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Mediation of the positive chronotropic effect of CGP 12177 and cyanopindolol in the pithed rat by atypical β-adrenoceptors, different from β_3-adrenoceptors

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1 The influence of β_1-, β_2-, and β_3-adrenoceptor agonists and of CGP 12177 and cyanopindolol on heart rate and diastolic blood pressure was studied in the pithed rat.

2 The β_1-adrenoceptor agonist, prenalterol, increased heart rate and the β_2-adrenoceptor agonist, fenoterol, caused a fall in blood pressure. The effect of prenalterol was antagonized by the β_1-adrenoceptor antagonist, CGP 20712 0.1 μmol kg^{-1} and the action of fenoterol was attenuated by the β_2-adrenoceptor antagonist, ICI 118551 0.1 μmol kg^{-1}. Both effects were markedly diminished by the non-selective β-adrenoceptor antagonist, bupranolol 0.1 μmol kg^{-1}.

3 The non-selective β-adrenoceptor agonist, isoprenaline, three β_3-agonists as well as CGP 12177 and cyanopindolol elicited a positive chronotropic effect, exhibiting the following pED_{A60} values (negative log values of the doses increasing heart rate by 60 beats min^{-1}): isoprenaline 10.4, CGP 12177 8.3, cyanopindolol 7.2, BRL 37344 6.9, ZD 2079 5.2 and CL 316243 < 5.

4 CGP 20712 0.1 μmol kg^{-1}, given together with ICI 118551 0.1 μmol kg^{-1}, markedly attenuated the positive chronotropic effect of isoprenaline, BRL 37344, ZD 2079 and CL 316243 without affecting the increase in heart rate produced by CGP 12177 and cyanopindolol.

5 The positive chronotropic effect of CGP 12177 and cyanopindolol was attenuated by CGP 20712, 1 and 10 μmol kg^{-1} and bupranolol, 10 μmol kg^{-1} but was not affected by ICI 118551, 10 μmol kg^{-1}. The effect of CGP 12177 was also not changed by BRL 37344 1 μmol kg^{-1}, ZD 2079 10 μmol kg^{-1}, CL 316243 10 μmol kg^{-1}, the α_1-adrenoceptor antagonist, prazosin 1μmol kg^{-1} and the 5-hydroxytryptamine $5\text{-}HT_{2A}$ receptor antagonist, ketanserin 3 μmol kg^{-1}.

6 CGP 12177 0.002 μmol kg^{-1} and cyanopindolol 0.003 μmol kg^{-1} shifted to the right the dose-response curve of prenalterol for its positive chronotropic effect. The −log values of the doses causing a twofold shift to the right were 9.6 and 9.5, respectively.

7 Isoprenaline 0.00001 − 0.001 μmol kg^{-1}, BRL 37344 0.01 − 1 μmol kg^{-1} and CGP 12177 0.1 μmol kg^{-1} caused a fall in diastolic blood pressure which was markedly attenuated by combined administration of CGP 20712 and ICI 118551, 0.1 μmol kg^{-1} each.

8 CGP 12177 0.01 and 0.1 μmol kg^{-1} and cyanopindolol 1 μmol kg^{-1} elicited an increase in diastolic blood pressure. CGP 20712, ICI 118551, bupranolol and, in the case of CGP 12177, also BRL 37344, ZD 2079, CL 316243, prazosin and ketanserin did not influence this effect.

9 In conclusion, the positive chronotropic effect of CGP 12177 and cyanopindolol is not mediated via β_1-, β_2-, β_3-, α_1-adrenoceptors or $5\text{-}HT_{2A}$ receptors. This effect may involve atypical β-adrenoceptors, similar or identical to those described by Kaumann (1989) in isolated heart preparations.

Keywords: pithed rat; positive chronotropic effect; atypical β-adrenoceptors; β_3-adrenoceptors; CGP 12177; cyanopindolol; BRL 37344; ZD 2079; CGP 20712; bupranolol

Introduction

During the last decade, β_3-adrenoceptors have been identified in various tissues, especially in white and brown adipocytes (for review, see Arch & Kaumann, 1993). Effects mediated via β_3-adrenoceptors are antagonized by β-adrenoceptor antagonists at very low affinities compared to their affinities for β_1- and/or β_2-adrenoceptors and are mimicked by selective β_3-adrenoceptor agonists (e.g., BRL 37344) and by high concentrations of the so-called non-conventional partial β-adrenoceptor agonists (e.g., CGP 12177) (for review, see Arch & Kaumann, 1993). More recently, β_3-adrenoceptors from human subjects (Emorine *et al.*, 1989) and rats (Granneman *et al.*, 1991) have also been cloned.

Little is known about the occurrence of β_3-adrenoceptors in the cardiovascular system. β_3-Adrenoceptor agonists and/or non-conventional agonists have been shown to have vasodilator effects in the dog *in vivo* (Tavernier *et al.*, 1992; Berlan *et al.*, 1994; Shen *et al.*, 1994). Furthermore, some non-conventional agonists exhibited cardiostimulant effects in isolated tissues of animals (e.g., cat; for review, see Kaumann, 1989; Arch & Kaumann, 1993) and of human subjects (Kaumann, 1995) at concentrations much higher than those necessary to block β_1- and/or β_2-adrenoceptors. It is not yet clear whether the receptors involved in the latter effect belong to the β_3-adrenoceptors or constitute a different class of atypical β-adrenoceptors (see Arch & Kaumann, 1993).

The present study, aimed at the identification of β_3- or other atypical β-adrenoceptors in the cardiovascular system of pithed rats, utilized the selective β_3-adrenoceptor agonists, BRL 37344, ZD 2079 and CL 316243, the non-conventional partial agonists CGP 12177 and cyanopindolol, the β_1-adrenoceptor agonist, prenalterol and the β_2-adrenoceptor agonist, fenoterol on heart rate and diastolic blood pressure. A preliminary ac-

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count of the present data was given at the XIIth congress of the Polish Pharmacological Society, Bydgoszcz, 1995 (Malinowska *et al.*, 1995).

Methods

Male Wistar rats weighing 180–320 g were anaesthetized with pentobarbitone, 300 μmol kg^{-1} i.p. and then injected i.p. with atropine, 2 μmol kg^{-1}. After cannulation of the trachea the animals were pithed and artificially ventilated with air (60 strokes min^{-1}) and bilaterally vagotomized. Heart rate was derived from the ECG recorded via subcutaneous electrodes. Diastolic blood pressure was measured from the right carotid artery via a pressor transducer (Gould P23ID). The electrodes for ECG and the pressor transducer were connected to the monitor Trendscope 8031 (SandW Vickers Ltd, Białystok, Poland). The left femoral vein was cannulated for i.v. injections of drugs administered in a volume of 0.5 ml kg^{-1}. Body temperature was kept constant at 37±1°C. Following pithing, blood pressure was routinely raised to 80–100 mmHg by infusion of vasopressin (0.04–0.4 i.u., kg^{-1} min^{-1}) into the right femoral vein (since vasopressor/vasodepressor effects are more marked at a higher level of blood pressure; see, e.g., Malinowska & Schlicker, 1993). After 15–30 min of equilibration, during which the cardiovascular parameters were allowed to stabilize, experiments were performed.

In most experiments, four increasing doses of agonists were injected to one rat with sufficient time for full recovery to the pre-injection value. In the case of prenalterol, BRL 37344, CGP 12177 and cyanopindolol, two or even three rats were used since the tachycardic response to the higher doses of these compounds declined very slowly (for details, see legends to the figures and to Table 3). In preliminary experiments we did not detect any differences in the response to the highest dose of, e.g., prenalterol or BRL 37344 given alone or after the three lower doses of the respective agonist.

Calculations and statistics

To assess the potency of the β-adrenoceptor agonists in increasing heart rate, the negative log values of the doses causing an increase in heart rate of 60 min^{-1} ($pED_{\Delta 60}$ values) were determined. The antagonistic potency of the β-adrenoceptor antagonists towards the tachycardic response to the β-adrenoceptor agonists was calculated according to the formula: apparent $pK_B = \log([E']/[E]-1) - \log[B]$, where [E'] and [E] are the $ED_{\Delta 60}$ values of the agonist with or without previous administration of the antagonist, respectively, and [B] is the dose of the antagonist. The apparent pK_B of ICI 118551 towards the effect of fenoterol on diastolic blood pressure has been determined in an analogous manner; [E'] and [E] are the doses of fenoterol causing a decrease in diastolic blood pressure by 15 mmHg with or without previous administration of ICI 118551, respectively. Results are given as means±s.e.mean throughout the paper (*n*: number of rats). For comparison of mean values the *t* test for paired or unpaired data was used. When two or more treatment groups were compared to the same control group, Bonferroni's procedure was used. The differences were considered as significant when $P<0.05$.

Drugs

The following drugs were used: isoprenaline bitartrate, atropine sulphate, prazosin hydrochloride, [Lys^8]-vasopressin (Sigma, Munich, Germany), fenoterol hydrobromide (Boehringer Ingelheim, Ingelheim, Germany), BRL 37344 (**R***, **R***)-(±)-4-(2'-[2-hydroxy-2-(3-chlorophenyl)ethy-lamino]propyl) phenoxyacetate (SmithKline Beecham, Epsom, U.K.), CGP 12177 (±)-4-(3-*t*-butylamino-2-hydroxypropoxy) benzimidazol-2-one, CGP 20712 (±)-1-[2-(3-carbamoyl-4-hydroxyphenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]-2-propanol (Ciba-Geigy, Basle, Switzerland), CL 316243 (**R, R**)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (American Cyanamid Company, Pearl River, U.S.A.), ICI 118551 erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol (ICI Pharmaceuticals, Macclesfield, U.K.), ZD 2079 (±)-1-phenyl-2-(2-(4-carboxymethylphenoxy)-ethylamino)-ethan-1-ol (Zeneca Pharmaceuticals, Cheshire, U.K.), cyanopindolol (Sandoz, Basle, Switzerland), bupranolol (Schwarz Pharma, Monheim, Germany), (±)-prenalterol hydrochloride (Hässle, Gothenburg, Sweden), ketanserin (Janssen, Beerse, Belgium), pentobarbitone (Biowet, Puławy, Poland). Drugs were dissolved in water (pentobarbitone, atropine), in saline (prenalterol, fenoterol, BRL 37344, CGP 12177, CL 316243, ICI 118551, cyanopindolol, bupranolol), in saline containing ascorbic acid 6 mol l^{-1} (isoprenaline), in a mixture of HCl 0.01 mol l^{-1} and dimethylsulphoxide (DMSO) 4:1 (prazosin), in a mixture of saline and HCl 0.01 mol l^{-1} (ketanserin) or in a mixture of saline and DMSO (16:1 CGP 20712, 27:1 ZD 2079). The solvents did not affect cardiovascular parameters.

Results

Influence of β_1- and β_2-adrenoceptor agonists on heart rate and diastolic blood pressure

The β_1-adrenoceptor agonist, prenalterol, increased heart rate (for basal value, see legend to Figure 2) in a dose-dependent manner (Figure 1a) but did not affect diastolic blood pressure (not shown). The β_1-adrenoceptor antagonist, CGP 20712 0.1 μmol kg^{-1} and the non-selective β-adrenoceptor antagonist, bupranolol 0.1 μmol kg^{-1} decreased the chronotropic effect of prenalterol (Figure 1a; for apparent pK_B values, see Table 1).

The β_2-adrenoceptor agonist, fenoterol caused a dose-dependent fall in diastolic blood pressure (Figure 1b; for basal value of diastolic blood pressure, see Table 3) and, at doses of 1 and 10 μmol kg^{-1}, increased heart rate by 14.2±3.2 and 79.0±6.0 beats min^{-1}, respectively. The β_2-adrenoceptor antagonist, ICI 118551 0.1 μmol kg^{-1} as well as bupranolol

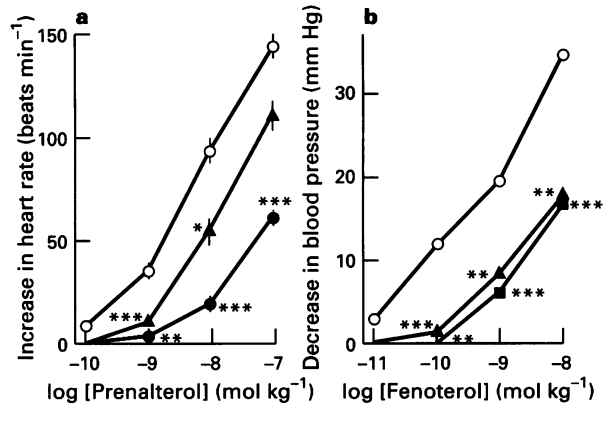

Figure 1 Increase in heart rate induced by prenalterol (a) and decrease in diastolic blood pressure elicited by fenoterol (b) in pithed and vagotomized rats. Four increasing doses of fenoterol (in all experiments) of prenalterol (only if a β-adrenoceptor antagonist was applied) were administered to one rat. If no β-adrenoceptor antagonist was injected, the three lower doses of prenalterol were given to one rat and the highest dose was injected in another animal. The first (or only) dose of agonist was applied 6 min after injection of saline (control; ○), bupranolol 0.1 μmol kg^{-1} (▲), CGP 20712 0.1 μmol kg^{-1} (●) or ICI 118551 0.1 μmol kg^{-1} (■). Means± s.e.mean of 4–9 rats. For the sake of clarity, no symbols have been used for values lower than 1 beat min^{-1} and 1 mmHg. For many points s.e.mean is contained within the symbols. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to the control group.

0.1 μmol kg^{-1} diminished the fall in blood pressure induced by the β_2-adrenoceptor agonist (Figure 1b; for apparent pK_B values, see Table 1). The chronotropic effect of fenoterol 1 and 10 μmol kg^{-1} was not affected by ICI 118551 but was attenuated by bupranolol by about 40 and 70%, respectively (data not shown). CGP 20712, ICI 118551 and bupranolol (each at 0.1 μmol kg^{-1}) did not change heart rate and blood pressure by themselves (not shown).

Influence of isoprenaline, β_3-adrenoceptor agonists and non-conventional partial agonists on heart rate

The non-selective β-adrenoceptor agonist, isoprenaline, the β_3-adrenoceptor agonists, BRL 37344, ZD 2079 and CL 316243 and the non-conventional partial agonists, CGP 12177 and cyanopindolol caused a dose-dependent increase in heart rate (Figure 2). The maximum effect could not be determined since a high percentage of the animals did not tolerate a heart rate exceeding about 470 beats min^{-1}. The rank order of potencies of the drugs based on their $pED_{\Delta 60}$ values (negative logarithms of the doses increasing heart rate by 60 beats min^{-1}) was: isoprenaline > > CGP 12177 > cyanopindolol > BRL 37344 > ZD 2079 > CL 316243 (the latter increased heart rate by less than 60 beats min^{-1}; Table 2).

The maximum positive chronotropic effect of the highest dose of the agonists was reached within 1 min for isoprenaline, BRL 37344 and ZD 2079, within 2 min for CL 316243, within 5 min for CGP 12177 and after 15 min for cyanopindolol (data not shown) and gradually subsided. The chronotropic effect induced by the highest dose of isoprenaline had disappeared about 10 min after administration of the drug. The increase in heart rate induced by the highest doses of ZD 2079, CL 316243, BRL 37344 and CGP 12177 amounted to 43, 53, 73 and 97% of the maximal value 15 min after administration of the respective compound.

CGP 20712 0.1 μmol kg^{-1} given together with ICI 118551 0.1 μmol kg^{-1} markedly attenuated the positive chronotropic effect of isoprenaline, BRL 37344, ZD 2079 and CL 316243 (Figure 3) but did not affect the positive chronotropic effect of CGP 12177 (Figure 4b) and cyanopindolol (Figure 5).

The dose-dependent increase in heart rate induced by CGP 12177 was only decreased by high doses of CGP 20712 (Figure 4a) and by a high dose of bupranolol (Figure 4b; for apparent pK_B values, see Table 1). The following compounds (doses given in μmol kg^{-1}) did not affect the positive chronotropic effect of CGP 12177: ICI 118551 (10) (Figure 4a), the α_1-adrenoceptor antagonist, prazosin (1) and the 5-HT_{2A} antagonist, ketanserin (3) as well as BRL 37344(1), ZD 2079(10) and CL 316243(10) (4–5 experiments each, results not shown). In the case of the latter three compounds, CGP 20712 (plus ICI 118551; 0.1 μmol kg^{-1} each) was infected 5 min beforehand in order to counteract their positive chronotropic effect (see above).

The dose-dependent increase in heart rate induced by cyanopindolol was decreased by CGP 20712 1 μmol kg^{-1} (Figure 4c; for the apparent pK_B value, see Table 1). As shown in Figure 5, the increase in heart rate due to cyanopindolol 1 μmol kg^{-1} was decreased by CGP 20712 10 μmol kg^{-1} and bupranolol 10 μmol kg^{-1} but not affected by ICI 118551 10 μmol kg^{-1}.

Heart rate was not affected by the combined administration of CGP 20712 plus ICI 118551 (0.1 μmol kg^{-1} each), by prazosin 1 μmol kg^{-1}or ketanserin 3 μmol kg^{-1} (not shown). CGP 20712 at a dose of 1 μmol kg^{-1} did not affect heart rate but at a dose of 10 μmol kg^{-1} decreased it by 31.7±3.2 beats min^{-1}. Six min after its injection (i.e. when the agonist under study was administered) basal heart rate was still slightly diminished by about 5%. ICI 118551 10 μmol kg^{-1} caused a short-lasting decrease in basal heart rate by 36.3±3.2 beats min^{-1} (which was reversible within 6 min). Bupranolol 10 μmol kg^{-1} elicited a prolonged decrease in heart rate (the maximal effect was 59.5±3.7 beats min^{-1}). Six minutes after its injection, basal heart rate was still diminished by about 7%.

We carried out additional experiments in order to evaluate the antagonistic potencies of CGP 12177 and cyanopindolol towards prenalterol. As shown in Figure 6, CGP 12177 0.002 μmol kg^{-1} and cyanopindolol 0.003 μmol kg^{-1} caused

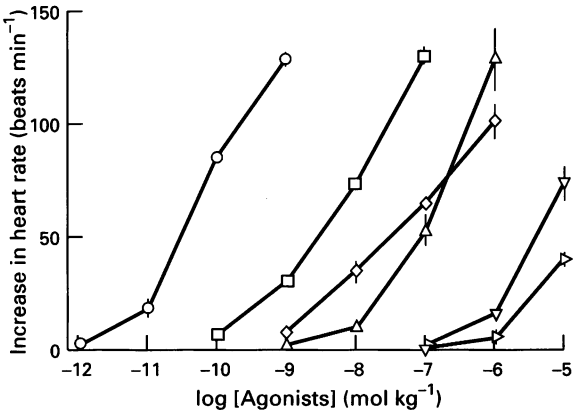

Figure 2 Effect of β-adrenoceptor agonists on heart rate in pithed and vagotomized rats. Four increasing doses of isoprenaline (○), ZD 2079 (▽) or CL 316243 (▷) were injected to one rat. In the case of CGP 12177 (□) and BRL 37344 (△), the three lower doses were administered to one rat, whereas the highest dose was studied in a separate animal. In the case of cyanopindolol (◇), the two lower doses were applied to the same rat whereas each of the higher doses was examined in separate animals. Basal heart rate was 320±5.3 beats min^{-1}. Means±s.e.mean of 4–19 rats. For many points s.e.mean is contained within the symbols.

Table 1 Antagonist potencies of CGP 20712, ICI 118551 and bupranolol towards cardiovascular effects mediated via three types of β-adrenoceptors in pithed and vagotomized rats

β-Adrenoceptor antagonist	*β_1-adrenoceptor-mediated increase in heart rate*	*Apparent pK_B towards the β_2-adrenoceptor-mediated decrease in diastolic blood pressure*	*increase in heart mediated via atypical β-adrenoceptors*
CGP 20712	8.6[1]	–	6.1[3], 5.8[4], 6.4[5]
ICI 118551	–	8.4[2]	<5[6]
Bupranolol	7.6[1]	8.3[2]	5.3[7]

[1]Determined from Figure 1a; [2]From Figure 1b; [3]From Figure 4a; agonist CGP 12177, antagonist CGP 20712 1 μmol kg^{-1}; [4]From Figure 4a; agonist CGP 12177, antagonist CGP 20712 10 μmol kg^{-1}; [5]From Figure 4c; agonist cyanopindolol, antagonist CGP 20712 1 μmol kg^{-1}; [6]From Figure 4a and [7]From Figure 4b.

rightward shifts of the dose-response curve for prenalterol (for the apparent pK_B values, see Table 2). The difference between agonist and antagonist potency was about 1.3 log units for CGP 12177 and about 2.3 log units for cyanopindolol (Table 2). CGP 12177 and cyanopindolol by themselves increased heart rate 11 min after injection (i.e., when prenalterol was administered) by 42.0±6.9 and 22.4±4.7 beats min^{-1}, respectively.

Influence of isoprenaline, β_3-adrenoceptor agonists and non-conventional partial agonists on diastolic blood pressure

Isoprenaline and BRL 37344 elicited a dose-dependent vasodepressor response, which was markedly attenuated by the β_1/β_2 blockade (Table 3). CGP 12177 0.01 μmol kg^{-1} caused a short-lasting (<1 min) hypertensive response by 6.3± 0.8 mmHg. CGP 12177 0.1 μmol kg^{-1} elicited a biphasic change in blood pressure. An initial short-lasting (<1 min) increase (by 8.4±0.8 mmHg) was followed by a decrease (by 7.4±1.2 mmHg, 5 min after injection of the agonist). The increase in blood pressure was not affected by the following drugs (doses in μmol kg^{-1}): CGP 20712 (0.1) plus ICI 118551 (0.1), CGP 20712 (1 and 10), ICI 118551 (10), bupranolol (10), BRL 37344 (1), ZD 2079 (10), CL 316243 (10), prazosin (1) and ketanserin (3) (data not shown). The decrease in blood pressure was markedly diminished by CGP 20712 0.1 μmol kg^{-1} plus ICI 118551 0.1 μmol kg^{-1}, ICI 118551 or bupranolol (each 10 μmol kg^{-1}). Cyanopindolol 1 μmol kg^{-1} induced a prolonged increase in blood pressure by 10.8±1.2 mmHg (10 min after its administration). This vasopressor effect was not changed by CGP 20712, ICI 118551 or bupranolol (not shown). ZD 2079, CL 316243 and the lowest doses of CGP 12177 and cyanopindolol did not change blood pressure (not shown).

The antagonists did not affect diastolic blood pressure by themselves, with two exceptions. ICI 118551 10 μmol kg^{-1} elicited a biphasic blood pressure response. An initial short-lasting (<2 min) decrease (by 45.6±1.7 mmHg) was followed by an increase (by 8.4±1.2 mmHg, 6 min after its injection). Bupranolol 10 μmol kg^{-1} produced a short-lived (<2 min) fall in blood pressure (by 33.4±1.2 mmHg).

Table 2 Agonistic and antagonistic potencies for different β-adrenoceptor agents in pithed and vagotomized rats

Drug	*$pED_{\Delta 60}$*[1]	*Apparent pK_B*[2]
Isoprenaline	10.4	ND[3]
CGP 12177	8.3	9.6
Cyanopindolol	7.2	9.5
BRL 37344	6.9	ND
ZD 2079	5.2	ND
CL 316243	<5	ND

[1]Determined from Figure 2; [2]From Figure 6; [3]ND, not determined.

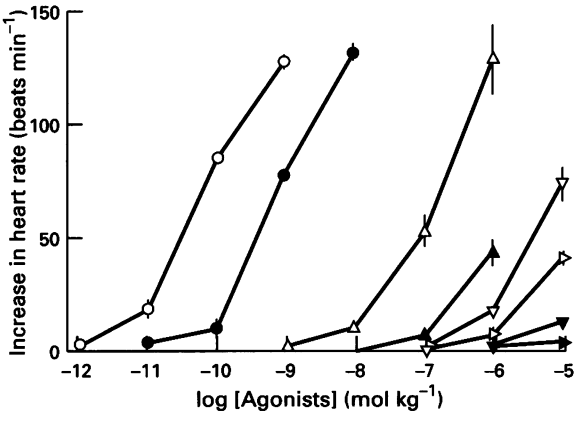

Figure 3 Effect of β-adrenoceptor agonists on heart rate in pithed and vagotomized rats and their interaction with a β_1- plus β_2-adrenoceptor antagonist. Four increasing doses of isoprenaline (○, ●), ZD 2079 (▼, ▽) or CL 316243 (▶, ▷) were injected into one rat. In the case of BRL 37344, four increasing doses were administered to the same animal if the β-adrenoceptor antagonists were injected (▲). If the β-adrenoceptor antagonists were not applied (△), the three lower doses of BRL 37344 were administered to one rat and the highest dose was studied in a separate animal. The first (or only) dose of agonist was given 6 min after injection of saline (control; open symbols) or CGP 20712 0.1 μmol kg^{-1} together with ICI 118551 0.1 μmol kg^{-1} (closed symbols). Means±s.e.mean of 4–19 rats. For many points s.e.mean is contained within the symbols. For the sake of clarity, no symbols have been used for values lower than 1 beat min^{-1}. The increase in heart rate in rats injected with the β-adrenoceptor antagonists was significantly different compared to the respective control; $P<0.001$.

Discussion

General remarks

The present study was aimed at the identification of β_3- and/or atypical β-adrenoceptors in the rat cardiovascular system *in situ*. The pithed rat preparation offers the opportunity to study effects of compounds on the cardiovascular system without interference with reflex loops; this is important since the β_3-adrenoceptor-mediated increase in heart rate in the anaesthetized dog is not a direct effect but occurs in response to the β_3-adrenoceptor-mediated decrease in blood pressure (Tavernier *et al.*, 1992; Berlan *et al.*, 1994). We used the unselective β-adrenoceptor agonist, isoprenaline, the selective β_3-adrenoceptor agonists, BRL 37344, ZD 2079 and CL 316243 and the 'non-conventional' partial β-adrenoceptor agonist, CGP 12177. This term, coined by Kaumann (1989) (and further explained below), was extended to cyanopindolol, which was examined in our study as well. Bupranolol was used since it acts as an antagonist both at β_3-adrenoceptors (Arch & Kaumann, 1993; Blin *et al.*, 1993) and atypical β-adrenoceptors (Kaumann, 1989; Arch & Kaumann, 1993), although at concentrations much higher than those necessary for the blockade of β_1- or β_2-adrenoceptors. We also used the β_1-adrenoceptor antagonist, CGP 20712 and the β_2-adrenoceptor antagonist, ICI 118551 in order to detect effects by the aforementioned agonists related to the activation of β_1- or β_2-adrenoceptors. CGP 20712 and ICI 118551 were frequently used in combination since it was not the primary aim of the study to differentiate between β_1- and β_2-adrenoceptor-mediated effects.

We are aware of the fact that the inability to construct whole dose-response curves somewhat impairs the reliability of the interpretation of responses. The $pED_{\Delta 60}$ values only represent rough estimates of the potencies of the β-adrenoceptor agonists with respect to their tachycardic effect. In particular, the use of $pED_{\Delta 60}$ values neglects the fact that the maximum effect obtainable (1) via β_1- versus atypical β-adrenoceptors and (2) by different agonists acting on the same β-adrenoceptor (e.g., CGP 12177 versus cyanopindolol) may be different.

Effects of β_3-adrenoceptor agonists and non-conventional partial agonists on heart rate

Isoprenaline, the β_3-adrenoceptor agonists and the non-conventional partial agonists increased heart rate. The tachycardic effect of isoprenaline and the β_3-adrenoceptor agonists was markedly attenuated by combined administration of CGP 20712 and ICI 118551 (used at doses markedly antagonizing the effect of a β_1- and β_2-adrenoceptor agonist, respectively). On the other hand, the tachycardia elicited by CGP 12177 and cyanopindolol was not affected. Is this latter effect mediated via β_3-adrenoceptors?

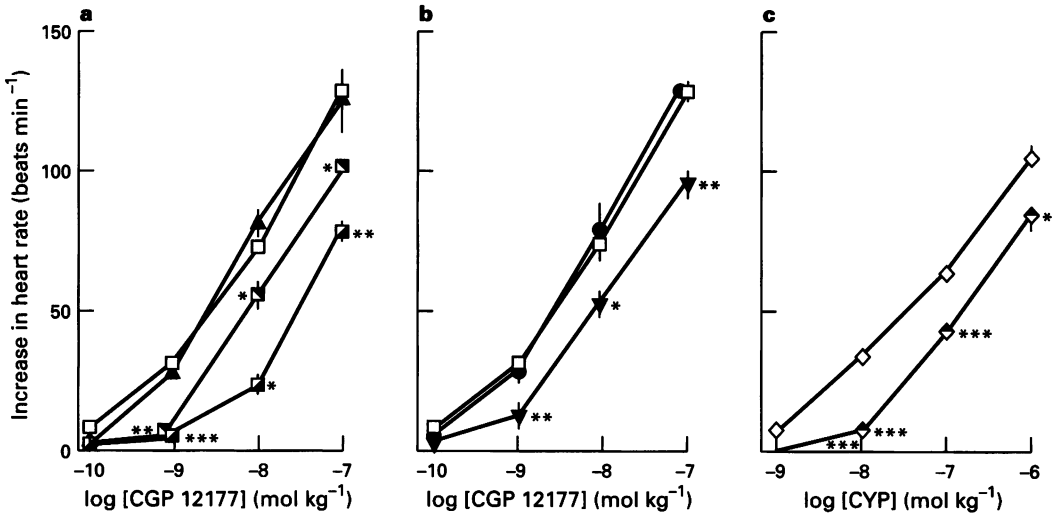

Figure 4 Effects of β-adrenoceptor antagonists on the positive chronotropic effects of CGP 12177 (a, b) and cyanopindolol (CYP; c) in pithed and vagotomized rats. The three lower doses of CGP 12177 were administered to one rat and the highest dose was studied in a separate animal. In the case of CYP, the two lower doses were applied to the same rat whereas each of the higher doses was examined in separate animals. The first or only dose of agonist was given 6 min after injection of saline (control; □, ◇), CGP 20712 1 (◹ ◆) or 10 μmol kg^{-1}(◪), ICI 118551 10 μmol kg^{-1} (▲), CGP 20712 0.1 μmol kg^{-1} given together with ICI 118551 0.1 μmol kg^{-1} (●) or bupranolol 10 μmol kg^{-1} (▼). Means ± s.e.mean of 4–19 rats. For many points, s.e.mean is contained within the symbols. For the sake of clarity, no symbol has been used for a value lower than 1 beat min^{-1}. *P<0.05, **P<0.01, ***P<0.001 compared to the respective controls.

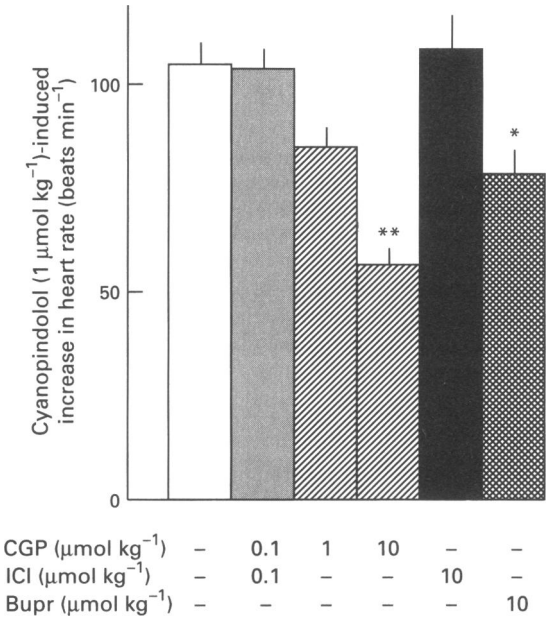

Figure 5 Effects of β-adrenoceptor antagonists on the positive chronotropic effect of cyanopindolol in pithed and vagotomized rats. Cyanopindolol 1 μmol kg^{-1} was given 6 min after injection of saline (control), CGP 20712 (CGP), ICI 118551 (ICI) or bupranolol (Bupr), at the doses indicated. Means ± s.e.mean of 4–11 rats. *P<0.05, **P<0.01 compared to the control group.

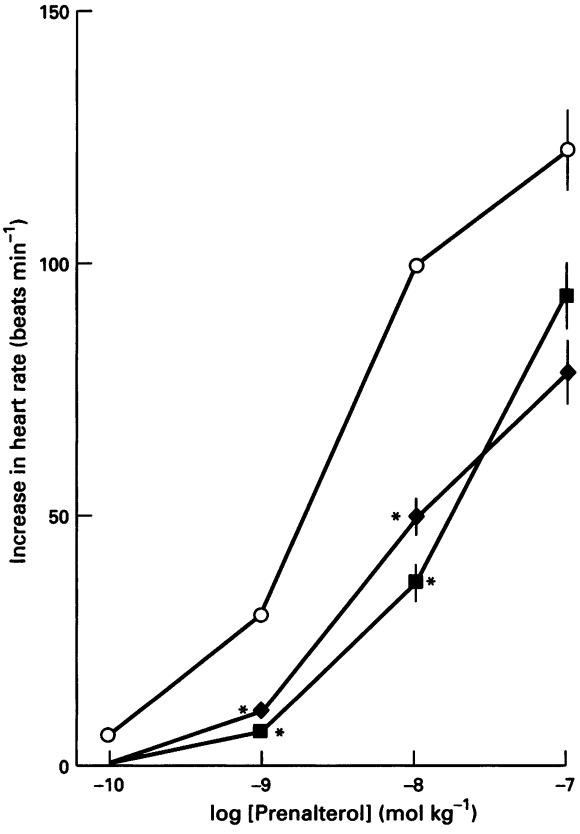

Figure 6 Interaction of CGP 12177 or cyanopindolol with the prenalterol-induced increase in heart rate in pithed and vagotomized rats. The three lower doses of prenalterol were given to one rat and the highest dose was studied in a separate animal. The first (or only) dose of prenalterol was applied 11 min after injection of saline (○; control), CGP 12177 0.002 μmol kg^{-1} (◆) or cyanopindolol 0.003 μmol kg^{-1} (■). Means ± s.e.mean of 4–9 rats. For many points s.e.mean is contained within the symbols. For the sake of clarity, no symbols have been used for values lower than 1 beat min^{-1}. *P<0.001 compared to the respective control.

According to Arch & Kaumann (1993), a β_3-adrenoceptor-mediated effect should be (i) mimicked by non-conventional partial agonists, (ii) antagonized by high concentrations of β-adrenoceptor antagonists and (iii) mimicked by selective β_3-adrenoceptor agonists. Both the first and the second criteria are fulfilled. Thus, the tachycardia in response to CGP 12177 and cyanopindolol was attenuated by high doses of bupranolol and CGP 20712. The possibility that the antagonistic effect of bupranolol and CGP 20712, 10 μmol kg^{-1} each, is related to

Table 3 Influence of isoprenaline and BRL 37344 on basal diastolic blood pressure in pithed and vagotomized rats

Antagonists (μmol kg^{-1})	*Basal blood pressure*[1] (mmHg)	*Agonist-induced decrease in blood pressure* (mmHg) *Isoprenaline (nmol kg^{-1})* 0.01	0.1	1	*BRL 37344 (nmol kg^{-1})* 10	100	1000
–	88.7±2.9	8.1±0.6	21.4±2.9	39.9±1.9	15.3±1.1	27.4±2.5	40.4±3.2
CGP 20712 0.1 + ICI 118551 0.1	87.0±2.1	1.3±0.5**	3.5±0.3*	18.8±0.5**	2.7±0.7**	8.7±1.5*	13.0±2.7**

Four increasing doses of isoprenaline (in all experiments) and BRL 37344 (in rats injected with β-adrenoceptor antagonists) were administered to one rat. In rats not treated with β-adrenoceptor antagonists, the three lower doses of BRL 37344 were applied to one rat and the highest dose was studied in a separate animal. The first (or only) dose of agonist was given 6 min after injection of saline (control) or CGP 20712 0.1 μmol kg^{-1} together with ICI 118551 0.1 μmol kg^{-1}. The lowest doses of isoprenaline (0.001 nmol kg^{-1}) and BRL 37344 (1 nmol kg^{-1}) did not change blood pressure (not shown). Means±s.e.mean of 4–9 rats. *$P<0.01$, **$P<0.001$, compared to the control group.

[1] Immediately before administration of the agonist under study.

their negative chronotropic effects is not very likely. Thus, CGP 20712 1 μmol kg^{-1} (which did not affect basal heart rate) had similar pK_B values to CGP 20712 10 μmol kg^{-1} (Table 1).

The third criterion is not fulfilled. Thus, tachycardia in response to the three β_3-adrenoceptor agonists cannot involve β_3-adrenoceptors since it was markedly reduced by combined β_1/β_2 blockade and occurred at very high doses only. In the pithed rat, the BRL 37344-induced and β_3-adrenoceptor-mediated increase in oxygen consumption and the elevation of brown adipose tissue temperature reach their maximum at 0.01 μmol kg^{-1} (Oriowo *et al.*, 1994). The same dose of BRL 37344 caused a tachycardic response in pithed rats which is only slightly higher than the threshold for this effect (Oriowo *et al.*, 1994; present study). The possibility that, in the present model, cardiac β_3-adrenoceptors occur, which are activated by CGP 12177 and cyanopindolol but *blocked* by BRL 37344, ZD 2079 and CL 316243 is very unlikely since high doses of the three β_3-adrenoceptor agonists did not affect the tachycardic response to CGP 12177. Apart from β_3-adrenoceptors, α_1-adrenoceptors and 5-HT$_{2A}$ receptors are also probably not involved in the tachycardic response to CGP 12177 and cyanopindolol (lack of effect of the respective antagonists, prazosin and ketanserin).

Do CGP 12177 and cyanopindolol act via the atypical β-adrenoceptor, identified mainly by Kaumann and coworkers in the heart (for review, see Kaumann, 1989; Arch & Kaumann, 1993)? These receptors resemble the β_3-adrenoceptors in that they are activated by non-conventional partial agonists and blocked only by high concentrations of β-adrenoceptor antagonists. The non-conventional partial agonists possess a 10 to 1000 fold lower affinity as agonists at atypical β-adrenoceptors than as antagonists at β_1- or β_2-adrenoceptors (Kaumann, 1989; Arch & Kaumann, 1993). We have determined the antagonistic potency of CGP 12177 and cyanopindolol against prenalterol for its β_1-adrenoceptor-mediated effect. CGP 12177 and cyanopindolol were 18 and 200 times more potent, respectively, as antagonists at the β_1-adrenoceptor than as agonists at the atypical β-adrenoceptor. These ratios even represent an underestimation. Thus, the pED$_{A60}$ values (as estimates of the agonist potency at the atypical β-adrenoceptor) are higher than the −log values of the dose *exactly* causing the half-maximum effect (which, due to problems inherent in the experimental model, could not be determined). One might argue that, in our model, CGP 12177 and cyanopindolol caused an increase in heart rate which had not returned to the baseline level within 11 min (i.e. until prenalterol was injected). However, the dose-response curve of prenalterol was identical in rats which had relatively low or relatively high basal levels of heart rate.

Thus, our data are compatible with the view that the tachycardia elicited by CGP 12177 and cyanopindolol is mediated via the atypical β-adrenoceptor found by Kaumann and coworkers *in vitro*. The atypical β- and the β_3-adrenoceptor are both very insensitive to blockade by β-adrenoceptor antagonists; however, the rank orders of potencies of three β-adrenoceptor antagonists at the two receptors markedly differ. Thus, at the human β_3-adrenoceptor, expressed in COS cells, the following rank order of affinities was found: bupranolol > ICI 118551 > CGP 20712 (Blin *et al.*, 1993). For the atypical β-adrenoceptor in the present study, the rank order of antagonistic potencies was: CGP 20712 > bupranolol > ICI 118551 (no antagonistic effect at 10 μmol kg^{-1}).

Effects of β_3-adrenoceptor agonists and non-conventional partial agonists on diastolic blood pressure

The effects of isoprenaline, the selective β_3-adrenoceptor agonists and the non-conventional partial agonists on diastolic blood pressure were, unlike those on heart rate, very heterogeneous. The *vasodepressor* response to isoprenaline, BRL 37344 and CGP 12177, which was markedly attenuated by the combined administration of ICI 118551 and CGP 20712, is most probably related to the activation of β_2-adrenoceptors. For the *vasopressor* response to high doses of the two non-conventional partial agonists the mechanism is unclear although the involvement of β_1- and β_2-adrenoceptors and, in the case of CGP 12177, also of β_3, α_1 and 5-HT$_{2A}$ receptors can be excluded. Our data show that in the pithed rat, unlike in the dog *in vivo* (Tavernier *et al.*, 1992; Berlan *et al.*, 1994), a β_3-adrenoceptor-mediated fall in blood pressure cannot be demonstrated.

Conclusions

The atypical β-adrenoceptor resembles that previously described by Kaumann and coworkers in the heart *in vitro*, inasmuch as it is activated by CGP 12177. In addition, the present study shows that cyanopindolol most probably acts via the same receptor, although at a lower potency and probably also at a lower intrinsic activity. This receptor is blocked by a very high dose of bupranolol, as in previous studies of Kaumann and coworkers (Walter *et al.*, 1984; Kaumann, 1995). In addition, we found that CGP 20712 is an antagonist at this receptor although at a dose much higher than that necessary for the blockade of β_1-adrenoceptors. The extent of the positive chronotropic effect of CGP 12177 and cyanopindolol is very pronounced, suggesting that the receptor mediating this effect may be more than a pharmacological peculiarity and may possess a role, at least under certain conditions.

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References

ARCH, J.R.S. & KAUMANN, A.J. (1993). β_3 and atypical β-adrenoceptors. *Med. Res. Rev.*, **13,** 663–729.

BERLAN, M., GALITZKY, J., BOUSQUET-MELOU, A., LAFONTAN, M. & MONTASTRUC, J.L. (1994). Beta-3 adrenoceptor-mediated increase in cutaneous blood flow in the dog. *J. Pharmacol. Exp. Ther.*, **268,** 1444–1451.

BLIN, N., CAMOIN, L., MAIGRET, B & STROSBERG, A.D. (1993). Structural and conformational features determining selective signal transduction in the β_3-adrenergic receptor. *Mol. Pharmacol.*, **44,** 1094–1104.

EMORINE, L.J., MARULLO, S., BRIEND-SUTREN, M.M., PATEY, G., TATE, K., DELAVIER-KLUTCHKO, C. & STROSBERG, A.D. (1989). Molecular characterization of the human β_3-adrenergic receptor. *Science*, **245,** 1118–1121.

GRANNEMAN, J.G., LAHNERS, K.N. & CHAUDHRY, A. (1991). Molecular cloning and expression of the rat β_3-adrenergic receptor. *Mol. Pharmacol.*, **40,** 895–899.

KAUMANN, A.J. (1989). Is there a third heart β-adrenoceptor? *Trends Pharmacol. Sci.*, **10,** 316–320.

KAUMANN, A.J. (1995). (−)-CGP 12177 increases human atrial contraction through a putative third β-adrenoceptor. *Br. J. Pharmacol.*, (in press).

MALINOWSKA, B., BUCZKO, W. & SCHLICKER, E. (1995). Atypical β-adrenoceptors, different from β_3-adrenoceptors, mediate the positive chronotropic effect of CGP 12177 and cyanopindolol in the pithed rat. *Pol. J. Pharmacol.*, **47, (Suppl.)** 38–39.

MALINOWSKA, B. & SCHLICKER, E. (1993). Identification of endothelial H_1, vascular H_2 and cardiac presynaptic H_3 receptors in the pithed rat. *Naunyn-Schmied. Arch. Pharmacol.*, **347,** 55–60.

ORIOWO, M.A., SENNITT, M.V., SMITH, S.A., RUFFOLO, R.R. & CAWTHORNE, M.A. (1994). The β-adrenoceptor selectivity profile of BRL 37344 in the pithed rat. *J. Auton. Pharmacol.*, **14,** 337–344.

SHEN, Y.T., ZHANG, H. & VATNER, S.F. (1994). Peripheral vascular effects of beta-3 adrenergic receptor stimulation in conscious dogs. *J. Pharmacol. Exp. Ther.*, **268,** 466–473.

TAVERNIER, G., GALITZKY, J., BOUSQUET-MELOU, A., MONTASTRUC, J.L. & BERLAN, M. (1992). The positive chronotropic effect induced by BRL 37344 and CGP 12177, two beta-3 adrenergic agonists, does not involve cardiac beta adrenoceptors but baroreflex mechanisms. *J. Pharmacol. Exp. Ther.*, **263,** 1083–1090.

WALTER, M., LEMOINE, H. & KAUMANN, A.J. (1984). Stimulant and blocking effects of optical isomers of pindolol on the sinoatrial node and trachea of guinea pig. Role of β-adrenoceptor subtypes in the dissociation between blockade and stimulation. *Naunyn-Schmied. Arch. Pharmacol.*, **327,** 159–175.

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