et al.*, 1986). Our results have confirmed these findings, and may reflect the distinct characteristics of the β_1 -adrenoceptors in guinea-pig right atria and left ventricular papillary muscle.

To date, noradrenaline, isoprenaline and selective β_2 -adrenoceptor agonists such as procaterol (Yabuuti *et al.*, 1977; Yamashita *et al.*, 1978) are frequently used to evaluate β -adrenoceptor-adenylate cyclase coupling, and to charac-

terize β -adrenoceptor subtypes. Noradrenaline is a β_1 -selective agonist with potent α -adrenoceptor agonist activity, and this activity has been reported to induce positive inotropic action (Schumann *et al.*, 1978; Bruchner *et al.*, 1984) and cardiac hypertrophy (Simpson, 1985). Recently, β_2 -adrenoceptors in the human heart have been found to couple to adenylyl cyclase and to mediate positive inotropic and chronotropic actions (Gille *et al.*, 1985; Kaumann & Lemoine, 1987). Isoprenaline in human right atrium has been reported to produce positive inotropic action via stimulation of β_1 - and β_2 -adrenoceptors (Zerkowski *et al.*, 1986). Since T-0509 was a highly selective and full β_1 -adrenoceptor agonist as mentioned above, like procaterol it may be useful in the study of β -adrenoceptor-adenylate cyclase coupling and in characterizing β -adrenoceptor subtypes in experimental animals and man.

In summary, T-0509 appears to be a highly selective and full β_1 -adrenoceptor agonist, having weak β_2 -adrenoceptor agonist and minimum α -adrenoceptor agonist actions. T-0509 may be useful as a standard tool in the study of β -adrenoceptors. Moreover, denopamine and xamoterol are also both selective β_1 -adrenoceptor partial agonists, but the intrinsic activity of denopamine is higher than that of xamoterol.

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