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Positive inotropic and lusitropic effects mediated *via* the low-affinity state of β_1-adrenoceptors in pithed rats

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1 Activation by CGP 12177 and cyanopindolol of the human and rat low-affinity state of β_1-adrenoceptors increases frequency and contractile force and hastens relaxation in isolated cardiac tissues, and probably relaxes isolated vessels. In order to identify the positive inotropic, positive lusitropic and vasodilator effects of both agonists also *in vivo*, we have determined their effects on the left ventricular systolic pressure (LVSP), the rate of intraventricular pressure rise ($+dP\,dt^{-1}_{max}$) and decline ($-dP\,dt^{-1}_{max}$), the diastolic blood pressure (DBP) and the mesenteric blood flow (MBF) in pithed and vagotomized rats.

2 CGP 12177 (0.1–100 nmol kg^{-1}) and cyanopindolol (1–1000 nmol kg^{-1}) dose-dependently enhanced all cardiac parameters. The nonselective β-adrenoceptor antagonist bupranolol 10 μmol kg^{-1} diminished the CGP 12177 (100 nmol kg^{-1})-stimulated increases in LVSP from 26.3 ± 8.2 to 13.1 ± 1.8 mmHg ($P<0.05$), $+dP\,dt^{-1}_{max}$ from 5287 ± 290 to 2439 ± 296 mmHg s^{-1} ($P<0.001$) and $-dP\,dt^{-1}_{max}$ from -3836 ± 301 to -2187 ± 443 mmHg s^{-1} ($P<0.05$), respectively. The β_1-adrenoceptor antagonist CGP 20712A 10 μmol kg^{-1} (known to block the low-affinity state of β_1-adrenoceptors at high doses) inhibited increases in $\pm dP\,dt^{-1}_{max}$ elicited by the highest dose of CGP 12177.

3 The highest doses of CGP 12177 and cyanopindolol increased DBP by about 10 mmHg and MBF by 1.4 ± 0.3 and 0.6 ± 0.3 ml min^{-1}, respectively. The vascular effects of CGP 12177 were not affected by bupranolol and CGP 20712A.

4 In conclusion, activation of the low-affinity state of β_1-adrenoceptors by CGP 12177 and cyanopindolol in pithed rats causes a positive inotropic and lusitropic effect. By contrast, the vascular effects of CGP 12177 and cyanopindolol are not mediated by these receptors and have only marginal influence under *in vivo* conditions.
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Abbreviations: CGP 12177, (±)-4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazole-2-one; CGP 20712A, (±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]-phenoxy]propyl]-amino]ethoxy]-benzamide monomethane sulphonate; DBP, diastolic blood pressure; $+dP\,dt^{-1}_{max}$, maximal rate of contraction; $-dP\,dt^{-1}_{max}$, maximal rate of relaxation; HR, heart rate; LVSP, left ventricular systolic pressure; MBF, mesenteric blood flow

Introduction

There is increasing evidence that, besides the classical β_1-adrenoceptor (high-affinity state of β_1-adrenoceptor), a second form of cardiostimulant β_1-adrenoceptor exists. This receptor, known previously as 'atypical β-adrenoceptor' or 'putative β_4-adrenoceptor' (Kaumann & Molenaar, 1997; Konkar *et al*., 2000a; Granneman, 2001), is now termed the 'low-affinity state of β_1-adrenoceptor' (Alexander *et al*., 2004). On the basis of radioligand-binding studies with ^{3}H-(±)-4-[3-[(1,1-dimethyl-ethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazole-2-one (CGP 12177) on rat left ventricular (Sarsero *et al*., 1999) and human right atrial membranes (Sarsero *et al*., 2003), the low-affinity state of β_1-adrenoceptor occurs at a four-fold higher density than the high-affinity state of the β_1-adrenoceptor plus the β_2-adrenoceptor. The affinity of ^{3}H-CGP 12177 for the low-affinity state of β_1-adrenoceptor in these two studies is about 100 times lower when compared to its affinity for the high-affinity state of β_1-adrenoceptor plus the β_2-adrenoceptor.

The low-affinity state of β_1-adrenoceptor, which is positively coupled to adenylyl cyclase (Pak & Fishman, 1996; Konkar *et al*., 2000b; Sarsero *et al*., 2003; Joseph *et al*., 2004), is activated by nonconventional partial agonists like CGP 12177, cyanopindolol, pindolol, bucindolol or alprenolol, that is, drugs which activate β_3- and the low-affinity state of β_1-adrenoceptors at concentrations much higher than those at which they block β_1- and/or β_2-adrenoceptors. This receptor is relatively resistant to blockade by propranolol and other classical β-blockers, but blocked with moderate affinity by bupranolol (Kaumann, 1996; Kaumann & Molenaar, 1996; Malinowska & Schlicker, 1996; 1997; Bundkirchen *et al*., 2002;

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Lowe *et al*., 2002; Baker *et al*., 2003; Sarsero *et al*., 2003; Lewis *et al*., 2004) and carvedilol (Lowe *et al*., 1999). The presence of the high-affinity state of β_1-adrenoceptor is obligatory for the cardiostimulant effect of CGP 12177 because this response was abolished in atria from double β_1-/β_2-adrenoceptor knockout, mice but remained intact in atria from β_2-adrenoceptor knockout mice (Kaumann *et al*., 2001).

Although the pharmacology of the low-affinity state of β_1-adrenoceptor partly resembles that of the β_3-adrenoceptor (i.e. both receptors are activated by nonconventional partial agonists, resistant to propranolol and blocked with moderate potency by bupranolol), they are distinct. Thus, the low-affinity state of β_1-adrenoceptor (1) is not stimulated by selective β_3-adrenoceptor agonists (e.g. CL 316243); (2) unlike the β_3-adrenoceptor is antagonized by high concentrations/doses of the β_1-adrenoceptor antagonist (±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]-phenoxy]propyl]-amino]ethoxy]-benzamide monomethane sulphonate (CGP 20712A) (Kaumann & Molenaar, 1996; Malinowska & Schlicker, 1996; 1997) and (3) is unaffected in the hearts of knockout mice with disruption of the β_3-adrenoceptor gene (Kaumann *et al*., 1998).

So far, effects mediated *via* the low-affinity state of β_1-adrenoceptor have been demonstrated mainly under *in vitro* conditions. Thus, their activation induces (i) sinoatrial tachycardia in rat (Kaumann & Molenaar, 1996), mouse (Kaumann *et al*., 1998; 2001), ferret (Lowe *et al*., 2002) and probably human (Kaumann, 1989) heart, (ii) increases contractile force in the heart of humans (Kaumann, 1996; Bundkirchen *et al*., 2002; Sarsero *et al*., 2003), rats (Kaumann & Molenaar, 1996), mice (Kaumann *et al*., 1998) and ferrets (Lowe *et al*., 1999; 2002), (iii) hastens relaxation of the human myocardium in most (Kaumann & Molenaar, 1997; Sarsero *et al*., 1999; 2003; Joseph *et al*., 2003; Lewis *et al*., 2004) but not all studies (Bundkirchen *et al*., 2002), (iv) shortens ventricular action potential in ferret ventricle (Lowe *et al*., 1998) and (v) elicits arrhythmias in atrial and ventricular myocytes of the rat (Sarsero *et al*., 1999) and mouse (Freestone *et al*., 1999). Low-affinity binding to human recombinant β_1-adrenoceptors has also been demonstrated (e.g. Baker *et al*., 2003; Joseph *et al*., 2004). By contrast, under *in vivo* conditions only the positive chronotropic effect of agonists at the low-affinity state of β_1-adrenoceptor was demonstrated in the pithed rat (Malinowska & Schlicker, 1996; 1997; Malinowska *et al*., 2003). Thus, the first aim of our study was to examine whether activation of the low-affinity state of β_1-adrenoceptor also exhibits positive inotropic and lusitropic actions in pithed rats.

With respect to blood vessels, the occurrence of vasorelaxant atypical β-adrenoceptors was proposed for a variety of vessels preconstricted with phenylephrine or noradrenaline, including the rat aorta (e.g. Brawley *et al*., 2000; Mallem *et al*., 2005), carotid artery (e.g. Oriowo, 1995), mesenteric artery (Kozłowska *et al*., 2003) and human internal mammary artery (Shafiei *et al*., 2000). These receptors were assumed to conform to the low-affinity state of β_1-adrenoceptors although, in the study on the rat mesenteric artery, the conclusion was reached that they may be different (Kozłowska *et al*., 2003). There is, however, increasing evidence from studies in the rat aorta (Brahmadevara *et al*., 2004), intrapulmonary artery (Leblais *et al*., 2004), mesenteric artery (Kozłowska *et al*., 2005) and human pulmonary artery (Kozłowska *et al*., 2005) that the vasorelaxant effects of some of the ligands at the low-affinity state of β_1-adrenoceptor result from their α_1-adrenolytic properties. On the other hand, when the rat mesenteric artery was preconstricted with *serotonin*, cyanopindolol still relaxed the vessel and this effect was counteracted by bupranolol (Kozłowska *et al*., 2003), suggesting that there may be vasorelaxant atypical β-adrenoceptors anyway. Therefore, the second aim of our study was to examine whether agonists of the low-affinity state of β_1-adrenoceptors given at doses which cause cardiostimulation affect vascular parameters under *in vivo* conditions. For direct comparison of cardiac and vascular effects, all parameters were measured simultaneously in the same pithed rats.

Methods

The experimental protocol and the use of laboratory animals were approved by the local Ethics Committee in Białystok (Poland). Male Wistar rats weighing 280–350 g were anaesthetized intraperitoneally (i.p.) with pentobarbitone sodium 300 μmol kg^{-1} and then injected with atropine 2 μmol kg^{-1}. After cannulation of the trachea, the animals were pithed by inserting a stainless-steel rod (1.5 mm diameter and 190 mm length) through the orbit and foramen magnum into the vertebral canal and artificially ventilated with air (1 ml 100 g^{-1}, 60 strokes min^{-1}) using a respiratory system (7025 Rodent respirator; Hugo Sachs Elektronik, March-Hugstetten, Germany). Both vagal nerves were cut. Diastolic blood pressure (DBP) was measured from the left carotid artery *via* a transducer (ISOTEC; Hugo Sachs Elektronik, March-Hugstetten, Germany). Heart rate (HR) was measured by a ratemeter triggered from the pressure record. To measure the peak left ventricular systolic pressure (LVSP), the intraventricular catheter-tip transducer (Millar SPR 407; Millar Inst., Houston, TX, U.S.A.) was inserted into the left ventricle through the right carotid artery. The maximum rates of intraventricular pressure rise and decline ($+dP\,dt^{-1}_{max}$ and $-dP\,dt^{-1}_{max}$) were obtained from LVSP. The left femoral vein was cannulated for i.v. injection of drugs administered in a volume of 0.5 ml kg^{-1}. Body temperature was kept constant at about 37°C using a heating pad (Bio-Sys-Tech, Białystok, Poland) and monitored by a rectal probe transducer (Physitemp BAT10, Clifton, NJ, U.S.A.). Following pithing, vasopressin (0.04–0.4 IU kg^{-1} min^{-1}) was routinely infused into the right femoral vein to raise DBP to about 80 mmHg (like in our previous studies; see Malinowska & Schlicker, 1996; 1997; Malinowska *et al*., 2003). Middle laparotomy was performed and the mesenteric artery was exposed where an ultrasonic flow probe (0.5 mm, V-series) was gently placed and mesenteric blood flow (MBF) continuously measured using a directional Ultrasonic Doppler flowmeter (Transonic Systems Inc., Ithaca, NY, U.S.A.).

After 30–40 min of equilibration, during which the cardiovascular parameters were allowed to stabilize, experiments were performed. Like in our previous studies (Malinowska & Schlicker, 1996; 1997; Malinowska *et al*., 2003), agonists were administered in a noncumulative manner. Since recovery from the effects was very slow, each dose of agonist was usually studied in a separate animal. Only in some cases, the two lowest doses of an agonist were injected to the same rat with sufficient time for full recovery to the preinjection value after

injection of the lowest dose. The first or only dose of agonist was injected 5 min after administration of the antagonist under study or vehicle.

Calculations and statistics

In order to assess the potency (pED_{50}) of CGP 12177 and cyanopindolol in increasing LVSP, $+dP\,dt^{-1}_{max}$, $-dP\,dt^{-1}_{max}$, HR, DBP and MBF, we determined the negative logarithms of the doses (in mol kg^{-1} body weight, i.v.) that increase LVSP by 15 and 10 mmHg, $+dP\,dt^{-1}_{max}$ by 2600 and 1400 mmHg s^{-1}, $-dP\,dt^{-1}_{max}$ by 1900 and 1100 mmHg s^{-1}, HR by 75 and 50 beats min^{-1}, DBP by 4 and 5 mmHg, and MBF by 0.7 and 0.3 ml min^{-1}, respectively. Strictly speaking, the term pED_{50} is only correct for LVSP, for which the maximal effect could be determined; in the case of the other parameters, those doses were used to determine the pED_{50} that causes an increase by about 50% of the effect obtained with the highest dose under study. Note that we say that the positive lusitropic drugs, which render the $-dP\,dt^{-1}_{max}$ value more negative, *increased* this value although, from a strict mathematical point of view, the reverse holds true.

Results are given as means ± s.e.m. (n = number of rats). For comparison of the mean values, the t-test for paired and unpaired data was used. When two or more treatment groups were compared to the same control, one-way analysis of variance (ANOVA) followed by the Dunnett test was used. Differences were considered as significant when $P<0.05$.

Drugs used

S-(−)-bupranolol hydrochloride (SchwarzPharma AG, Monheim, Germany); prenalterol hydrochloride (Hässle, Gothenburg, Sweden); atropine sulphate, [Lys^8]-vasopressin, CGP 20712A (Sigma, Munich, Germany); cyanopindolol, CGP 12177 (Tocris-Cookson, Bristol, U.K.); pentobarbitone sodium (Biowet, Puławy, Poland). All drugs were dissolved in saline, with the exception of CGP 20712A, which was dissolved in a mixture of saline and DMSO (16 : 1). None of the vehicles affected the cardiovascular parameters.

Results

General

In pithed and bilaterally vagotomized rats, the basal cardiovascular parameters measured at the beginning of the experiments (i.e. immediately prior to injection of bupranolol, CGP 20712A or their vehicles) were: LVSP 104 ± 3 mmHg; positive and negative $dP\,dt^{-1}_{max}$ 4414 ± 155 and 4775 ± 208 mmHg s^{-1}, respectively; HR 325 ± 3 beats min^{-1}; DBP 77 ± 1 mmHg (maintained by i.v. infusion of vasopressin 0.04–0.4 IU kg^{-1} min^{-1}) and MBF 4.1 ± 0.1 ml min^{-1} (in all cases $n=82$). Bupranolol and CGP 20712A (10 μmol kg^{-1} each) reduced HR and increased MBF; the effects were maximal 15–30 s after administration of the particular antagonist. After 5 min, that is, when CGP 12177 or cyanopindolol was injected, HR was still decreased by about 5 and 6% and MBF was still increased by about 5 and 12% in the groups treated with bupranolol and CGP 20712A, respectively. The latter two drugs produced short-lasting decreases of the other cardiovascular parameters (LVSP, $+dP\,dt^{-1}_{max}$, $-dP\,dt^{-1}_{max}$ and DBP), which, however, fully recovered within 5 min.

Influence of nonconventional partial β-adrenoceptor agonists and prenalterol on cardiac parameters

Two agonists of the low-affinity state of β_1-adrenoceptors CGP 12177 (0.1–100 nmol kg^{-1}) and cyanopindolol (1–1000 nmol kg^{-1}) dose-dependently increased myocardial contractility as reflected by LVSP (Figure 1a) and by $+dP\,dt^{-1}_{max}$ (Figure 2a), the rate of relaxation as reflected by $-dP\,dt^{-1}_{max}$ (Figure 2a) and HR (Figure 3a). Original tracings of the effect of CGP 12177 10 nmol kg^{-1} on LVSP and HR are shown in Figure 4. The maximal changes evoked by CGP 12177 amounted to about 26, 106, 71 and 40% of the basal values of LVSP, $+dP\,dt^{-1}_{max}$, $-dP\,dt^{-1}_{max}$ and HR, respectively. The potency of CGP 12177 exceeded that of cyanopindolol by about 1.0–1.2 (LVSP, $+dP\,dt^{-1}_{max}$, $-dP\,dt^{-1}_{max}$) and 0.5 (HR) log units (for pED_{50} values, see Table 1 and for their definition, see Methods). In addition, the effects induced by the highest dose of CGP 12177 100 nmol kg^{-1} were higher than those elicited by cyanopindolol 1000 nmol kg^{-1} by about 27% (LVSP), 47% ($+dP\,dt^{-1}_{max}$), 42% ($-dP\,dt^{-1}_{max}$) and 35% (HR). Thus, we selected CGP 12177 for the interaction experiments with bupranolol and CGP 20712A.

The nonselective β-adrenoceptor antagonist bupranolol 10 μmol kg^{-1} shifted to the right the dose–response curves of CGP 12177 for its positive inotropic, lusitropic and chronotropic effects and reduced all maximal effects (Figures 1b, 2b and 3b). Original tracings of the effect of CGP 12177 10 nmol kg^{-1} on LVSP and HR after the administration of bupranolol 10 μmol kg^{-1} are shown in Figure 4. In addition, CGP 20712A 10 μmol kg^{-1} (i.e. used here at a dose capable of blocking the low-affinity state of β_1-adrenoceptors) diminished the CGP 12177 (100 nmol kg^{-1})-induced increase in $+dP\,dt^{-1}_{max}$, $-dP\,dt^{-1}_{max}$ and HR and tended to reduce LVSP (Figures 1b, 2b and 3b).

The β_1-adrenoceptor agonist prenalterol 10 nmol kg^{-1}, used here as a reference drug, also exhibited positive inotropic, lusitropic and chronotropic effects (Figures 1a, 2a and 3a). Compared to prenalterol, the effects of the same doses of CGP 12177 and cyanopindolol amounted to only about 66 and 8% (LVSP), 66 and 8% ($+dP\,dt^{-1}_{max}$), 84 and 10% ($-dP\,dt^{-1}_{max}$), and 79 and 39% (HR), respectively.

Figure 5 allows to compare the time-course of changes induced by CGP 12177, cyanopindolol and prenalterol given at doses that produced roughly similar changes. The maximal increases in all four cardiac parameters induced by CGP 12177 100 nmol kg^{-1}, cyanopindolol 1000 nmol kg^{-1} and prenalterol 10 nmol kg^{-1} were reached within 5 min. The positive chronotropic action of both nonconventional β-adrenoceptor agonists lasted longer than their positive inotropic effect. Thus, 30 min after their administration, the increase in HR stimulated by CGP 12177 was diminished only by about 7% and the one induced by cyanopindolol was not changed at all. By contrast, at the same time-point, the increase in LVSP and $+dP\,dt^{-1}_{max}$ stimulated by CGP 12177 was reduced by about 64 and 47%, respectively. In the case of cyanopindolol, enhancement in LVSP and $+dP\,dt^{-1}_{max}$ was diminished already at 15 min after its injection by 94 and 45%, respectively. The positive lusitropic effect (increase in $-dP\,dt^{-1}_{max}$) of CGP 12177

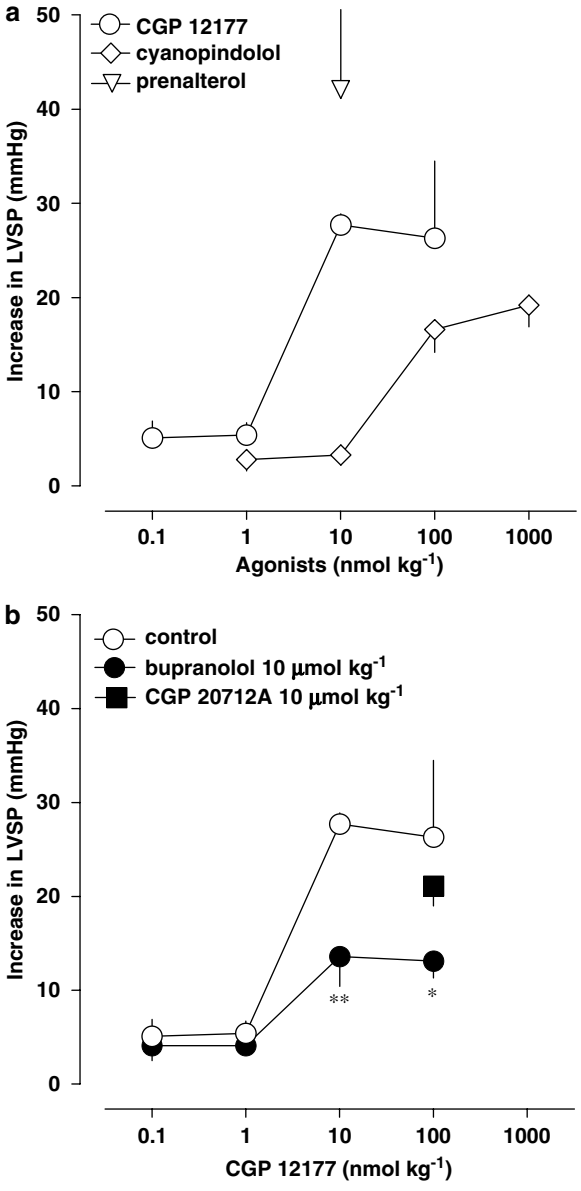

Figure 1 Effects of CGP 12177, cyanopindolol and prenalterol on the LVSP (a) and interaction of CGP 12177 with bupranolol and CGP 20712A (b) in pithed and vagotomized rats. Each dose of β-adrenoceptor agonist (CGP 12177, cyanopindolol or prenalterol) was studied in separate rats although, in some cases, the two lower doses were applied to the same animal. The first or only dose was given 5 min after injection of vehicle (control) or β-adrenoceptor antagonist (bupranolol or CGP 20712A). Means ± s.e.m. of 3–8 rats. $^*P<0.05$, $^{**}P<0.01$, compared to control. For some points s.e.m. is contained within the symbols.

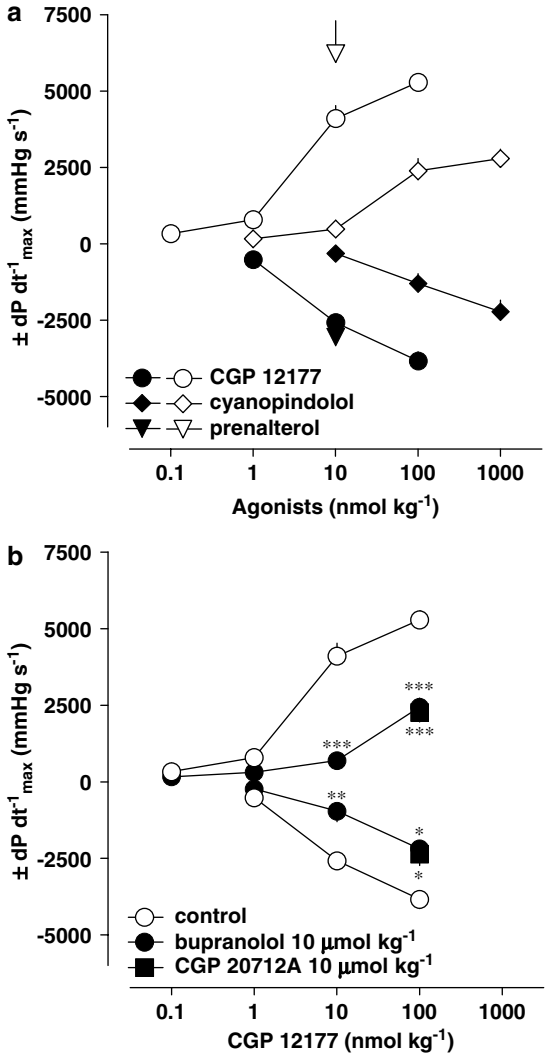

Figure 2 Effects of CGP 12177, cyanopindolol and prenalterol on $+\mathrm{d}P\,\mathrm{d}t^{-1}_{max}$ (upper half; a) and $-\mathrm{d}P\,\mathrm{d}t^{-1}_{max}$ (lower half; a) and interaction of CGP 12177 with bupranolol and CGP 20712A (b) in pithed and vagotomized rats. Each dose of β-adrenoceptor agonist (CGP 12177, cyanopindolol or prenalterol) was studied in separate rats although, in some cases, the two lower doses were applied to the same animal. The first or only dose was given 5 min after injection of vehicle (control) or β-adrenoceptor antagonist (bupranolol or CGP 20712A). Means ± s.e.m. of 3–8 rats. $^*P<0.05$; $^{**}P<0.01$, $^{***}P<0.001$ compared to control. For many points s.e.m. is contained within the symbols.

was not changed 30 min after its administration, whereas that elicited by cyanopindolol was reduced by about 74% at the same time-point.

In contrast to the agonists of the low-affinity state of β_1-adrenoceptors, all changes evoked by prenalterol lasted shorter. Thus, the prenalterol-induced increase in HR was reduced by about 44 and 59% at 15 and 30 min, respectively, whereas the increases in LVSP, $+\mathrm{d}P\,\mathrm{d}t^{-1}_{max}$ and $-\mathrm{d}P\,\mathrm{d}t^{-1}_{max}$ returned to the basal values within 15 min after its injection (Figure 5).

Influence of nonconventional partial β*-adrenoceptor agonists and prenalterol on vascular parameters*

CGP 12177 and cyanopindolol increased both DBP (Figure 6) and MBF (Figure 7). The increases in DBP elicited by CGP 12177 100 nmol kg^{-1} and cyanopindolol 1000 nmol kg^{-1} amounted to about 9 and 13% of the basal values, respectively (Figure 6a). Maximal changes were noticed 15–30 s after administration of the drug; they fully recovered toward baseline within 10 min. The elevation in MBF evoked by CGP 12177 100 nmol kg^{-1} and cyanopindolol 1000 nmol kg^{-1} amounted to about 31 and 13% of the basal values, respectively (Figure 7a), and occurred after 3 min; after 30 min MBF was still increased by about 22 and 10% of the

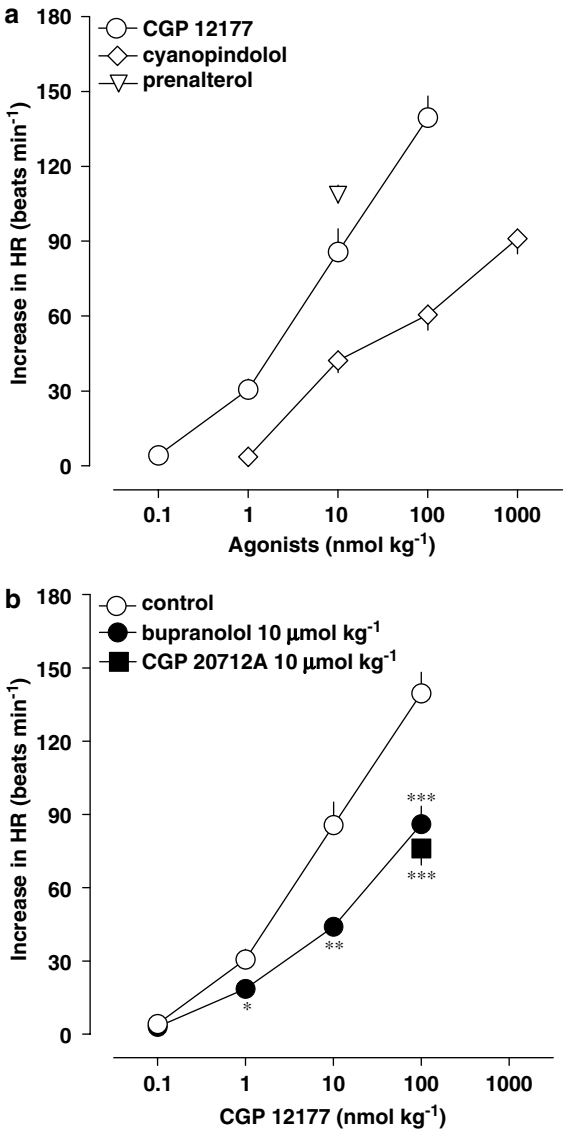

Figure 3 Effects of CGP 12177, cyanopindolol and prenalterol on HR (a) and interaction of CGP 12177 with bupranolol and CGP 20712A (b) in pithed and vagotomized rats. Each dose of β-adrenoceptor agonist (CGP 12177, cyanopindolol or prenalterol) was studied in separate rats although, in some cases, the two lower doses were applied to the same animal. The first or only dose was given 5 min after injection of vehicle (control) or β-adrenoceptor antagonist (bupranolol or CGP 20712A). Means ± s.e.m. of 3–8 rats. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to control. For many points s.e.m. is contained within the symbols.

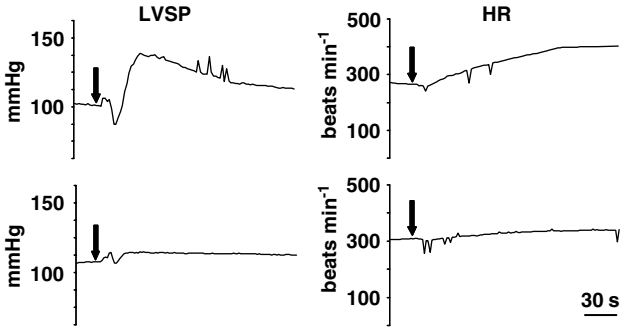

Figure 4 Effect of CGP 12177 10 nmol kg^{-1} on the LVSP (left panels) and on HR (right panels) and interaction with bupranolol 10 µmol kg^{-1} (lower panels) in pithed and vagotomized rats. The moment of administration of CGP 12177 is marked by the arrows; this occurred 5 min after injection of vehicle (control) or bupranolol. Note that the maximum increase in LVSP and HR was obtained 1 and 4 min after injection of CGP 12177, respectively. This type of experiment was repeated five times (upper panels) and six times (lower panels).

Table 1 Agonistic potencies of nonconventional partial β-adrenoceptors in pithed and vagotomized rats

	pED_{50}	
Parameter	*CGP 12177*	*Cyanopindolol*
Left ventricular systolic pressure (LVSP)	8.6	7.4
Rate of intraventricular pressure rise ($+dP\,dt^{-1}_{max}$)	8.4	7.4
Rate of ventricular relaxation ($-dP\,dt^{-1}_{max}$)	8.3	7.2
Heart rate (HR)	8.2	7.7
Diastolic blood pressure (DBP)	8.5	7.4
Mesenteric blood flow (MBF)	7.5	6.4

As pED_{50} for CGP 12177 and cyanopindolol, that dose was determined graphically from Figures 1–3 and 6 and 7 which caused an increase in LVSP by 15 and 10 mmHg, in $+dP\,dt^{-1}_{max}$ by 2600 and 1400 mmHg s^{-1}, in $-dP\,dt^{-1}_{max}$ by 1900 and 1100 mmHg s^{-1}, in HR by 75 and 50 beats min^{-1}, in DBP by 4 and 5 mmHg and in MBF by 0.7 and 0.3 ml min^{-1}, respectively.

basal value, respectively. The effects of both drugs on DBP occurred in a tenfold lower dose range than the effects on MBF. With respect to both vascular parameters, the potency of CGP 12177 exceeded that of cyanopindolol by 1.1 log units (for pED_{50} values, see Table 1 and for their definition, see Methods). Bupranolol and CGP 20712A failed to affect the CGP 12177-induced changes in both vascular parameters (Figures 6b and 7b).

Compared to CGP 12177 and cyanopindolol, the same dose of 10 nmol kg^{-1} of prenalterol produced higher increases in DBP and MBF, that is, the effects of CGP 12177 and cyanopindolol amounted to only about 71 and 16% (DBP) and 21 and 4% (MBF) of the effects of prenalterol, respectively (Figures 6a and 7a). Both changes induced by prenalterol were obtained 30–60 s after its administration. However, in contrast to the agonists of the low-affinity state of β_1-adrenoceptor, the increases in DBP and MBF were followed by decreases in both parameters. Thus, at 30 min they were diminished by about 26 and 48% of the basal value, respectively (data not shown).

Discussion

The aim of the present study was the direct comparison of cardiac and vascular effects of ligands at the low-affinity state of β_1-adrenoceptors in the same experiment, namely in the pithed and vagotomized rat. This model, already used by us previously (Malinowska & Schlicker, 1996; 1997; Malinowska *et al*., 2003), offers the opportunity to study drug effects on the peripheral cardiovascular system without interference with

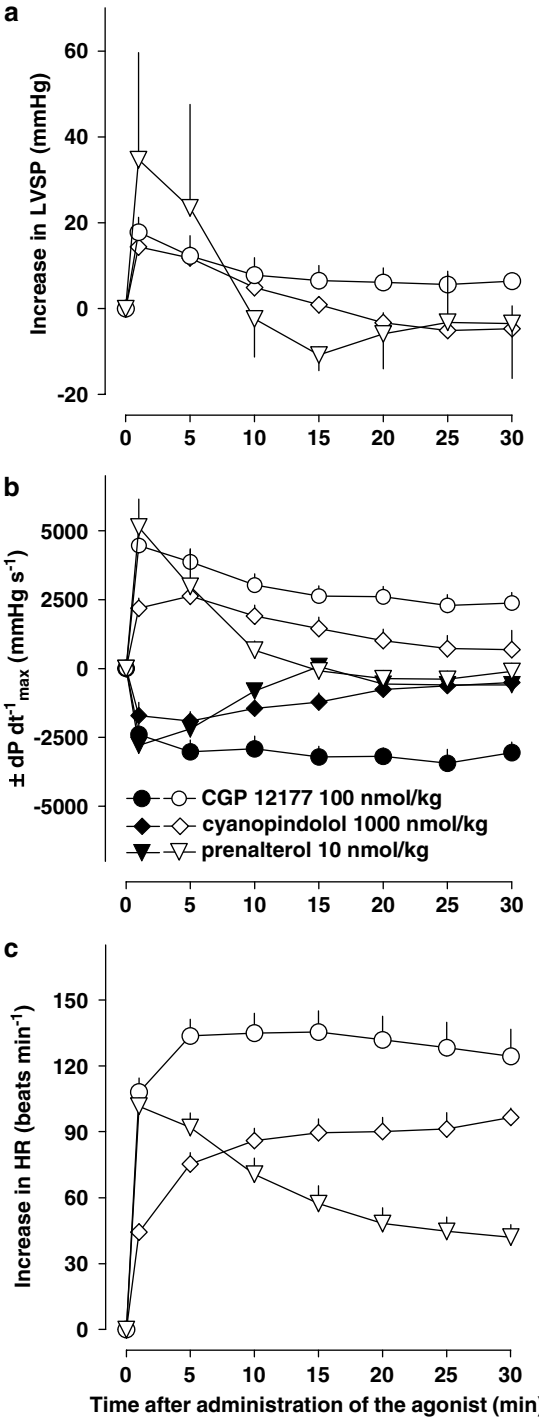

Figure 5 Time course of changes in LVSP (a), $+dP\,dt^{-1}_{max}$ (upper half of b), $-dP\,dt^{-1}_{max}$ (lower half of b) and HR (c) induced by CGP 12177, cyanopindolol and prenalterol in pithed and vagotomized rats. Means ± s.e.m. of 3–8 rats. For many points, s.e.m. is contained within the symbols.

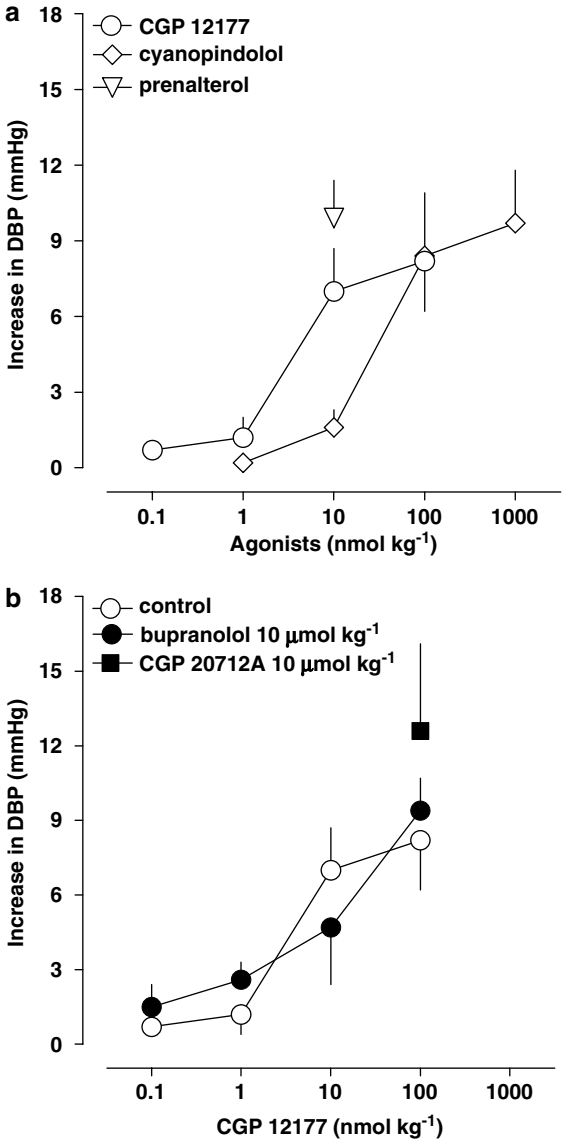

Figure 6 Effects of CGP 12177, cyanopindolol and prenalterol on DBP (a) and interaction of CGP 12177 with bupranolol and CGP 20712A (b) in pithed and vagotomized rats. Each dose of β-adrenoceptor agonist (CGP 12177, cyanopindolol or prenalterol) was studied in separate rats although, in some cases, the two lower doses were applied to the same animal. The first or only dose was given 5 min after injection of vehicle (control) or β-adrenoceptor antagonist (bupranolol or CGP 20712A). Means ± s.e.m. of 3–8 rats. For some points s.e.m. is contained within the symbols.

reflex loops implicating the central nervous system. The level of basal DBP was increased by infusion of vasopressin to allow for comparisons with our former studies and since changes in vascular parameters are more marked at a higher baseline level (e.g. Malinowska & Schlicker, 1993). We analyzed four cardiac parameters, (i) HR (examined by us in detail previously), (ii) the maximal inotropic action assessed by the LVSP and (iii) $+dP\,dt^{-1}_{max}$ and (iv) $-dP\,dt^{-1}_{max}$, a measure of the lusitropic action. Simultaneously, we studied two vascular parameters, namely (i) the DBP and (ii) the MBF. The latter parameter was considered since we have previously examined the vasodilator effects of ligands at the low-affinity state of β_1-adrenoceptors in isolated rat mesenteric arteries (Kozłowska *et al*., 2003; 2005).

We used two agonists (CGP 12177 and cyanopindolol) and two antagonists (bupranolol and CGP 20712A) of the low-affinity state of β_1-adrenoceptors. For the sake of comparison, the selective partial β_1-adrenoceptor agonist prenalterol has been studied as well. CGP 12177 and cyanopindolol belong to the so-called nonconventional partial β-adrenoceptor agonists, which activate the low-affinity state of β_1-adrenoceptors at

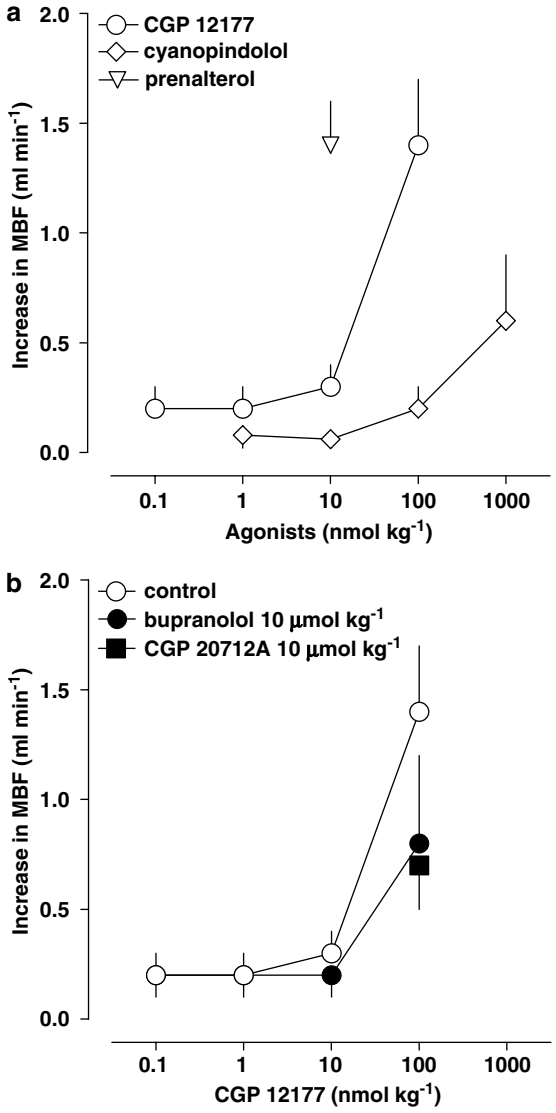

Figure 7 Effects of CGP 12177, cyanopindolol and prenalterol on MBF (a) and interaction of CGP 12177 with bupranolol and CGP 20712A (b) in pithed and vagotomized rats. Each dose of β-adrenoceptor agonist (CGP 12177, cyanopindolol or prenalterol) was studied in separate rats although, in some cases, the two lower doses were applied to the same animal. The first or only dose was given 5 min after injection of vehicle (control) or β-adrenoceptor antagonist (bupranolol or CGP 20712A). Means $\pm$ s.e.m. of 3–8 rats.

concentrations/doses much higher than those at which they block β_1- and/or β_2-adrenoceptors. The nonselective antagonist of β_1-, β_2- and β_3-adrenoceptors, bupranolol, still remains the best available tool to study the interaction of ligands with the low-affinity sites of both native and recombinant β_1-adrenoceptors (Malinowska *et al*., 2003; Joseph *et al*., 2004), and has also been shown to block these receptors in the isolated human atrium (Kaumann, 1996). In addition, we applied CGP 20712A at a high dose, at which this compound is capable of antagonizing the low-affinity β_1-adrenoceptor-mediated positive chronotropic and inotropic actions in the rat heart (e.g. Kaumann & Molenaar, 1996; Malinowska & Schlicker, 1996; 1997) and the agonistic response stimulated by these receptors in CHO cells expressing human β_1-adrenoceptors (Baker *et al*., 2003), but failed to counteract the β_3-adrenoceptor-mediated relaxation of rat colon (Kaumann & Molenaar, 1996) or thermogenesis in brown adipose tissue in pithed rats (Malinowska & Schlicker, 1997). Maximum agonistic effects could be determined for LVSP only. For the other parameters, the pED_{50} values correspond to the doses producing 50% of the effect elicited by the highest dose of the agonist and only represent rough estimates of the potencies of CGP 12177 and cyanopindolol.

Influence of nonconventional partial β*-adrenoceptor agonists on cardiac parameters*

The present study shows that CGP 12177 and cyanopindolol produced a strong and dose-dependent increase in myocardial contractility, as reflected by the enhancement of the LVSP and $+dP\,dt^{-1}_{max}$. In addition, the rate of ventricular relaxation reflected by $-dP\,dt^{-1}_{max}$ was also increased. Our results suggest that these effects are mediated *via* the cardiac low-affinity state of β_1-adrenoceptors. First, the effects of CGP 12177 were attenuated by bupranolol and CGP 20712A. Second, the efficacy of CGP 12177 exceeded that of cyanopindolol. Third, CGP 12177 displayed a higher potency than cyanopindolol. Virtually identical properties had been obtained for both drugs with respect to the increase in HR, which also involves the low-affinity state of β_1-adrenoceptors and was characterized in our previous papers (Malinowska & Schlicker, 1996; 1997). Admittedly, interaction experiments of cyanopindolol with bupranolol and CGP 20712A have not been carried out in the present study. However, the fact that the two antagonists counteracted the positive chronotropic effect of cyanopindolol (Malinowska & Schlicker, 1996; 1997) suggests that its positive inotropic and lusitropic effects involve the low-affinity state of β_1-adrenoceptors as well.

To our knowledge, this is the first paper demonstrating the positive inotropic and lusitropic effects mediated *via* the low-affinity state of β_1-adrenoceptor under *in vivo* conditions. We are aware of the fact that the parameter $-dP\,dt^{-1}_{max}$ depends on the actual ventricular pressure maximum and, therefore, does not characterize the lusitropic condition of heart independently from inotropic changes (Langer *et al*., 2005). On the other hand, our *in vivo* data conform well to previous results obtained on isolated human cardiac tissue, on which the positive lusitropic effect has been shown, using different experimental approaches (Kaumann & Molenaar, 1997; Sarsero *et al*., 1999; 2003; Joseph *et al*., 2003; Lewis *et al*., 2004).

The dependence of cardiac muscle contraction on heart frequency plays an important role in modulating heart function (Georgakopoulos & Kass, 2001). Thus, the question arises whether the changes in contraction observed in our study are dependent on the simultaneous, strong increase in HR stimulated by CGP 12177 and cyanopindolol? Such a dependence is, however, relatively unlikely in small rodents (including the rat), which display a flat force–frequency dependence at physiologically relevant frequencies (Georgakopoulos & Kass, 2001). One piece of evidence that the positive chronotropic and inotropic effects mediated *via* the low-affinity state of β_1-adrenoceptor are relatively independent from each other is our finding that the positive chronotropic effect of both agonists was stable, whereas their positive inotropic action (both LVSP and $+dP\,dt^{-1}_{max}$) was reduced by about 50% over a time period of 30 min.

In additional experiments we examined the cardiac effects of prenalterol, an agonist (partial) at the high-affinity state of β_1-adrenoceptors. The dose–response relationship for this drug for its positive chronotropic effect in the pithed rat had been examined in one of our previous papers (Malinowska & Schlicker, 1996). Qualitatively, the cardiac effects of prenalterol, CGP 12177 and cyanopindolol are very similar. They share positive chronotropic, inotropic and lusitropic effects. In addition, they resemble each other with respect to the time course of the three effects; thus, the positive inotropic and lusitropic effects are faster than the positive chronotropic effect (with one exception, namely, the positive lusitropic effect of CGP 12177 had the same time course like its positive chronotropic effect). The cardiac effects elicited by the nonconventional partial agonists showed much slower kinetics than the corresponding effects induced by prenalterol. It would be an intriguing idea to assume that the discrepancy reflects different velocities in signal transduction between the two cardiostimulatory β-adrenoceptors; however, the possibility that it simply reflects different pharmacokinetic properties of the three drugs is at least as likely.

A potential clinical significance of the low-affinity state of β_1-adrenoceptors has to be considered. There is increasing evidence that activation of these receptors, which elicit ventricular and atrial arrhythmias (Lowe *et al*., 1998; Freestone *et al*., 1999; Sarsero *et al*., 1999), contributes to the intrinsic sympathomimetic activity (ISA) shown by pindolol and alprenolol in the human heart (Lowe *et al*., 2002). It has been suggested that activation of the low-affinity state of β_1-adrenoceptors by the latter two compounds and by bucindolol might be implicated in the failure of these β-adrenoceptor antagonists to increase survival when taken by patients after myocardial infarction (Bundkirchen *et al*., 2002; Lowe *et al*., 2002). Indeed, the therapeutic plasma level of pindolol conforms to the dissociation constant of this drug for its positive inotropic and/or chronotropic effects in the isolated heart from humans (Joseph *et al*., 2003), guinea-pigs (Kaumann, 1989) and ferrets (Lowe *et al*., 2002).

Influence of nonconventional partial β*-adrenoceptor agonists on vascular parameters*

In contrast to the four cardiac parameters, CGP 12177 and cyanopindolol influenced DBP only slightly, that is, at the highest doses increased this parameter by about 10%. Although the potencies of CGP 12177 and cyanopindolol for inducing the marginal vasopressor effect and the strong cardiac stimulation were identical, the former effect is not mediated by the low-affinity state of β_1-adrenoceptors, since it was not affected by bupranolol or CGP 20712A. The latter two drugs also failed to alter the increasing effect of CGP 12177 on MBF, suggesting that the effects of CGP 12177 and cyanopindolol on MBF are also not related to the activation of the low-affinity state of β_1-adrenoceptors. The effects of CGP 12177 and cyanopindolol on MBF occurred in a ten-fold higher dose range when compared to their effects on the other cardiovascular parameters, suggesting that the effects on MBF do not markedly contribute to the overall effect of the two drugs.

We have previously shown that CGP 12177 and cyanopindolol caused complete relaxation of the isolated rat mesenteric artery preconstricted with phenylephrine mainly through their α_1-adrenolytic properties (Kozłowska *et al*., 2005). At first glance, the same mechanism might also explain the increase in MBF in the pithed rat; the increase in DBP (although occurring in a lower dose range) might be due to a partial agonism at α_1-adrenoceptors. However, blockade of, or partial agonism at, α_1-adrenoceptors can be excluded on the basis of two observations. (i) Compared to the present *in vivo* study, an opposite rank order of potency (cyanopindolol > CGP 12177) was obtained for the isolated vessels (Brawley *et al*., 2000; Kozłowska *et al*., 2003; 2005). (ii) Preliminary studies from our laboratory show that the increasing effects of CGP 12177 on DBP and MBF were not altered by combined administration of the α_1-adrenoceptor antagonist prazosin and the α_2-adrenoceptor antagonist rauwolscine. Additional experiments will be necessary to clarify the exact mechanism of action of CGP 12177 and cyanopindolol and of prenalterol, which showed a biphasic effect both on DBP and MBF.

Conclusions

The present study reveals that the activation of the low-affinity state of β_1-adrenoceptors by CGP 12177 and cyanopindolol causes positive inotropic and lusitropic effects also *in vivo*, which had been shown so far under *in vitro* conditions only. In addition, we found that the vascular effects of CGP 12177 and cyanopindolol, which are marginal under *in vivo* conditions, are not related to the activation of the low-affinity state of β_1-adrenoceptors. Both observations might possess clinical significances since well-known β-adrenoceptor antagonists such as pindolol, bucindolol or alprenolol have been demonstrated to bind to the low-affinity state of β_1-adrenoceptors.

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