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Phosphodiesterase PDE3 blunts the positive inotropic and cyclic AMP enhancing effects of CGP12177 but not of noradrenaline in rat ventricle

[1]Maria Luisa Vargas, [1]Jesus Hernandez & *,[2]Alberto J. Kaumann

[1]Department of Pharmacology, University of Murcia, Murcia, Spain and [2]Department of Physiology, University of Cambridge, Downing Street, Cambridge CB2 3EG

1 The cardiostimulant effects of CGP12177, mediated through a β_1-adrenoceptor site with low affinity for (−)-propranolol, are potentiated by the nonselective PDE inhibitor IBMX but the role of PDE isoenzymes is unknown. We studied the effects of the PDE3-selective inhibitor cilostamide (300 nM) and PDE4-selective inhibitor rolipram (1 μM) on the positive inotropic and cyclic AMP-enhancing effects of CGP12177 and noradrenaline in right ventricular strips of rat.

2 CGP12177 (under (−)-propranolol 200 nM) only increased contractile force in the presence of either cilostamide or rolipram with $-\log EC_{50}M$ 6.7 ($E_{max} = 23\%$ over basal) and 7.1 ($E_{max} = 50\%$) respectively. The combination of cilostamide and rolipram caused CGP12177 to enhance contractile force with $-\log EC_{50}M = 7.7$ and $E_{max} = 178\%$.

3 The positive inotropic effects of noradrenaline ($-\log EC_{50}M = 6.9$) were potentiated by rolipram ($-\log EC_{50}M = 7.4$) but not by cilostamide ($-\log EC_{50}M = 7.0$).

4 In the presence of rolipram and (−)-propranolol, noradrenaline (2 μM) and CGP12177 (10 μM) produced matching inotropic effects but failed to increase cyclic AMP levels. 20 μM (−)-noradrenaline increased cyclic AMP levels, a response further enhanced by rolipram.

5 Both PDE3 and PDE4 of rat ventricle appear to hydrolyse cyclic AMP generated through the low-affinity β_1-adrenoceptor site, thereby preventing inotropic responses of CGP12177. When (−)-noradrenaline interacts with the β_1-adrenoceptor, the generated cyclic AMP is hydrolysed only by PDE4, thereby reducing cardiostimulation.
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Abbreviations: CGP12177, (±)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimidazole-2-one); IBMX, isobutyl-methyl-xanthine; ICI118551, (±)-1-(2,3-(dihidro-7-methyl-1H-inden-4-yl)oxy)-3-((1-methylethyl)amino)-2-butanol hydrochloride; PDE, phosphodiesterase

Introduction

Two binding sites of functional relevance have been identified at cardiac (Sarsero *et al*., 1998; 1999; 2003; Kaumann *et al*., 2001) and recombinant β_1-adrenoceptors (Joseph *et al*., 2004a, b). One site mediates the effects of both catecholamines and conventional partial agonists and has high affinity (H-site) for β-blockers (e.g. (−)-propranolol). The other site (L-site) has low affinity for β-blockers and mediates the agonist effects of some β-blockers, coined non-conventional partial agonists (Kaumann, 1989). The cardiostimulant effects of non-conventional partial agonists, previously attributed to be mediated through a putative β_4-adrenoceptor, are now accepted to be mediated through the L-site of the β_1-adrenoceptor (Kaumann, 2000; Granneman, 2001; Kaumann *et al*., 2001; Joseph *et al*., 2003; Lewis *et al*., 2004) and resistant to blockade by (−)-propranolol (Kaumann, 1989).

An experimental prototype of nonconventional partial agonist is CGP12177, a hydrophilic compound (Staehelin *et al*., 1983), which has cardiostimulant effects in several species (Kaumann, 1983; Kaumann & Molenaar, 1996; Kaumann *et al*., 1998; Lowe *et al*., 2002) including man (Kaumann, 1996; Sarsero *et al*., 2003), shown to be mediated through the L-site of the β_1-adrenoceptor of both cardiac tissues (Sarsero *et al*., 1998; 1999; 2003; Joseph *et al*., 2003) and recombinant receptors (Pak & Fishman, 1996; Konkar *et al*., 2000; Baker *et al*., 2003; Joseph *et al*., 2003; 2004a, b). CGP12177 enhances cyclic AMP (Kaumann *et al*., 1997) and stimulates cyclic AMP-dependent protein kinase (PKA) in rat (Kaumann & Lynham, 1997) and human heart (Sarsero *et al*., 2003). The cardiostimulant effects of CGP12177 are potentiated by the nonselective phosphodiesterase (PDE) inhibitor isobutyl-methyl-xanthine (IBMX) (Kaumann & Lynham, 1997: Sarsero *et al*., 2003), but it is unknown which PDE isoenzymes hydrolyse the cyclic AMP generated through activation of the low-affinity site of the β_1-adrenoceptor. The PDE isoenzymes that hydrolyse cyclic AMP in the rat heart are mainly PDE3 and PDE4 (Nicholson *et al*., 1991; Verde *et al*., 1999). We therefore investigated the influence of the PDE3 inhibitor cilostamide (Beavo, 1995) and PDE4 inhibitor rolipram (Beavo, 1995) on the effects of CGP12177 on contractile force and cyclic AMP levels in strips of rat right ventricle.

*Author for correspondence; E-mail: ajk41@hermes.cam.ac.uk

Methods

Paced ventricular strips

Male Sprague–Dawley rats (250–350 g) were stunned and exsanguinated. The chest was opened and the heart rapidly removed and placed in Tyrode solution saturated with 95% O_2/5% CO_2 and the free wall of the right ventricle was excised. All procedures were performed in the presence of Tyrode solution of the following composition (mM): NaCl 136.9, KCl 5.0, $CaCl_2$ 1.8, $MgCl_2$ 1.5, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and dextrose 5.0, pyruvate 5, and ascorbate 0.2. Right venticular strips (2 mm wide, 10 mm long) were mounted longitudinally between two platinum electrodes in a 30 ml organ bath under 1 g tension in Tyrode's solution maintained at 37°C and gassed with 95% O_2/5% CO_2. The preparations were electrically stimulated (Grass SD-9 stimulator) with square wave pulses (1 Hz, 1 ms duration, just over threshold voltage). Contractions were measured using a force-displacement transducer (Grass FT-03) and displayed on the screen of a computer by using a Stemtech amplifier (Stemtech Inc., Houston, Texas, U.S.A.) and the ACODAS computer software (Dataq Instruments, Ohio, U.S.A.). Tissues were allowed to equilibrate for 1 h before drug challenge.

Contractility studies with CGP12177 and (−)-noradrenaline

All experiments were performed in the presence of phentolamine (1 μM), desipramine (2 μM), corticosterone (30 μM) to block α-adrenoceptors, and to inhibit neuronal and extraneuronal uptakes, respectively. Experiments with (−)-noradrenaline were carried out also in the presence of ICI118551 (50 nM) to block β_2 adrenoceptors or (−)-propranolol (200 nM) to obtain a condition identical to that used for studies with CGP12177. Experiments with CGP12177 were carried out in the presence of (−)-propranolol (200 nM) to block the high-affinity site of the β_1-adrenoceptor (Joseph *et al*., 2004a). Concentration–effects curves to the agonists were determined by cumulative addition of half-log increments in concentration.

The concentrations of cilostamide and rolipram used in this study are known to potentiate the effects of some cAMP-dependent inotropic agents (Verde *et al*., 1999). The PDE inhibitors were left in contact with the tissue for 15 min before an agonist was added. $-\log EC_{50}$ values were calculated by nonlinear regression analyses of concentration–responses curves, using Prism (Graph Pad Software).

Measurement of cyclic AMP

In order to investigate whether contractile responses correlated with cyclic AMP levels in the same preparation, basal force and agonist-induced force were measured and the tissues frozen in liquid nitrogen immediately 3 min after the agonist addition. To allow systematic conditions, all experiments were carried out in the presence of (−)-propranolol (200 nM). A maximum inotropically effective concentration of CGP12177 (10 μM) was used. Concentrations of (−)-noradrenaline (200 nM, 2 μM and 20 μM) were chosen to gradually surmount the blockade by (−)-propranolol in order to estimate from interpolation matching inotropic effects with the effects of CGP12177.

Levels of cyclic AMP were measured by radioimmunoassay [^{125}I]TME-S-cAMP; Diagnostic Pasteur, France), according to the manufacture's instructions. Incubation time with the PDE inhibitors was 15 min, similar to that used by other authors (Katano & Endoh, 1992; Verde *et al*., 1999). After freezing, the tissue was weighed and homogenized in cold perchloric acid 1.5 ml (0.3 M) with a Polytron homogenizer (setting 4 for 30 s) and centrifuged (10,000 × *g*, 4°C, 15 min). The supernatants were treated with potassium hydroxide until pH 6.2 was reached. The sensitivity of the assay was 2 pmol ml^{-1}. Intra and inter-assay coefficients of variation were 7.7 and 8.2%, respectively. The antibody cross-reacted 100% with 3′, 5′-cyclic AMP and <0.3% with other nucleotides. Cyclic AMP concentrations were expressed as nmol g^{-1} of tissue.

Drugs

ICI118551 was purchased from Tocris (London, U.K.). (±)-CGP12177, (−)-noradrenaline, (−)-propranolol, corticosterone, rolipram and cilostamide were purchased from Sigma Chemicals (Madrid, Spain). Corticosterone, rolipram y cilostamide were dissolved in dimethylsulfoxide and Tyrode solution (20% dimethylsulfoxide in Tyrode's solution). Stock solutions (3 mM) were diluted into prewarmed and preoxygenated bathing solution at the desired concentrations. Drugs were added to the organ bath so that the concentration of dimethylsulfoxide was <0.1%, which by itself did not modify contractile force.

Statistical analysis

Results are expressed as mean ± s.e.m. values. Significance between differences was assessed with two-way ANOVA, followed by Bonferroni correction, or paired Student's *t*-tests. $P<0.05$ was considered significant. Number of experiments refers to number of rats.

Results

Cilostamide and rolipram induce inotropic effects of CGP12177

CGP12177 alone did not produce positive inotropic effects. However, in the presence of either cilostamide or rolipram CGP12177 caused concentration-dependent increases in contractile force with $-\log EC_{50}M$ of 6.66 ± 0.19 ($n=6$) and 7.10 ± 0.09 ($n=6$) and E_{max} (% increase over basal) of 23 ± 4 and 50 ± 11%, respectively (Figure 1). The effects of CGP12177 were potentiated in the presence of both cilostamide and rolipram ($-\log EC_{50}M = 7.66 \pm 0.17$, $E_{max} = 178 \pm 12\%$, $n=6$).

Potentiation of the contractile effects of (−)-noradrenaline by rolipram but not by cilostamide

The positive inotropic effects of (−)-noradrenaline in the presence of ICI118551 (50 nM) ($-\log EC_{50} = 6.90 \pm 0.09$, $n=6$) were potentiated by rolipram ($-\log EC_{50}M = 7.41 \pm 0.06$, $n=6$,

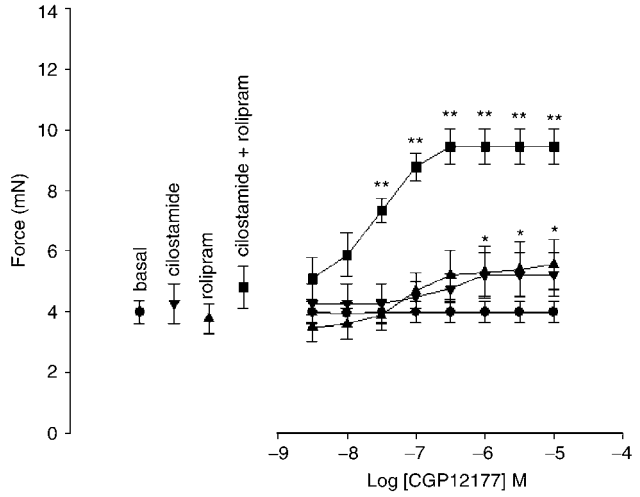

Figure 1 Cilostamide (300 nM) and rolipram (1 μM), administrated separatedly and in combination, uncover positive inotropic effects of CGP12177. *$P<0.05$ with respect to cilostamide or rolipram alone. **$P<0.01$ with respect to the combination of cilostamide + rolipram alone. Statistics were by paired Student's t-test. Results from six to nine rats for each group.

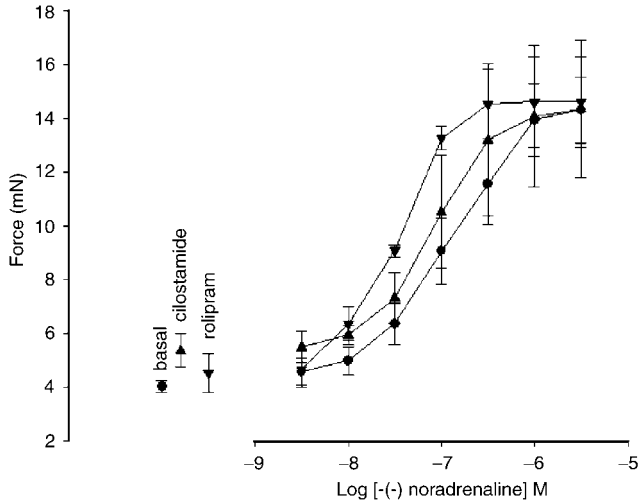

Figure 2 Potentiation of the positive inotropic effects of (−)-noradrenaline by rolipram but not by cilostamide. Results from six rats for each group. For further details see text.

$P<0.01$) but not by cilostamide ($-\log EC_{50}M = 7.00 \pm 0.18$; $n=6$) (Figure 2).

Effects of cilostamide and rolipram on the inotropic and cyclic AMP responses to (−)-noradrenaline and CGP12177

The inotropic effects of non-accumulative additions of (−)-noradrenaline (0.2, 2 and 20 μM) and of CGP12177 (10 μM) (Figure 3a) were investigated in the presence of propranolol (200 nM) and the corresponding cyclic AMP levels compared to basal levels in the absence of the agonists (Figure 3b). As observed with cumulative additions in the absence of (−)-propranolol (Figure 2), the effects of 2 and 20 μM (−)-noradrenaline were also significantly ($P<0.05$) increased by rolipram but not by cilostamide. Cilostamide and rolipram, administered together, potentiated the inotropic effects of (−)-noradrenaline, which were maximal at 2 and 20 μM (Figure 3a).

Only the inotropic effects of 20 μM (−)-noradrenaline, but not of lower concentrations, was accompanied by a significant increase in cyclic AMP level (Figure 3b). Rolipram, but not cilostamide, significantly ($P<0.05$) further increased cyclic AMP levels induced by 20 μM (−)-noradrenaline (Figure 3b). Cilostamide and rolipram, administered together, caused (−)-noradrenaline to markedly increase cyclic AMP in a concentration-dependent way (Figure 3b). Neither cilostamide nor rolipram, administered alone or in combination, changed significantly basal contractile force (Figure 3a). Neither cilostamide nor rolipram, administered separatedly or in combination, produced significant changes in cyclic AMP levels (Figure 3b).

As observed in Figure 1, CGP12177 (10 μM) only enhanced force in the presence of rolipram ($P<0.05$) or cilostamide ($P<0.05$) (Figure 3a). Rolipram induced 10 μM CGP12177 and 2 μM (−)-noradrenaline to produce matching increases in contractile force (Figure 3a) without measurable increases in cyclic AMP levels (Figure 3b). The simultaneous administra-

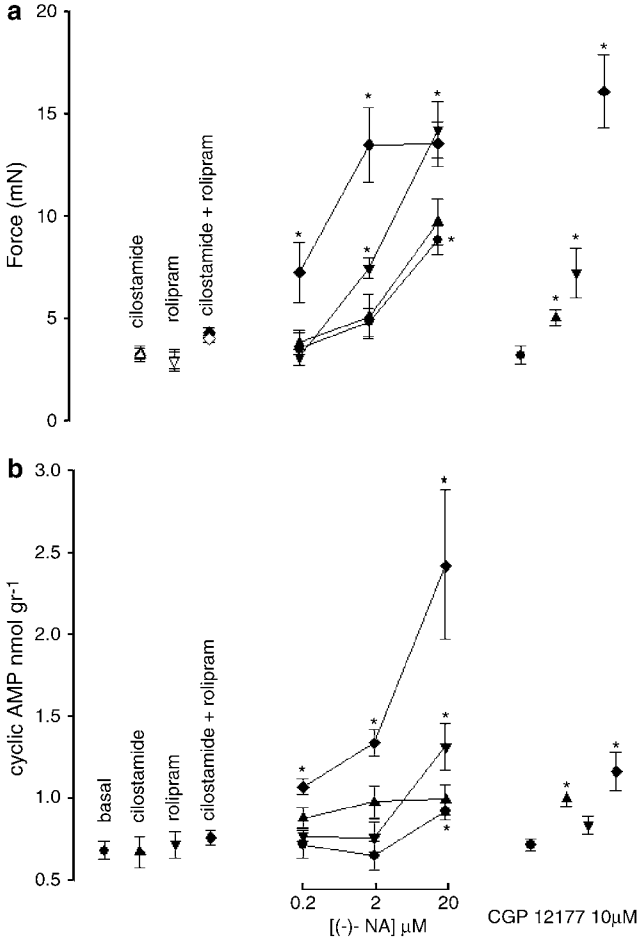

Figure 3 Comparison of the effects of cilostamide (300 nM) and rolipram (1 μM), administrated separatedly and in combination, on the positive inotropic (a) and cyclic AMP-elevating (b) effects of (−)-noradrenaline (0.2, 2 and 20 μM) and CGP12177 (10 μM). *$P<0.05$ with respect to agonist in the absence of PDE inhibitor. Statistcs were by two-way ANOVA with Bonferroni correction. Results from six to nine rats for each group with agonists. Basal cyclic AMP values were from 12 rats with a contractile force of 4.0 ± 0.37 mN.

tion of cilostamide and rolipram caused 10 μM CGP12177 to increase contractile force to an extent not significantly different from the force increase elicited by 2 and 20 μM (−)-noradrenaline under the same condition. The increase in cyclic AMP levels evoked by 10 μM CGP12177 with the combination of cilostamide and rolipram was similar to that caused by 2 μM (−)-noradrenaline but was significantly lower than that caused by 20 μM (−)-noradrenaline (Figure 3b). A lower concentration (1 μM) of CGP12177 also produced a maximal increase in contractile force and an increase in cyclic AMP not different from the effects of 10 μM CGP12177. Contractile force was increased from a basal of 4.0 ± 0.5 mN to 12.2 ± 1.3 mN with CGP12177 (1 μM) and cyclic AMP from 0.68 ± 0.05 to 1.26 ± 0.10 nmol g^{-1} in the presence of both cilostamide and rolipram (results not shown).

Discussion

Our results indicate for the rat ventricle that both PDE3 and PDE4 hydrolyse cyclic AMP generated through CGP12177-evoked activation of the low-affinity β_1-adrenoceptor site (Figure 4), thereby preventing cardiostimulation. In contrast, only PDE4 but not PDE3, hydrolyses cyclic AMP generated through (−)-noradrenaline-evoked activation of the high-affinity β_1-adrenoceptor site (Figure 4), thereby reducing cardiostimulation.

Activation of the low-affinity site but not of the high-affinity site of the β_1-adrenoceptor site enhances cyclic AMP in a PDE3 compartment

The involvement of PDE3 is supported by the appearance of both inotropic stimulation and a cyclic AMP signal produced by CGP12177 in the presence of the PDE3-selective inhibitor cilostamide (300 nM). Half maximal inhibition (IC_{50} values) of PDE3 and PDE4 by cilostamide occurs at around 50 nM (72 nM, Beavo 1995; 27 nM, Sudo *et al*., 2000), and 89 μM (Sudo *et al*., 2000) respectively. Therefore, under our conditions, using 300 nM cilostamide, approximately 86% of PDE3 would be inhibited with negligible (<0.4%) inhibiton of PDE4. In contrast to CGP12177, the inotropic and cyclic AMP-elevating responses to (−)-noradrenaline were unaffected by PDE3 inhibition with cilostamide. These results are consistent with a failure of cilostamide to enhance the inotropic and cyclic AMP-elevating responses of dobutamide, mediated through rat ventricular β_1-adrenoceptors (Juan-Fita *et al*., 2005). Furthermore, in agreement with our results of cilostamide vs (−)-noradrenaline, the PDE3-selective inhibitor milrinone also failed to enhance the inotropic and cyclic AMP responses to isoprenaline in rat ventricle (Katano & Endoh, 1992). Our results with (−)-noradrenaline are in line with a recent study on ventricular myocytes from neonatal rats, showing that the cyclic AMP pool that is activated in response to β_1-adrenoceptor stimulation is functionally coupled to PDE4 but not to PDE3, although PDE3 hydrolyses a large part of the cyclic AMP generated by forskolin in this system (Mongillo *et al*., 2004).

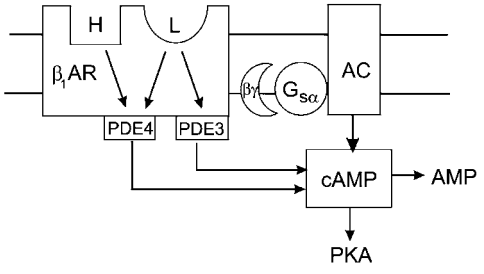

Figure 4 Scheme of different signals from the high-affinity (H) and low-affinity (L) sites to phosphodiesterases.

Our results suggest that part of the cyclic AMP, produced through CGP12177-evoked activation of the low-affinity β_1-adrenoceptor site, accumulates in a compartment close to the location of PDE3. PDE3 is located both in the cytosol and at the sarcoplasmic reticulum (Kauffman *et al*., 1986; Movsesian *et al*., 1991) and internal membranes (Mongillo *et al*., 2004). Part of the cyclic AMP produced through CGP12177-evoked β_1-adrenoceptor activation may reach these loci but is hydrolysed by PDE3. However, when PDE3 is inhibited by cilostamide or IBMX, it is likely that CGP12177 enhances contractility in part through PKA-catalysed phosphorylation of phospholamban, thereby stimulating the calcium pump and thus making more calcium available for release to enhance contractility. Consistent with this mechanism are the observations that in the presence of IBMX, CGP12177 hastens the onset of relaxation of human ventricular preparations through increases of cyclic AMP and PKA activation (Sarsero *et al*., 2003).

Although PDE3-selective inhibitors do not affect responses to (−)-noradrenaline (this report), isoprenaline (Katano & Endoh, 1992) and dobutamine (Juan-Fita *et al*., 2005), PDE3 appears to hydrolyse cyclic AMP generated through the glucagon receptor in rat ventricle. Cilostamide potentiates the positive responses to glucagon and causes glucagon to increase cyclic AMP levels (Juan-Fita *et al*., 2005). Thus, the cyclic AMP formed through activation of the glucagon receptor, and through the low-affinity site but not high-affinity site of the β_1-adrenoceptor, reaches the microdomain where PDE3 is located.

PDE4 limits responses to both (−)-noradrenaline and CGP12177

The involvement of PDE4 is supported by the appearance of inotropic stimulation in the presence of the PDE4-selective inhitor rolipram (1 μM). IC_{50} values for the inhibition by rolipram of PDE4 and PDE3 are around 1 μM (2 μM, Beavo, 1995; 0.45 μM, Sudo *et al*., 2000) and 242 μM (Collado *et al*., 1998) respectively. Therefore, under our conditions using 1 μM rolipram, PDE4 was likely to be inhibited by approximately 50% with negligible inhibition (0.4%) of PDE3. Inhibition of PDE4 activity by rolipram would be expected to reduce hydrolysis of cyclic AMP, when the second messenger is enhanced through β_1-adrenoceptor stimulation, as indeed observed in rat ventricular myocardium with isoprenaline (Katano & Endoh, 1992) and dobutamine (Juan-Fita *et al*., 2004). However, CGP12177 failed to enhance cyclic AMP levels in the presence of rolipram. Furthermore, in the presence of both (−)-propranolol and rolipram, the inotropic responses to CGP12177 (10 μM) and (−)-noradrenaline (2 μM) were similar but both agonists failed to elevate cyclic AMP levels (Figure 3a and b). Although these findings are inconsistent

with inhibition of PDE4, they do not rule out that rolipram enhanced the cylic AMP signal produced by both CGP12177 and low noradrenaline concentrations. A large increase of cyclic AMP may have occurred in a restricted sarcoplasmic membrane compartment (Jurevicius & Fischmeister, 1996) but was undetectable due to limited resolution of the cyclic AMP assay (Rich *et al*., 2001). However, under our conditions, the high concentration of 20 μM (−)-noradrenaline did elevate the cyclic AMP, an effect further increased by rolipram ($P<0.05$, Figure 3b), perhaps due to overflow of cyclic AMP from the sarcomere microdomain into the cytosol (Rich *et al*., 2001), where in turn it might be hydrolysed by PDE3. Consistent with this last interpretation is our observation that the inhibition both PDE34 and PDE3 potentiated the positive inotropic and cyclic AMP-enhancing effects of both (−)-noradrenaline and CGP12177 (Figures 1–3).

We conclude that CGP12177, acting through the low-affinity β_1-adrenoceptor site, causes the adenylyl cyclase to spill cyclic AMP into two distinct cell compartments, one affected by PDE3 the other by PDE4. Both enzymes prevent increases in contractility by CGP12177. In contrast, (−)-noradrenaline, acting through the high-affinity β_1-adrenoceptor site, produces a pool of cyclic AMP mainly hydrolysed by PDE4, thereby reducing the contractile responses.

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References

BAKER, J.G., HALL, I.P. & HILL, S.J. (2003). Agonist actions of β-blockers provide evidence for two agonist-activation sites or conformations of the human β_1-adrenoceptor. *Mol. Pharmacol.,* **63,** 1312–1321.

BEAVO, J.A. (1995). Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. *Physiol. Rev.,* **75,** 725–748.

COLLADO, M.C., BELETA, J., MARTINEZ, E., MIRALPEIX, M., DOMENECH, T., PALACIOS, J.M. & HERNANDEZ, J. (1998). Functional and biochemical evidence for diazepam as a cyclic nucleotide phosphodiesterase type 4 inhibitor. *Br. J. Pharmacol.,* **123,** 1047–1054.

GRANNEMAN, J.G. (2001). The putative β_4-adrenoceptor is a novel state of the β_1-adrenergic receptor. *Am. J. Physiol.,* **43,** E199–E202.

JOSEPH, S.S., LYNHAM, J.A., COLLEDGE, W. & KAUMANN, A.K. (2004a). Binding of (−)-[^{3}H]-CGP12177 at two sites in recombinant human β_1-adrenoceptors and interaction with β-blockers. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **369,** 525–532.

JOSEPH, S.S., LYNHAM, J.A., GRACE, A.A., COLLEDGE, W.H. & KAUMANN, A.J. (2004b). Markedly reduced effects of (−)-isoprenaline but not of (−)-CGP12177 and unchanged affinity of β-blockers at Gly389-β_1-adrenoceptors compared to Arg389-β_1-adrenoceptors. *Br. J. Pharmacol.,* **142,** 51–56.

JOSEPH, S.S., LYNHAM, J.A., MOLENAAR, P., GRACE, A.A., COLLEDGE, W.H. & KAUMANN, A.J. (2003). Intrinsic sympathetic activity of (−)-pindolol mediated through a (−)-propranolol-resistant site of the β_1-adrenoceptor in human atrium and recombinant receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **368,** 496–503.

JUAN-FITA, M.J., VARGAS, M.L. & HERNANDEZ, J. (2005). The phosphodiesterase 3 inhibitor cilostamide enhances inotropic responses to glucagon but not to dobutamine in rat ventricular myocardium. *Eur. J. Pharmacol.,* **512,** 207–213.

JUAN-FITA, M.J., VARGAS, M.L., KAUMANN, A.J. & HERNANDEZ-CASCALES, J. (2004). Rolipram reduces the inotropic tachyphylaxis of glucagon in rat ventricular myocardium. *Naunyn-Schmiedebergś Arch. Pharmacol.,* **370,** 324–329.

JUREVICIUS, J. & FISCHMEISTER, R. (1996). cAMP compartmentation is responsable for a local activation of cardiac Ca^{2+} channels by β-adrenergic agonists. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 295–299.

KATANO, Y. & ENDOH, M. (1992). Effects of a cardiotonic quinolinone derivative Y-20487 on the isoproterenol-induced positive inotropic action and cyclic AMP accumulation in rat ventricular myocardium: comparison with rolipram, Ro 20-1724, milrinone and isobutylmethylxanthine. *J. Cardiovasc. Pharmacol.,* **20,** 715–722.

KAUFFMAN, R.F., CROWE, V.G., UTTERBACK, B.G. & ROBERTSON, D.W. (1986). LY195114: a potent, selective inhibitor of cyclic nucleotide phosphodiesterases located in the sarcoplasmic reticulum. *Mol. Pharmacol.,* **30,** 609–616.

KAUMANN, A.J. (1983). Cardiac β-adrenoceptors – experimental viewpoints. *Z. Kardiol.,* **72,** 63–82.

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

KAUMANN, A.J. (1996). CGP12177-induced increase of human atrial contraction through a putative third β-adrenoceptor. *Br. J. Pharmacol.,* **117,** 93–98.

KAUMANN, A.J. (2000). G_s protein-coupled receptors in human heart. In: *The Pharmacology of Functional, Biochemical, and Recombinant Receptor Systems. Handbook of Experimental Pharmacology,* eds. Kenakin, T. & Angus, J.A. Vol. 148, pp. 73–116. Berlin, Heidelberg, New York: Springer.

KAUMANN, A.J., ENGELHARDT, S., MOLENAAR, P. & LOHSE, M. (2001). Abolition of (−)-CGP12177-evoked cardiostimulation in double β_1/β_2-adrenoceptor knockout mice. Obligatory role of β_1-adrenoceptor for putative β_4-adrenoceptor pharmacology. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **363,** 87–93.

KAUMANN, A.J. & LYNHAM, J.A. (1997). Stimulation of cyclic AMP-dependent protein kinase in rat atria by (−)-CGP12177 through an atypical β-adrenoceptor. *Br. J. Pharmacol.,* **129,** 1187–1189.

KAUMANN, A.J., LYNHAM, J.A., SARSERO, D. & MOLENAAR, P. (1997). The atypical cardiostimulant β-adrenoceptor is distinct from the β_3-adrenoceptor and is coupled to a cyclic AMP-dependent pathway in rat and human myocardium. *Br. J. Pharmacol.,* **120,** 102p.

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.

KAUMANN, A.J., PREITNER, F., SARSERO, D., MOLENAAR, P., REVELLI, J.P., GIACOBINO, J.P. & KAUMANN, A.J. (1998). CGP12177 causes cardiostimulation and binds to cardiac putative β_4-adrenoceptors in both wild-type and β_3-adrenoceptor knock out mice. *Mol. Pharmacol.,* **53,** 670–675.

KONKAR, A.A., ZHU, Z. & GRANNEMAN, J.G. (2000). Aryloxypropanolamine and catecholamine ligand interactions with the β_1-adrenergic receptor: evidence for interactions with distinct conformations of β_1-adrenergic receptors. *J. Pharmacol. Expl. Ther.,* **294,** 923–932.

LEWIS, C.J., GONG, H., BROWN, M.J. & HARDING, S.E. (2004). Overexpression of β_1-adrenoceptors in adult rat ventricular myocytes enhances CGP12177A cardiostimulation: implications for putative β_4-adrenoceptor pharmacology. *Br. J. Pharmacol.,* **141,** 813–824.

LOWE, M., LYNHAM, J.A., GRACE, A.A. & KAUMANN, A.J. (2002). Comparison of the affinity of β-blockers for two states of the β_1-adrenoceptor in ferret ventricular myocardial myocardium. *Br. J. Pharmacol.,* **135,** 451–461.

MONGILLO, M., MCSORLEY, T., EVELLIN, S., SOOD, A., LISSANDRON, V., TERRIN, A., HUSTON, E., HANNAWACKER, A., LOHSE, M.J., POZZAN, T., HOUSLAY, M.D. & ZACCOLO, M. (2004). Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases. *Circ. Res.,* **95,** 67–75.

MOVSESIAN, M.A., SMITH, C.J., KRALL, J., BRISTOW, M.R. & MANGANIELLO, V.C. (1991). Sarcoplasmic reticulum-associated cyclic adenosine 5′-monophosphate phosphodiesterase activity in normal and failing human hearts. *J. Clin. Invest.,* **88,** 15–19.

NICHOLSON, D., CHALLISS, R.A. & SHAHID, M. (1991). Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes. *Trends Pharmacol. Sci.,* **12,** 19–27.

PAK, M.D. & FISHMAN, P.H. (1996). Anormalous behavior of CGP12177 on β_1-adrenoceptors. *J. Recept. Signal Transduct. Res.,* **16,** 1–23.

RICH, T.C., FAGAN, K.A., TSE, T.E., SCHAACK, J., COOPER, D.M.F. & KARPEN, J.W. (2001). A uniform extracellular stimulus triggers distinct cAMP signals in different compartments of a simple cell. *Proc. Natl. Acad. Sci. U.S.A.,* **98,** 13049–13054.

SARSERO, D., MOLENAAR, P. & KAUMANN, A.J. (1998). Validity of (−)-[^{3}H]- CGP12177 as a radioligand for the putative β_4-adrenoceptor in rat atrium. *Br. J. Pharmacol.,* **123,** 371–380.

SARSERO, D., MOLENAAR, P., KAUMANN, A.J. & FREESTONE, N.S. (1999). Putative β_4-adrenoceptor in rat ventricle mediate increases in contractile force and cell Ca^{2+}: comparison with atrial receptors with atrial receptors and relationship to (−)-[^{3}H]-CGP12177 binding. *Br. J. Pharmacol.,* **128,** 1445–1460.

SARSERO, D., RUSSEL, F.D., RABNOTT, G., YANG, I., FONG, K.M., LI, L., KAUMANN, A.J. & MOLENAAR, P. (2003). CGP12177 increases contractile force and hastens relaxation of human myocardial preparations through a propranolol-resistant state of the β_1-adrenoceptor. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **367,** 10–21.

STAEHELIN, M., SIMONS, P., JAEGGI, K. & WIGGER, H. (1983). A hydrophillic beta-adrenergic radioligand reveals high affinity binding of agonists to intact cells. *J. Biol. Chem.,* **258,** 3496–3502.

SUDO, T., TACHIBANA, K., TOGA, K., TOCHIZAWA, S., INOUE, Y., KIMURA, Y. & HIDAKA, H. (2000). Potent effects of novel antiplatelet aggregatory cilostamide analogues on recombinant cyclic nucleotide phosphodiesterase isozyme activity. *Biochem. Pharmacol.,* **59,** 347–356.

VERDE, I., VANDECASTEELE, G., LEZOUALC'H, F. & FISCHMEISTER, R. (1999). Characterization of the cylic nucleotide phosphodiesterases subtypes in the regulation of the L-type calcium current in rat ventricular myocytes. *Br. J. Pharmacol.,* **127,** 65–74.

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