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SPECIAL REPORT

Stimulation of cyclic AMP-dependent protein kinase in rat atria by (−)-CGP 12177 through an atypical β-adrenoceptor

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Mammalian hearts possess an atypical β-adrenoceptor (non-β_1, non-β_2, non-β_3) through which (−)-4-(3-t-butylamino-2-hydroxypropoxy)benzimidazol-2-one ((−)-CGP 12177) causes cardiostimulant effects. Here we showed that (−)-CGP 12177 increased the activity of adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-dependent protein kinase in the presence of 200 nM (−)-propranolol in rat atria at a concentration (10 μM) that elicits maximum positive chronotropic and inotropic effects. The phosphodiesterase inhibitor 3-isobutyl-l-methylxanthine (IBMX) potentiated the positive chronotropic and inotropic effects of (−)-CGP 12177. We suggest that the atypical β-adrenoceptor is coupled positively to the Gs protein-adenylyl cyclase system.

Keywords: Atypical β-adrenoceptor; rat atrium, (−)-CGP 12177, cyclic AMP-dependent protein kinase; contractile force; sinoatrial rate

Introduction In several mammalian hearts, including human, both β_1- and β_2-adrenoceptors can mediate cardiostimulant effects through an adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-dependent pathway. Both cardiac β_1- and β_2-adrenoceptors are usually coupled to the Gs protein-adenylyl cyclase system, although the latter more tightly than the former, at least in human ventricle and atrium (Kaumann & Lemoine, 1987). Recently Gauthier *et al.* (1996) provided evidence for human ventricular β_3-adrenoceptors mediating reductions in contractile strength and action potential duration elicited by β_3-adrenoceptor-selective agonists. The cardioinhibitory effects of a β_3-adrenoceptor agonist were reduced by pertussis toxin, suggesting that human ventricular β_3-adrenoceptors are coupled to a Gi protein (Gauthier *et al.*, 1996).

In addition to β_1-, β_2- and β_3-adrenoceptors, the existence of an atypical β-adrenoceptor has been proposed to exist in mammalian heart (Kaumann, 1989), including human atrium (Kaumann, 1996). Although this receptor has certain properties in common with the β_3-adrenoceptor (Kaumann, 1989), it is neither activated nor blocked by agonists selective for the β_3-adrenoceptor (Malinowska & Schlicker, 1996; Kaumann & Molenaar, 1996) and is also resistant to blockade by a β_3-adrenoceptor-selective antagonist (Kaumann & Molenaar, 1996) indicating that it is different from the β_3-adrenoceptor. Because the atypical β-adrenoceptor mediates cardiostimulant effects of a series of non-conventional partial agonists (Kaumann, 1989), we have now tested the hypothesis that these effects occur through a cyclic AMP-dependent pathway. We used the non-conventional partial agonist (−)-CGP 12177 to investigate its effects in rat atria on the activity of the cyclic AMP-dependent protein kinase (PKA) at a concentration that causes maximally positive chronotropic and inotropic effects through the atypical β-adrenoceptor (Kaumann & Molenaar, 1996).

Methods *Isolated atria* Experiments were carried out at 37°C on spontaneously beating right atria and paced left atria (2 Hz) of Wistar rats of either sex (200–350 g), suspended in modified Krebs solution, and beating rate and contractile force were measured as described by Kaumann & Molenaar (1996). To block both β_1- and β_2-adrenoceptors all experiments were carried out in the presence of 200 nM (−)-propranolol, a concentration that does not modify the cardiostimulant effects of (−)-CGP 12177 (Kaumann, 1996; Kaumann & Molenaar, 1996). A single cumulative concentration-effect curve to (−)-CGP 12177 was carried out per tissue, either in the absence or presence of 3-isobutyl-l-methylxanthine (IBMX), preincubated for 12 min. Chronotropic data were expressed in beats min^{-1}. Inotropic data were expressed in mN contractile force.

PKA assay PKA activity levels were determined in freeze-clamped atria that had been exposed or not exposed to 200 μM (−)-isoprenaline or 10 μM (−)-CGP 12177 for 2 min, to 10 μM IBMX for 12 min alone or 10 μM IBMX for 10 min followed by 10 μM (−)-CGP 12177 for 2 min. PKA activity was assayed as described by Sanders *et al.* (1996) and is expressed as an activity ratio. This was obtained by dividing the PKA activity determined in the absence of exogenously added cyclic AMP by that obtained in the presence of 1 μM exogenously added cyclic AMP, calculated to activate fully the tissue PKA.

Data analysis The data are expressed as mean ± s.e.mean. Student's unpaired *t* test or one-way analysis of variance followed by Tukey's method for multiple-comparisons were used with the help of Minitab. $P<0.05$ and $P<0.01$ were used as the limit for statistical significance.

Drugs (−)-4-(3-t-butylamino-2-hydroxypropoxy)benzimidazol-2-one ((−)-CGP 12177) hydrochloride was a gift of Dr Lee Beeley (SmithKline Beecham, Epsom, U.K.). (−)-Propranolol, (−)-isoprenaline and IBMX were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.) and [γ-^{32}P]-ATP from NEN-Dupont (Stevenage, U.K.). The PKA substrate malantide was synthesized at the Babraham Institute.

Results PKA data are shown in Table 1. (−)-CGP 12177 (10 μM) increased PKA activity in right atria and left atria. IBMX (10 μM) increased basal PKA activity in both right and left atria. In the presence of IBMX, (−)-CGP 12177 caused further significant increases of PKA activity in both right and left atria. (−)-Isoprenaline (200 μM) increased PKA activity 3 to 4 times more than (−)-CGP 12177, in atria both treated and not treated with IBMX. (−)-Isoprenaline (40 nM), a concentration that in the presence of (−)-propranolol 200 nM causes matching positive chronotropic and inotropic effects to those of (−)-CGP 12177 (10 μM), caused an increase in PKA

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Table 1 Cardiostimulant effects and protein kinase A (PKA) activation by (−)-CGP 12177 (CGP), (−)-isoprenaline (Iso) and IBMX

	Right atrium				*Left atrium*			
Condition	*Rate* (beats min^{-1})	n	*PKA ratio*	n	*Force (mN)*	n	*PKA ratio*	n
Basal	285±4	73	0.185±0.006	15	2.2±0.1	74	0.159±0.007	17
CGP 10 μM	372±1.0[a]	12	0.247±0.015[a]	12	6.4±0.6[a]	13	0.214±0.010[a]	13
Iso 40 nM	378±13[a]	7	0.254±0.011[a]	7	5.9±0.8[a]	7	0.235±0.006[a]	7
Iso 200 μM	459±13[a]	9	0.400±0.018[a]	9	8.5±1.0[a]	9	0.323±0.014[a]	9
IBMX 10 μM	337±9[a]	18	0.312±0.006[a]	6	3.9±0.4[a]	17	0.263±0.006[a]	6
IBMX 10 μM +CGP 10 μM	410±5[c]	6	0.402±0.026[b]	6	7.1±0.7[c]	5	0.374±0.030[c]	5
IBMX 10 μM +Iso 200 μM	471±16[c]	6	0.636±0.030[c]	6	9.5±0.9[c]	6	0.619±0.022[c]	6

All experiments were carried out in the presence of 200 nM (−)-propranolol. Data are presented as means±s.e.mean. [a]$P<0.01$ compared to basal. [b]$P<0.05$ compared to IBMX. [c]$P<0.01$ compared to IBMX.

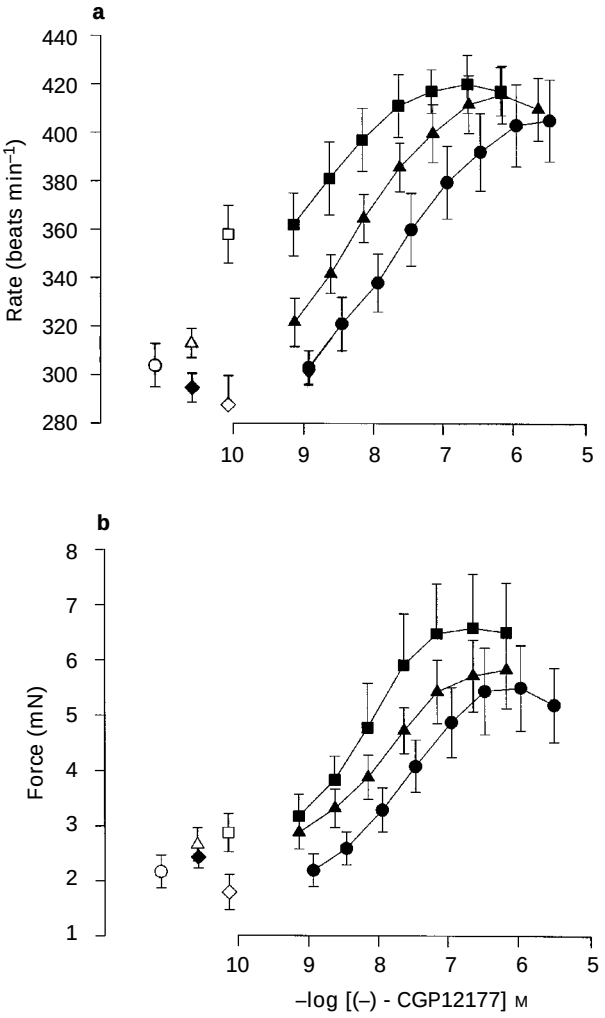

Figure 1 Enhancement of the effects of (−)-CGP 12177 by IBMX. (a) Right atria; (b) left atria. Concentration-effect curves to (−)-CGP 12177 in the absence (●) and presence of IBMX 1 μM (▲) and 10 μM (■) are shown; the corresponding basal values before the administration of (−)-CGP 12177 (○) and IBMX 1 μM (◆), 10 μM (◇), shown on the left, and the effects of IBMX 1 μM (△) and 10 μM (□) alone. Data shown are mean of 4–8 tissues; vertical lines indicate s.e.mean.

activity similar to and not statistically different from that produced by (−)-CGP 12177.

IBMX (10 μM) elicited positive chronotropic and inotropic effects (Figure 1 and Table 1). IBMX (1 μM) only caused marginal effects (Figure 1). Both concentrations of IBMX shifted the concentration-effect curves to (−)-CGP 12177 to the left (Figure 1). The chronotropic -log EC_{50} of (−)-CGP 12177 was 7.60±0.07 in the absence of IBMX and 8.09±0.06 ($P<0.001$) and 8.30±0.14 ($P<0.005$) in the presence of IBMX 1 and 10 μM, respectively. The inotropic -log EC_{50} was 7.67±0.03 in the absence of IBMX and 7.98±0.06 ($P<0.005$) and 8.23±0.10 ($P<0.005$) in the presence of IBMX 1 and 10 μM, respectively.

Discussion Our experiments suggest that the cardiostimulant effects of (−)-CGP 12177 occur through a cyclic AMP-dependent pathway and may result from PKA-dependent phosphorylation of ion channels and contractile proteins leading to increased sinoatrial rate and enhanced contractile force. The involvement of a cyclic AMP-dependent pathway is also supported by synergy of the positive chronotropic and inotropic effects of (−)-CGP 12177 and IBMX, as shown by the sensitization of the atria to (−)-CGP 12177 by IBMX. For matching positive chronotropic and inotropic effects in the presence of 200 nM (−)-propranolol, (−)-isoprenaline at 40 nM and (−)-CGP 12177 at 10 μM also produced matching increases in PKA activity, suggesting, but not proving, that the atypical β-adrenoceptor could be coupled as tightly to effectors as the β_1-adrenoceptor. The positive chronotropic effects of catecholamines in rat atrium are mediated mainly through β_1-adrenoceptors and only to a small extent through β_2-adrenoceptors (Kaumann, 1986). (−)-Isoprenaline 40 nM and 200 μM is assumed to surmount partially and completely, respectively, the blockade of both β_1- and β_2-adrenoceptors by (−)-propranolol (200 nM). The stimulation of PKA activity by (−)-isoprenaline 40 nM (in the presence of (−)-propranolol 200 nM) appears therefore to be mainly the result of an interaction with β_1-adrenoceptors, although a small contribution of β_2-adrenoceptors cannot be ruled out. The stimulation of PKA activity by (−)-CGP 12177, on the other hand, appears mostly due to activation of the atypical β-adrenoceptor. We have recently labelled with (−)-[^{3}H]-CGP 12177 the rat atrial atypical β-adrenoceptor for which catecholamines compete in stereoselective manner. The density of the atypical β-adrenoceptors is 2 fold higher than that of β_1-adrenoceptors (also labelled with (−)-[^{3}H]-CGP 12177), perhaps suggesting that the latter are coupled more tightly than the former to the Gs protein-adenylyl cyclase system (Sarsero *et al.*, 1997).

Our findings that the cardiac atypical β-adrenoceptor is likely to be coupled to the Gs protein-adenylyl cyclase system may be a useful landmark for the cloning of this receptor.

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