British Journal of Pharmacology (1997) 122, 1307–1314

Further evidence for differences between cardiac atypical β-adrenoceptors and brown adipose tissue β_3-adrenoceptors in the pithed rat

Barbara Malinowska & *,[1]Eberhard Schlicker

[1]Zakład Farmakodynamiki, Akademia Medyczna, Mickiewicza 2C, 15-230 Białystok 8, Poland and *Institut für Pharmakologie und Toxikologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Reuterstr. 2b, D-531.13 Bonn, Germany

1 We have previously shown (Malinowska & Schlicker, 1996) that the atypical β-adrenoceptor involved in the positive chronotropic effect of the so-called non-conventional partial β-adrenoceptor agonists CGP 12177 and cyanopindolol in the pithed rat possesses properties markedly different from those observed for β_3-adrenoceptors in the literature. In the present study, we have directly compared the pharmacological properties of the atypical cardiostimulant β-adrenoceptor and of the β_3-adrenoceptor mediating the thermogenic response in the brown adipose tissue in pithed and vagotomized rats.

2 Heart rate was dose-dependently increased by CGP 12177 and cyanopindolol by maximally 150 and 100 beats min^{-1}, yielding pED_{50} values of 8.0 and 7.3, respectively (pED_{50}, $-\log_{10}$ of the dose in mol kg^{-1} body weight i.v. causing the half-maximum effect), but not affected by the selective β_3-adrenoceptor agonist CL 316243 ($pED_{50} > 6.0$).

3 CGP 12177, cyanopindolol and CL 316243 increased temperature in the brown adipose tissue by maximally 1°C (pED_{50} values 7.4, 6.3 and 8.6, respectively).

4 The β_1-adrenoceptor antagonist CGP 20712 10 μmol kg^{-1}, attenuated the cardiostimulatory effect of CGP 12177 and, at a still higher dose (30 μmol kg^{-1}), also antagonized its thermogenic effect. The $-\log_{10}$ values of the doses causing a two fold shift of the dose-response curves (DRCs) of CGP 12177 to the right were 6.1 and 5.2, respectively, and were much lower than the corresponding value for the antagonism of CGP 20712 against the β_1-adrenoceptor-mediated positive chronotropic effect which was 8.6.

5 The cardiostimulant and the thermogenic effect of CGP 12177 were not affected by the β_2-adrenoceptor antagonist ICI 118551 10 μmol kg^{-1}.

6 The β_3-adrenoceptor antagonist SR 59230A (which, by itself, caused a β_1-adrenoceptor-mediated increase in heart rate and, for this reason, was studied after administration of a low dose of CGP 20712) attenuated the cardiostimulant and the thermogenic effect of CGP 12177 to a similar extent. The $-\log_{10}$ values of the doses causing two fold rightward shifts of the DRCs of CGP 12177 were 5.9 and 5.7, respectively.

7 The non-selective β-adrenoceptor antagonist bupranolol diminished the cardiostimulant and thermogenic response to a very similar extent. The $-\log_{10}$ values causing two fold rightward shifts of the DRCs of CGP 12177 were 5.6 and 5.7, respectively, and were much lower than the corresponding values for the antagonism of bupranolol against the β_1-adrenoceptor-mediated positive chronotropic effect and the β_2-adrenoceptor-mediated decrease in diastolic blood pressure which were 7.6 and 8.3, respectively.

8 The rank order of agonistic potencies for the cardiostimulant effect (CGP 12177 > cyanopindolol > CL 316243) differs from that for the thermogenic response in the brown adipose tissue (CL 316243 > CGP 12177 > cyanopindolol); furthermore, there is a difference with respect to the rank orders of antagonistic potencies for cardiostimulation (CGP 20712 $\geqslant$ SR 59230A $\geqslant$ bupranolol > ICI 118551) and thermogenesis (SR 59230A = bupranolol > CGP20712 > ICI 118551).

9 In conclusion, our study provides further evidence that the atypical cardiostimulant β-adrenoceptors (causing an increase in heart rate) and β_3-adrenoceptors are pharmacologically different.

Keywords: Pithed rat; positive chronotropic effect; brown adipose tissue thermogenesis; atypical β-adrenoceptors; β_3-adrenoceptors; CGP 12177; CL 316243; CGP 20712; SR 59230A; bupranolol

Introduction

Two cardiac atypical β-adrenoceptors (i.e. β-adrenoceptors different from β_1- and β_2-adrenoceptors) have been identified recently. One of these receptors is cardioinhibitory, i.e. causes a negative inotropic effect, and was found in human ventricular biopsies (Gauthier *et al*., 1996). The second atypical β-adrenoceptor causes a positive inotropic and chronotropic effect; this cardiostimulant β-adrenoceptor has been identified *in vitro* in cardiac preparations of rats, guinea-pigs, cats (for review, see Kaumann, 1989; 1997) and man (Kaumann, 1996) and *in situ* in the pithed rat (Malinowska & Schlicker, 1996).

With respect to the pharmacological properties of these two atypical β-adrenoceptors, the situation is very clear for the cardioinhibitory β-adrenoceptor. This receptor fulfills the criteria for a β_3-adrenoceptor as suggested by Arch & Kaumann (1993): The negative inotropic effect is (i) blocked only by a high concentration of a β-adrenoceptor antagonist (bupranolol), (ii) mimicked by selective β_3-adrenoceptor agonists (BRL 37344, CL 316243 and SR 58611) and (iii) mimicked by CGP 12177 (belonging to the socalled non-conventional partial

[1] Author for correspondence.

β-adrenoceptor agonists (Kaumann, 1989), i.e. drugs which block β_1- and/or β_2-adrenoceptors at concentrations much lower than those at which they activate cardiostimulant atypical β-adrenoceptors) (Gauthier *et al.*, 1996). On the other hand, the situation is more complex with the atypical cardiostimulant β-adrenoceptor. Although the effect is mimicked by non-conventional partial β-adrenoceptor agonists and blocked by high concentrations of bupranolol, it is not mimicked by selective β_3-adrenoceptor agonists; the selective β_3-adrenoceptor agonists also fail to block the cardiostimulant effect caused by CGP 12177 (Kaumann & Molenaar, 1996; Malinowska & Schlicker, 1996). Hence, the conclusion was reached that the atypical cardiostimulant β-adrenoceptor is different from β_3-adrenoceptors (Kaumann & Molenaar, 1996; Malinowska & Schlicker, 1996).

Our present study was aimed to find further evidence for differences between atypical cardiostimulant β-adrenoceptors and β_3-adrenoceptors. In our previous study (Malinowska & Schlicker, 1996), we had examined the properties of the atypical cardiostimulant β-adrenoceptor in pithed and vagotomized rats by studying the increase in heart rate caused by the non-conventional partial β-adrenoceptor agonists CGP 12177 and cyanopindolol. In the present study, we have simultaneously recorded a β_3-adrenoceptor-mediated effect as well. For this purpose, we have chosen thermogenesis in the brown adipose tissue (BAT), an effect studied previously in pithed (Oriowo *et al.*, 1994) and anaesthetized rats (Manara *et al.*, 1996).

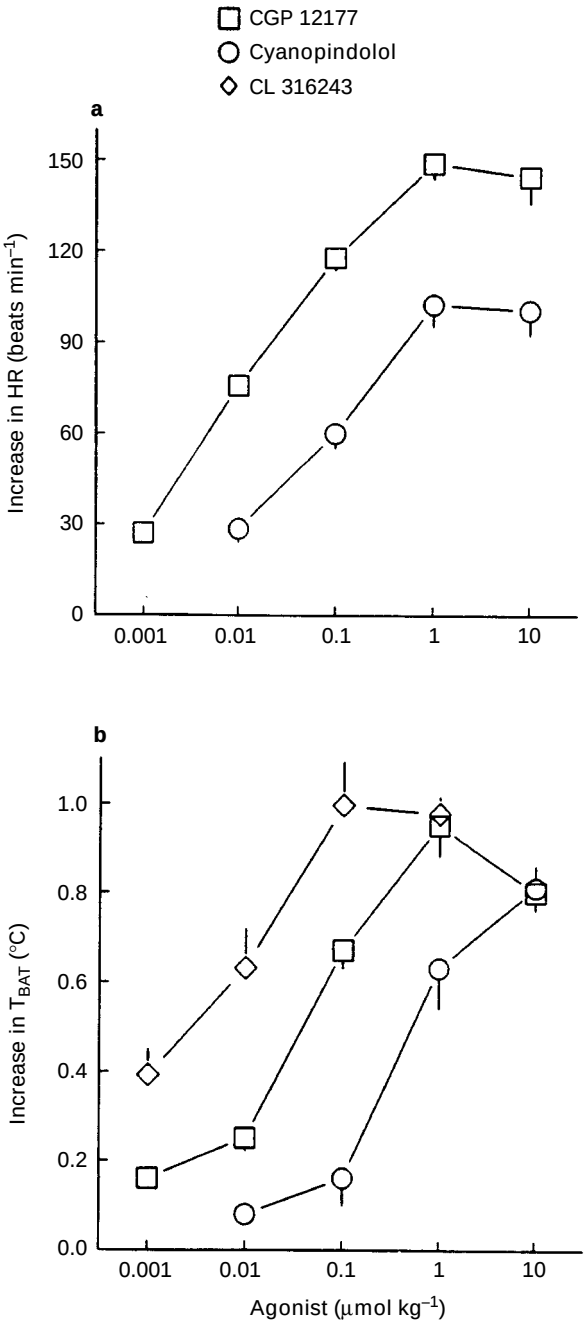

Figure 1 Effects of three β-adrenoceptor ligands on heart rate (HR; a) and brown adipose tissue temperature (T_{BAT}; b) in pithed and vagotomized rats. Each dose of CL 316243 and cyanopindolol was examined in a separate animal. In the case of CGP 12177, the two lower doses were applied to one rat, whereas each of the higher doses was studied in separate animals. The first or only dose was given 5 min after injection of saline. CL 316243 failed to affect heart rate. Means of 3–11 rats; vertical lines show s.e.mean. For many points s.e.mean is contained within the symbols.

Methods

Male Wistar rats weighing 130–190 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 artifically ventilated with air (60 strokes min^{-1}) by use of a respiratory system (Medipan, Warsaw, Poland). Both vagal nerves were cut. Heart rate was derived from the ECG recorded via subcutaneous electrodes. Diastolic blood pressure was measured from the right carotid artery via a pressure transducer (Gould P23ID). The electrodes for the ECG and the pressure transducer were connected to the monitor Trendscope 8031 (S&W 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} (1.2 ml kg^{-1} in the case of SR 59230A). Following pithing, diastolic 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.

Rats were placed ventrally on a thermostatically controlled heating table. Body temperature was kept constant at approximately 36°C and monitored by a rectal probe transducer connected to the monitor Trendscope 8031. A small incision was made in the intrascapular region. A 'miniature' thermistor was placed under the intrascapular brown pad to monitor its temperature (T_{BAT}; the studied temperature) and a similar rectal probe for rats was inserted into the rectum (monitor of T_{CORE}, the reference temperature). Both thermistors were

Table 1 Agonistic potencies of three β-adrenoceptor ligands in pithed and vagotomized rats

	pED_{50}	
Drug	*Increase in heart rate*[1]	*Brown adipose tissue thermogenesis*[2]
CL 316243	<6	8.6[3]
CGP 12177	8.0[3]	7.4[3]
	8.1[4]	7.3[4]
Cyanopindolol	7.3[3]	6.3[3]

[1]As pED_{50} that dose was determined graphically from Figure 1 or 7 which caused an increase in heart rate (beats min^{-1}) by 75 (CGP 12177) and 50 (cyanopindolol). [2]As pED_{50} that dose was determined graphically from Figure 1 or 7 which caused an increase in temperature in brown adipose tissue by 0.5°C. In the case of cyanopindolol (for which a complete dose-response curve could not be constructed) we assumed that the same maximum would be reached as in the case of CL 316243 or CGP 12177. [3]Determined from Figure 1. [4]Determined from Figure 7 in the presence of CGP 20712 0.1 μmol kg^{-1} and ethylene glycol.

connected to the multipurpose thermometer BAT-10 (Physitemp Instruments Inc., New Jersey, U.S.A.). After 20–30 min of equilibration, during which both the cardiovascular and temperature parameters were allowed to stabilize, experiments were performed.

Experimental protocol

Each dose of β-adrenoceptor agonist was studied in a separate animal (only in the case of CGP 12177, the two lower doses were administered to one rat). The first or only dose of β-adrenoceptor agonist was given 5 min after injection of saline

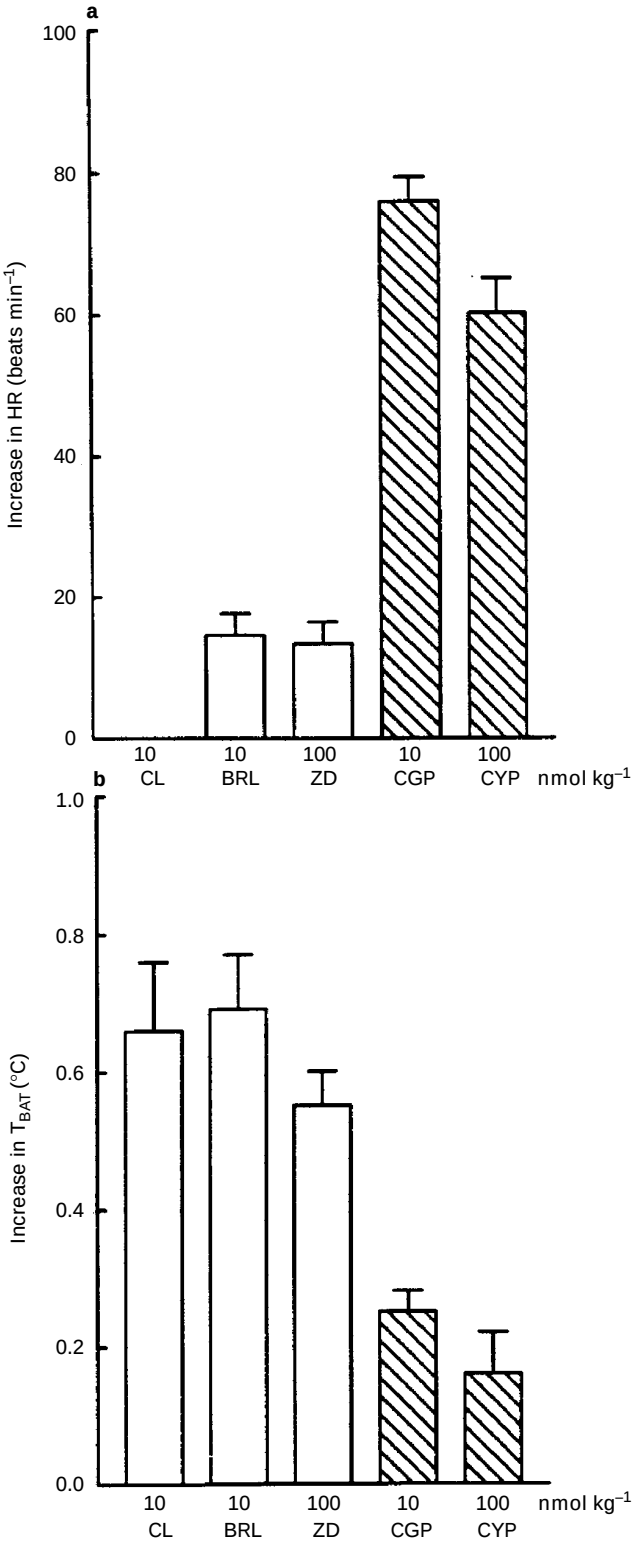

Figure 2 Effects of the three β_3-adrenoceptor agonists CL 316243 (CL), BRL 37344 (BRL) and ZD 2079 (ZD) and of the two non-conventional partial β-adrenoceptor agonists CGP 12177 (CGP) and cyanopindolol (CYP) on heart rate (HR; a) and brown adipose tissue temperature (T_{BAT}; b) in pithed and vagotomized rats. The β-adrenoceptor ligand under study was applied 5 min after saline. Means ± s.e.mean of 5–10 rats.

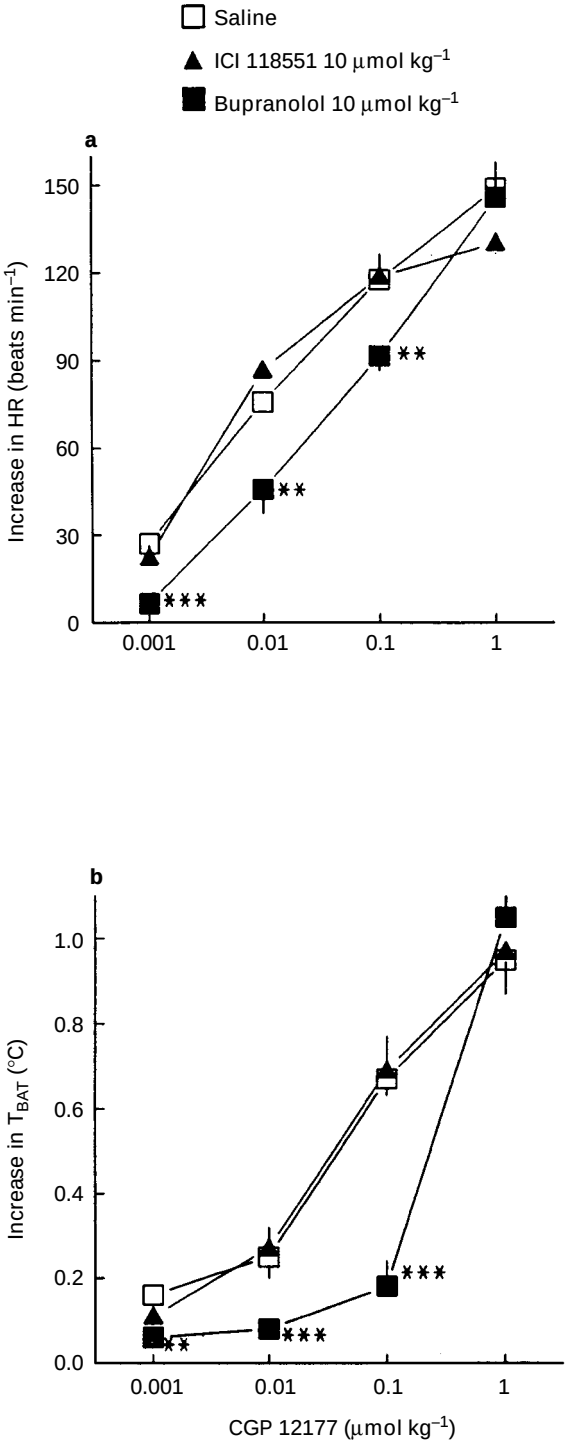

Figure 3 Effects of CGP 12177 on heart rate (HR; a) and brown adipose tissue temperature (T_{BAT}; b) in pithed and vagotomized rats and its interaction with the β_2-adrenoceptor antagonist ICI 118551 and the non-selective β-adrenoceptor antagonist bupranolol. The two lower doses of CGP 12177 were applied to one rat, whereas each of the higher doses was studied in separate animals. The first or only dose was given 5 min after injection of saline (control), ICI 118551 10 μmol kg^{-1} or bupranolol 10 μmol kg^{-1}. Means of 4–11 rats; vertical lines show s.e.mean. For many points s.e.mean is contained within the symbols. $^{**}P<0.01$, $^{***}P<0.001$ compared to the corresponding value without bupranolol.

(control) or one of the β-adrenoceptor antagonists. In some experiments, β-adrenoceptor antagonists (SR 59230A and bupranolol or their vehicles ethylene glycol and saline, respectively) were administered immediately after injection of CGP 20712 0.1 μmol kg^{-1} and 5 min before the first dose of CGP 12177. The β-adrenoceptor agonist-induced changes in the temperature of the brown adipose tissue (BAT), heart rate and blood pressure were recorded simultaneously in the same rat.

Calculations and statistics

To determine the potency (pED_{50}) of the non-conventional partial β-adrenoceptor agonists in increasing heart rate, the negative $\log_{10}$ values of the doses (in mol kg^{-1} body weight, i.v.) causing an increase in heart rate of 75 beats min^{-1} (CGP 12177) or 50 beats min^{-1} (cyanopindolol) were calculated. To determine the potency of CGP 12177, cyanopindolol and CL 316243 for their thermogenic effect, the $-\log_{10}$ values of the doses causing an increase in BAT temperature of 0.5°C were calculated. The antagonistic potency of the β-adrenoceptor antagonists towards the tachycardic and thermogenic responses to the β-adrenoceptor agonists was calculated according to the formula: $\log_{10}$ ([E′]/[E] - 1) $-$ $\log_{10}$ [B], where [E′] and [E] are the ED_{50} values of the agonist with or without previous administration of the antagonist, respectively, and [B] is the dose of the antagonist. Results are given as means $\pm$ s.e.mean throughout the paper (*n*: number of rats). For comparison of mean values, the *t* test for unpaired or paired 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 used

Atropine sulphate, [Lys^8]-vasopressin (Sigma, Munich, Germany), BRL 37344 ((**R*****R***)-(±)-4-(2′-[2-hydroxy-2-(3-chlorophenyl)-ethylamino]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, Basel, 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, Macclesfield, U.K.), SR 59230A (3-(2-ethylphenoxy) - 1 - [(1S) - 1,2,3,4-tetrahydronaphth-1-ylamino]-2S-2-propanol oxalate; Sanofi, Milan, Italy), cyanopindolol (Sandoz Pharma Ltd, Basel, Switzerland), bupranolol (Schwarz Pharma AG, Monheim, Germany), pentobarbitone (Biowet, Puławy, Poland).

Drugs were dissolved in water (pentobarbitone, atropine), in saline (BRL 37344, CGP 12177, CL 316243, ZD 2079, ICI 118551, cyanopindolol, bupranolol), in a mixture of saline and dimethylsulphoxide (DMSO) (16 : 1 CGP 20712, 27 : 1 ZD 2079) or in a mixture of saline and ethylene glycol (1 : 1 SR 59230A). Since the vehicle for SR 59230A caused a short-lasting decrease in blood pressure, this drug or its vehicle were administered very slowly over a time period of 30 – 60 s; thus, the fall in blood pressure (which recovered within 5 min, i.e. before the agonist under study was administered) did not exceed 20 mmHg. Other solvents did not affect cardiovascular parameters. None of the solvents affected temperature.

Results

General

Basal heart rate, immediately before administration of one of the β-adrenoceptor agonists, ranged between 300 and 400 beats min^{-1}. Before the injection of β-adrenoceptor ligands, the temperature in the BAT exceeded that in the rectum by 0.9 to 1.3°C (rectal temperature was not affected by any of the β-adrenoceptor ligands used in the present study).

Influence of non-conventional partial β-adrenoceptor agonists and β_3-adrenoceptor agonists on heart rate and brown adipose tissue thermogenesis

CGP 12177 and cyanopindolol increased heart rate and BAT temperature in a dose-dependent manner (Figure 1). The maximal effects of CGP 12177, obtained at a dose of 1 μmol kg^{-1}, were 148.5 ± 5.0 beats min^{-1} and 0.95 ± 0.07°C, respectively. The maximal positive chronotropic effect to cyanopindolol (obtained at 1 μmol kg^{-1}) was by about 30% ($P<0.001$) lower than that elicited by CGP 12177. (With respect to the thermogenic response to cyanopindolol, the exact maximum effect could not be determined since a complete dose-response curve could not be constructed). The β_3-adrenoceptor agonist CL 316243 also caused a dose-dependent increase in BAT temperature, maximally by 1.04 ± 0.10°C (Figure 1), but failed to affect heart rate at doses up to 1 μmol kg^{-1}. The agonistic potencies of the three compounds for their cardiostimulant and thermogenic effects are given in Table 1.

Figure 2 shows that CGP 12177 and cyanopindolol at doses roughly corresponding to their ED_{50} values for their positive chronotropic effect increased BAT temperature only slightly. Conversely, CL 316243 and another two β_3-adrenoceptor agonists, BRL 37344 and ZD 2079, at doses causing an increase in BAT temperature by about 0.6 – 0.7°C (i.e. more than

Table 2 Antagonistic potencies of four β-adrenoceptor antagonists towards effects mediated via four types of β-adrenoceptors in pithed and vagotomized rats

	Negative log of the dose of the antagonist causing a two fold rightward shift of the dose-response curve of the respective agonist[1]			
β-adrenoceptor antagonist	*β_1-adrenoceptor-mediated increase in heart rate*	*β_2-adrenoceptor-mediated decrease in diastolic blood pressure*	*Atypical β-adrenoceptor mediating increase in heart rate*	*β_3-adrenoceptor-mediated increase in brown adipose tissue temperature*
CGP 20712	8.6[2]	ND	6.2[3], 6.0[4], 6.3[5]	5.2[4]
ICI 118551	ND	8.4[2]	< 5[6]	< 5[6]
SR 59230A	ND	ND	5.9[7]	5.7[7]
Bupranolol	7.6[2]	8.3[2]	5.6[6]	5.7[6]

[1]The following agonists were used: prenalterol (β_1-adrenoceptor), fenoterol (β_2-adrenoceptor), CGP 12177 (atypical β-adrenoceptor and β_3-adrenoceptor, footnotes 3, 4, 6 and 7), cyanopindolol (atypical β-adrenoceptor, footnote 5). [2]From Malinowska & Schlicker (1996), [3]Determined from Figure 4, CGP 20712 dose 10 μmol kg^{-1}. [4]From Figure 4, CGP 20712 dose 30 μmol kg^{-1}. [5]From Figure 5; [6]From Figure 3; [7]From Figure 7. ND, not determined.

the half-maximum effect of CL 316243) failed to affect (CL 316243) or only very slightly increased heart rate (BRL 37344 and ZD 2079) (Figure 2).

Influence of β-adrenoceptor antagonists on the changes in heart rate and brown adipose tissue temperature induced by non-conventional partial β-adrenoceptor agonists and/or CL 316243

The non-selective β-adrenoceptor antagonist bupranolol 10 μmol kg^{-1} attenuated the positive chronotropic effect and the increase in BAT temperature in response to CGP 12177 to a similar extent (Figure 3; for antagonistic potencies, see Table 2). A high dose of the β_1-adrenoceptor antagonist CGP 20712 (10 μmol kg^{-1}) diminished the increase in heart rate induced by CGP 12177 (Figure 4a) and cyanopindolol (Figure 5a) but had no effect on the increase in BAT temperature elicited by CGP 12177 (Figure 4b), cyanopindolol (Figure 5b) and CL 316243 (Figure 6). At a higher dose, 30 μmol kg^{-1}, CGP 12177 also reduced the increase in BAT temperature induced by CGP 12177 (Figure 4b; for antagonistic potencies, see Table 2). As shown in Figure 3, a high dose of the β_2-adrenoceptor antagonist ICI 118551 (10 μmol kg^{-1}) failed to affect the CGP 12177-induced changes both in heart rate and BAT temperature.

The β_3-adrenoceptor antagonist SR 59230A 10 μmol kg^{-1} increased heart rate by itself by 28.5$\pm$4.8 beats min^{-1} (n = 4); this effect was abolished by the β_1-adrenoceptor antagonist

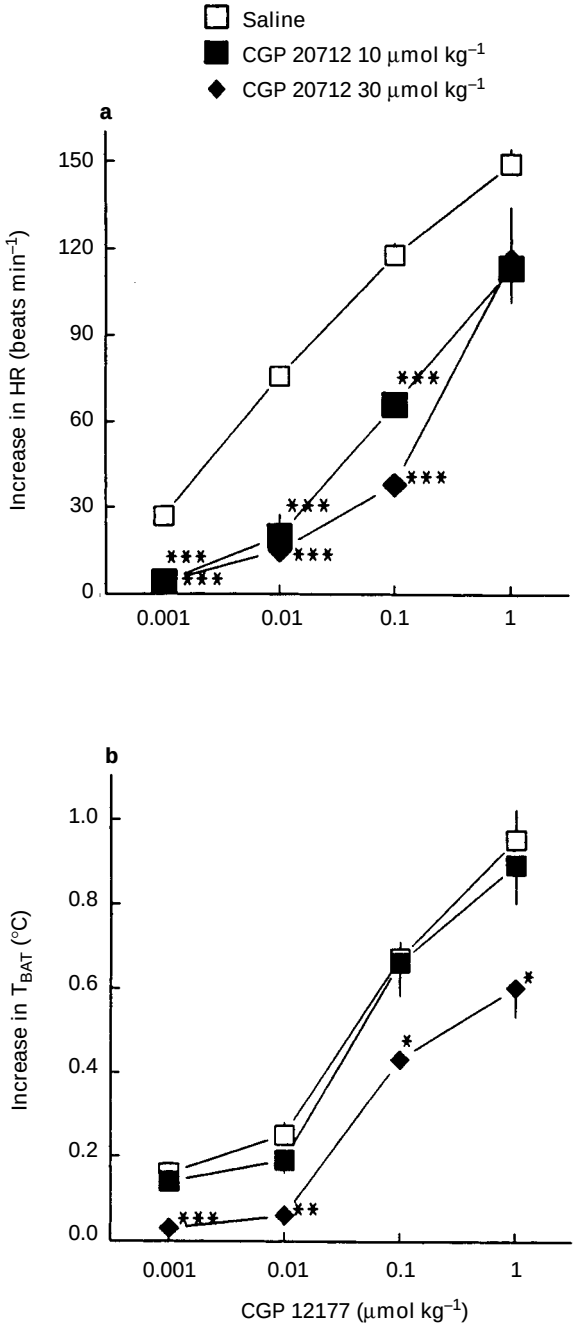

Figure 4 Effects of CGP 12177 on heart rate (HR; a) and brown adipose tissue temperature (T_{BAT}; b) in pithed and vagotomized rats and its interaction with the β_1-adrenoceptor antagonist CGP 20712. The two lower doses of CGP 12177 were applied to one rat, whereas each of the higher doses was studied in separate animals. The first or only dose was given 5 min after injection of saline (control), CGP 20712 10 μmol kg^{-1} or 30 μmol kg^{-1}. Means of 4–11 rats; vertical lines show s.e.mean. For many points s.e.mean is contained within the symbols. *P<0.05, **P<0.01, ***P<0.001 compared to the corresponding value without CGP 20712.

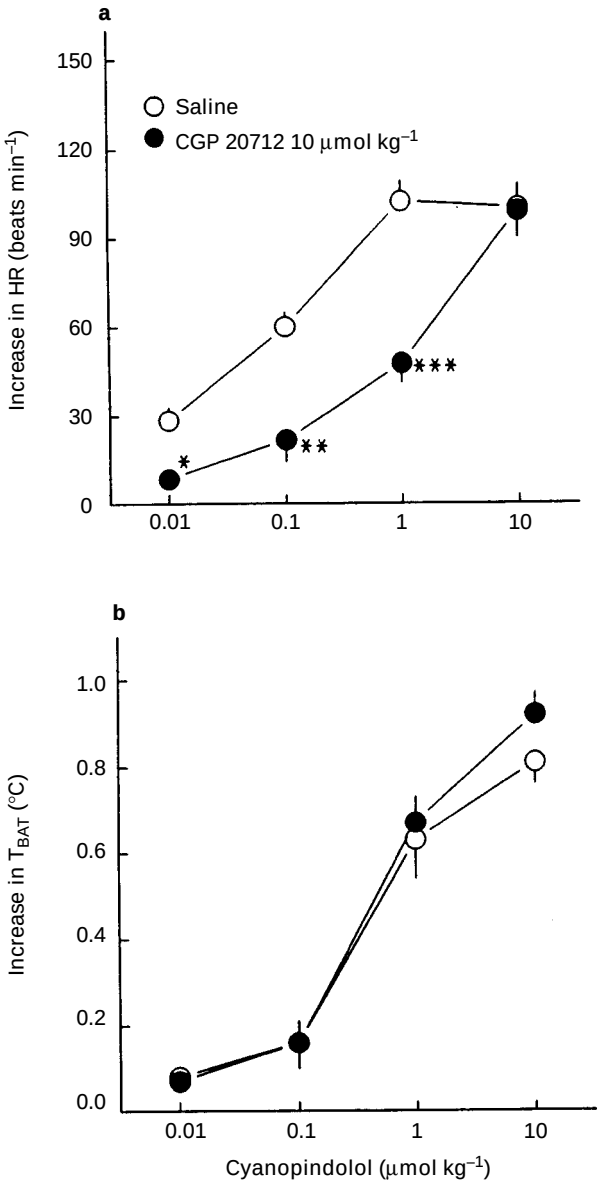

Figure 5 Effects of cyanopindolol on heart rate (HR; a) and brown adipose tissue temperature (T_{BAT}; b) in pithed and vagotomized rats and its interaction with the β_1-adrenoceptor antagonist CGP 20712. Each dose of cyanopindolol was studied in separate animals; it was given 5 min after injection of saline (control) or CGP 20712 10 μmol kg^{-1}. Means of 4–5 rats; vertical lines show s.e.mean. For some points s.e.mean is contained within the symbols. *P<0.05, **P<0.01, ***P<0.001 compared to the corresponding value without CGP 20712.

CGP 20712 0.1 μmol kg^{-1} (n = 6, not shown). The influence of SR 59230A on the CGP 12177-induced changes in heart rate and BAT temperature was, therefore, examined in the presence of the β_1-adrenoceptor antagonist CGP 20712 0.1 μmol kg^{-1}. Administration of CGP 20712 0.1 μmol kg^{-1} had no influence on the CGP 12177-stimulated increase in heart rate and BAT temperature (Figure 7) or the degree of inhibition by bupranolol of the CGP 12177-induced effects (compare Figures 3 and 7). The vehicle for SR 59230A (ethylene glycol) also did not affect the CGP 12177-induced positive chronotropic and thermogenic response (Figure 7). SR 59230A 10 μmol kg^{-1} attenuated the CGP 12177-evoked increase in heart rate and BAT temperature to a similar degree (Figure 7; for antagonistic potencies, see Table 2).

Basal heart rate, determined at the moment of administration of the agonist, was not affected by CGP 20712 1 μmol kg^{-1} but was decreased by CGP 20712 10 and 30 μmol kg^{-1} (by 5 and 15%, respectively) and by bupranolol 10 μmol kg^{-1} (by 7%). ICI 118551 10 μmol kg^{-1} caused a short-lived decrease in heart rate which already had recovered when the agonist under study was administered (Malinowska & Schlicker, 1996). None of the β-adrenoceptor antagonists induced changes in BAT temperature (data not shown).

Discussion

In the present study, the properties of the β_3-adrenoceptor increasing BAT temperature (for review, see Arch & Kaumann, 1993; Galitzky *et al*., 1995; Strosberg & Pietri-Rouxel, 1996) and the atypical β-adrenoceptor increasing heart rate were directly compared in pithed and vagotomized rats. In our experiments, the level of basal blood pressure was increased by [Lys^8]-vasopressin in order to allow comparisons with our previous study (Malinowska & Schlicker, 1996); furthermore, a high level of blood pressure proved favourable for measuring thermogenesis in BAT. As in our previous study, we used non-conventional partial β-adrenoceptor agonists (CGP 12177, cyanopindolol), selective β_3-adrenoceptor agonists (CL 316243, BRL 37344, ZD 2079), the selective β_1- and β_2-adrenoceptor antagonists CGP 20712 and ICI 118551, respectively, and the non-selective β-adrenoceptor antagonist bupranolol. In addition, the recently described β_3-adrenoceptor antagonist SR 59230A (Manara *et al*., 1995) was used.

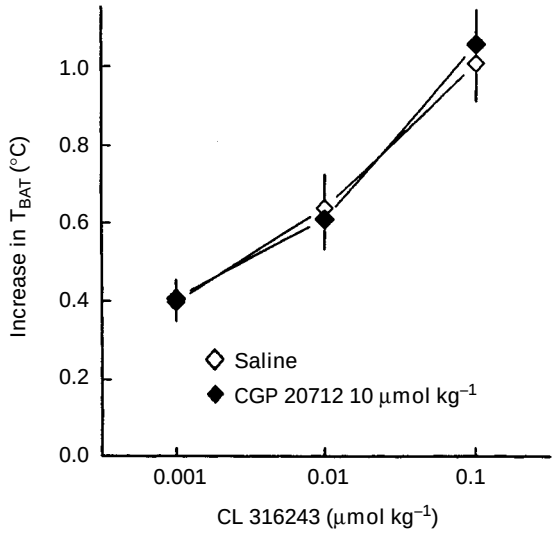

Figure 6 Effects of CL 316243 on brown adipose tissue temperature (T_{BAT}) in pithed and vagotomized rats and its interaction with the β_1-adrenoceptor antagonist CGP 20712. Each dose of CL 316243 was studied in separate animals. It was given 5 min after injection of saline (control) or CGP 20712 10 μmol kg^{-1}. Means of 3–5 rats; vertical lines show s.e.mean.

The non-conventional partial β-adrenoceptor agonist cyanopindolol increased heart rate at a lower potency than CGP 12177 (Table 1). The maximal positive chronotropic effect of cyanopindolol was about 30% lower than that of CGP 12177.

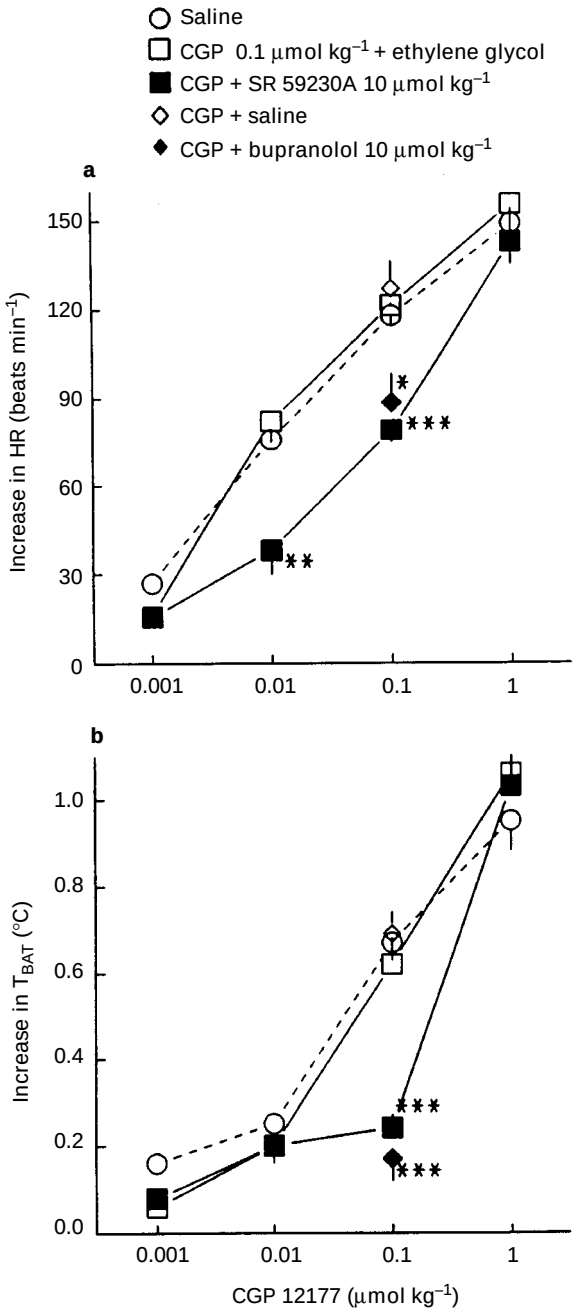

Figure 7 Effects of CGP 12177 on heart rate (HR; a) and brown adipose tissue temperature (T_{BAT}; b) in pithed and vagotomized rats and its interaction with β-adrenoceptor antagonists. The two lower doses of CGP 12177 were applied to one rat, whereas each of the higher doses was studied in separate animals. In most of the experiments, the first or only dose of CGP 12177 was given 5 min after injection of the β_1-adrenoceptor antagonist CGP 20712 0.1 μmol kg^{-1} (CGP) co-administered either with the β_3-adrenoceptor antagonist SR 59230A 10 μmol kg^{-1} (or its vehicle ethylene glycol) or with the non-selective β-adrenoceptor antagonist bupranolol 10 μmol kg^{-1} (or its vehicle saline). For comparison, the CGP 12177-induced changes in heart rate and BAT temperature are also shown for rats which received saline but no CGP 20712. Means of 4–6 rats; vertical lines show s.e.mean. For many points s.e.mean is contained within the symbols. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to the corresponding value without SR 59230A or bupranolol.

Cyanopindolol and CGP 12177 have been shown to evoke a positive chronotropic and, in addition, a positive inotropic effect on rat isolated cardiac tissue and were approximately equipotent in this respect (Kaumann & Molenaar, 1996). Also in the latter study, cyanopindolol elicited a lower maximal response than CGP 12177 (around 50% compared to CGP 12177). Thus, we suppose that cyanopindolol is a partial agonist at the atypical β-adrenoceptor in the heart.

The selective β_3-adrenoceptor agonist CL 316243 (Dolan *et al*., 1994) elicited a thermogenic response in the BAT at doses lower than those of CGP 12177 and cyanopindolol. Doses of CGP 12177 and cyanopindolol that roughly correspond to their ED_{50} values for their cardiostimulant effect only slightly increased BAT temperature. On the other hand, the three β_3-adrenoceptor agonists at doses causing about two thirds of the maximal thermogenic response of CL 316243 did not affect heart rate (CL 316243) or only marginally increased it (BRL 37344, ZD 2079). Note that the latter increase in heart rate is related to the activation of β_1- and/or β_2-adrenoceptors (Oriowo *et al*., 1994; Cohen *et al*., 1995; Malinowska & Schlicker, 1996).

Among the β-adrenoceptor antagonists, bupranolol, at a dose much higher than that antagonizing β_1- and β_2-adrenoceptor-mediated effects (Table 2), counteracted the tachycardia and the thermogenic response caused by CGP 12177 at a very similar potency. Bupranolol, which is a weak antagonist at the cardiostimulant atypical β-adrenoceptor (Kaumann, 1989; 1997; Arch & Kaumann, 1993) and at the β_3-adrenoceptor (Arch & Kaumann, 1993; Blin *et al*., 1993; Strosberg & Pietri-Rouxel, 1996; Kaumann, 1997), also antagonized the β_3-adrenoceptor-mediated relaxation of the rat colon and, at least equipotently, the positive inotropic and chronotropic effects mediated via the atypical β-adrenoceptor in the rat heart *in vitro* (Kaumann & Molenaar, 1996).

CGP 20712, at a dose much higher than that antagonizing β_1-adrenoceptor-mediated effects (Table 2), antagonized the tachycardia mediated via the atypical β-adrenoceptor and, at a still higher dose, counteracted the thermogenic response mediated via β_3-adrenoceptors. These findings can be reconciled with those obtained by Kaumann & Molenaar (1996) for the atypical β-adrenoceptor and the β_3-adrenoceptor in the rat isolated heart and colon, respectively. CGP 20712 10 and 30 μmol kg^{-1} and bupranolol 10 μmol kg^{-1} decreased heart rate by themselves. The possibility that this effect interferes with the ability of both drugs to antagonize the cardiostimulant effect is unlikely since the estimates of antagonistic potency based on the doses of 10 and 30 μmol kg^{-1} CGP 20712 did not differ from that of the dose of 1 μmol kg^{-1} CGP 20712, which did not affect heart rate by itself (Malinowska & Schlicker, 1996).

ICI 118551, at doses/concentrations much higher than those antagonizing effects mediated via β_2-adrenoceptors (Table 2), failed to affect the atypical cardiac β-adrenoceptor and the β_3-adrenoceptor, both *in situ* (present study) and *in vitro* (Kaumann & Molenaar, 1996).

SR 59230A counteracted the β_3-adrenoceptor-mediated thermogenic response in the present experiments on pithed rats as expected from the paper by Manara *et al*. (1996), in which it antagonized the β_3-adrenoceptor-mediated increase in BAT temperature and inhibition of colonic motility in the anaesthetized rat. Surprisingly, SR 59230A equipotently antagonized the tachycardia mediated via the atypical β-adrenoceptor and the β_3-adrenoceptor-mediated thermogenesis in the present study. We cannot satisfactorily explain this phenomenon, which is in marked contrast with the *in vitro* findings of Kaumann & Molenaar (1996), who found that the potency of SR 59230A at the β_3-adrenoceptor exceeded that at the cardiac atypical β-adrenoceptor by at least one log unit. SR 59230A increased heart rate by itself and this effect was abolished by a low dose of CGP 20712, i.e. is mediated via β_1-adrenoceptors. CGP 20712 0.1 μmol kg^{-1} was, therefore, used in all experiments with SR 59230A. The possibility that the presence of CGP 20712 was the reason for the antagonistic effect of SR 59230A at the atypical β-adrenoceptor can be excluded, since another β blocker, bupranolol, attenuated the tachycardia of CGP 12177 to the same extent, regardless of whether CGP 20712 0.1 μmol kg^{-1} was administered or not. In addition, the solvent for SR 59230A, ethylene glycol, cannot account for the discrepancy since this solvent, given alone, had no influence on the response mediated via the atypical cardiac β-adrenoceptor.

Our findings provide direct evidence that, also *in situ*, the atypical cardiostimulant β-adrenoceptor and the β_3-adrenoceptor can be differentiated pharmacologically. This is reflected by the different rank orders both of agonistic and antagonistic potencies. With respect to the agonists, the rank order was CGP 12177 > cyanopindolol > CL 316243 for the cardiostimulant β-adrenoceptor and CL 316243 > CGP 12177 > cyanopindolol for the β_3-adrenoceptor (Table 1). With respect to the antagonists, the rank orders were CGP 20712 $\geqslant$ SR 59230A $\geqslant$ bupranolol > ICI 118551 (cardiostimulatory β-adrenoceptor) and SR 59230A = bupranolol > CGP 20712 > > ICI 118551 (β_3-adrenoceptor) (Table 2). Note that the difference in the rank orders of potency between heart rate and BAT temperature is caused by only one drug, both with respect to the agonists (CL 316243) and antagonists (CGP 20712).

According to the findings of the present study, the atypical cardiac β-adrenoceptors and the BAT β_3-adrenoceptors differ in three respects. Firstly, selective β_3-adrenoceptor agonists induce BAT thermogenesis at doses lower than 1 μmol kg^{-1} but do not exert any specific effects at atypical cardiac β-adrenoceptors. Secondly, the non-conventional partial β-adrenoceptor agonists are more potent at the atypical cardiostimulant β-adrenoceptor than at the β_3-adrenoceptor. Thirdly, CGP 20712 is markedly more potent in diminishing the positive chronotropic effect than in attenuating the β_3-adrenoceptor-mediated BAT thermogenic response.

In conclusion, the present *in situ* study and the *in vitro* study by Kaumann & Molenaar (1996), in both of which the properties of the atypical cardiostimulant β-adrenoceptor were directly compared to those of a β_3-adrenoceptor, clearly show that the two receptors are pharmacologically different. The differences between the study of Kaumann & Molenaar (1996) and our own study with respect to the cardiostimulant β-adrenoceptor should not be overinterpreted; they may be, at least partially, explained by the more complex situation for a drug *in situ* than *in vitro*. The atypical cardiostimulant β-adrenoceptor has so far not attracted much attention; such a receptor has not yet been cloned and its physiological/pathophysiological role is unclear. The activation of this receptor by catecholamines also remains to be established. However, in this context, unpublished binding data obtained by Kaumann (1997) are of interest. In rat atrial membranes, (-)-[^{3}H]-CGP 12177 labels binding sites, different from β_3-sites, which are stereoselectively inhibited by catecholamines and, in addition, are displaced by β-adrenoceptor ligands at affinities fitting to their potencies at the cardiostimulant β-adrenoceptor.

This work was supported by the Polish Government (KBN No 113884). The authors are grateful to Professor Jan Górski (Department of Physiology, Medical Academy in Białystok, Poland) for helpful advice considering BAT physiology and to Professor Zenon Mariak (Clinic of Neurosurgery, Medical Academy in Białystok) for lending the equipment for precise temperature measurements. We are also indebted to the Alexander von Humboldt-Stiftung (Bonn, Germany) for generously providing some of the equipment, to S&W Vickers, Ltd. (Białystok) for adapting the equipment for our experiments and the pharmaceutical companies Ciba-Geigy, Cyanamid, ICI Pharmaceuticals, Sandoz, Sanofi, Schwarz Pharma AG, SmithKline Beecham and Zeneca for gifts of drugs.

References

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

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.

COHEN, M.L., SARAZAN, R.D., KLOTZ, U., BLOOMQUIST, W. & PALKOWITZ, A. (1995). Effect of β_3-selective agonists, BRL 37,344 and CL 316,243 on blood pressure and heart rate in the rat. *J. Drug Dev. Clin. Pract.*, **7,** 37 – 44.

DOLAN, J.A., MUENKEL, H.E., BURNS, M.G., PELLEGRINO, S.M., FRASER, C.M., PIETRI, F., STROSBERG, A.D., LARGIS, E.E., DUTIA, M.D., BLOOM, J.D., BASS, A.S., TANIKELLA, T.K., COBUZZI, A., LAI, F.M. & CLAUS, T.H. (1994). Beta-3 adrenoceptor selectivity of the dioxolane dicarboxylate phenethanolamines. *J. Pharmacol. Exp. Ther.*, **269,** 1000 – 1006.

GALITZKY, J., CARPÉNÉ, C., BOUSQUET-MÉLOU, A., BERLAN, M. & LAFONTAN, M. (1995). Differential activation of β_1-, β_2- and β_3-adrenoceptors by catecholamines in white and brown adipocytes. *Fundam. Clin. Pharmacol.*, **9,** 324 – 331.

GAUTHIER, C., TAVERNIER, G., CHARPENTIER, F., LANGIN, D. & LE MAREC, H. (1996). Functional β_3-adrenoceptor in the human heart. *J. Clin. Invest.*, **98,** 556 – 562.

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

KAUMANN, A.J. (1996). (-)-CGP 12177-induced increase of human atrial contraction through a putative third β-adrenoceptor. *Br. J. Pharmacol.*, **117,** 93 – 98.

KAUMANN, A.J. (1997). Four β-adrenoceptor subtypes in the mammalian heart. *Trends Pharmacol. Sci.*, **18,** 70 – 76.

KAUMANN, A.J. & MOLENAAR, P. (1996). Differences between the third cardiac β-adrenoceptor and the colonic β_3-adrenoceptor in the rat. *Br. J. Pharmacol.*, **118,** 2085 – 2098.

MALINOWSKA, B. & SCHLICKER, E. (1996). Atypical β-adrenoceptors, different from β_3-adrenoceptors, mediate the positive chronotropic effect of CGP 12177 and cyanopindolol in the pithed rat. *Br. J. Pharmacol.*, **117,** 943 – 949.

MANARA, L., BADONE, D., BARONI, M., BOCCARDI, G., CECCHI, R., CROCI, T., GIUDICE, A., GUZZI, U., LANDI, M. & LE FUR, G. (1995). Aryloxypropanolaminotetralins are the first selective antagonists for atypical (β_3) β-adrenoceptors. *Pharmacol. Comm.*, **6,** 253 – 258.

MANARA, L., BADONE, D., BARONI, M., BOCCARDI, G., CECCHI, R., CROCI, T., GIUDICE, A., GUZZI, U., LANDI, M. & LE FUR, G. (1996). Functional identification of rat atypical β-adrenoceptors by the first β_3-selective antagonists, aryloxypropanolaminotetralins. *Br. J. Pharmacol.*, **117,** 435 – 442.

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.

STROSBERG, A.D. & PIETRI-ROUXEL, F. (1996). Function and regulation of the β_3-adrenoceptor. *Trends Pharmacol. Sci.*, **17,** 373 – 381.

(Received June 2, 1997
Revised August 26, 1997
Accepted September 1, 1997)