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SPECIAL REPORT

Action potential shortening through the putative β_4-adrenoceptor in ferret ventricle: comparison with β_1- and β_2-adrenoceptor-mediated effects

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The electrophysiological responses to (−)-CGP 12177 ((−)-4-(3-tertiarybutylamino-2-hydroxypropoxy) benzimidazol-2-one), an agonist for the putative β_4-adrenoceptor, were investigated on isolated perfused ferret hearts paced at 100 min^{-1} and compared to those of (−)-noradrenaline and (−)-adrenaline, mediated through β_1- and β_2-adrenoceptors respectively. The three agonists decreased ventricular monophasic action potential duration but prolonged the action potential plateau; β_3-adrenoceptor-selective agonists had no effect. (−)-CGP 12177 was the most potent, but (−)-noradrenaline the most efficacious; both agonists caused ventricular extra-systoles. Because only (−)-noradrenaline but not (−)-CGP 12177 elicited shortening of the refractory period, the mechanism of arrhythmias mediated through β_1- and putative β_4-adrenoceptors may be different.

Keywords: Ferret heart; action potentials; β_1-, β_2- β_3- and putative β_4-adrenoceptors; arrhythmias; ventricular pressure; (−)-noradrenaline, (−)-adrenaline and (−)-CGP 12177

Introduction A putative β_4-adrenoceptor has been proposed that mediates positive inotropic and chronotropic effects in mammalian heart (Kaumann, 1997). The receptor interacts with so called non-conventional partial agonists, e.g. (−)-CGP 12177, that cause cardiostimulant effects at concentrations considerably higher than those that block β_1- and β_2-adrenoceptors. It has been labelled with (−)-[^{3}H]-CGP 12177 and shown to bind to non-conventional partial agonists and stereoselectively to catecholamines (Sarsero *et al*., 1998). In addition, the receptor mediates increases in Ca^{2+} transients in ventricular myocytes and induces arrhythmias (Kaumann & Freestone, 1997). It resembles the β_3-adrenoceptor through which non-conventional agonists, including CGP 12177, mediate lipolysis and gut relaxation (Arch & Kaumann, 1993), but is not activated by selective β_3-adrenoceptor agonists (Kaumann & Molenaar, 1996), and remains functional in the hearts of β_3-adrenoceptor knockout mice (Kaumann *et al*., 1998).

To gain insight into the electrophysiological responses following stimulation of the putative β_4-adrenoceptor we have measured and analysed monophasic action potentials and ventricular effective refractory periods from ferret ventricle during perfusion with (−)-CGP 12177. Drug-induced changes in left ventricular developed pressure were assessed simultaneously. As both β_1- and β_2-adrenoceptors mediate experimental arrhythmias in isolated human atrial preparations (Kaumann & Sanders, 1993), the β_1- and β_2-adrenoceptor-mediated effects of (−)-noradrenaline and (−)-adrenaline, respectively, were studied for comparison. We also investigated the effects of the β_3-adrenoceptor-selective agonists BRL 37344 and CL 316243, and the β_3-adrenoceptor-selective antagonist SR 59230A (Kaumann & Molenaar, 1996).

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Methods Experiments were carried out on *Langendorff*-perfused ferret hearts. Ferrets of either sex, 3–12 months of age, were anaesthetised with sodium pentobarbitone (250 $mg.kg^{-1}$ i.p.). The hearts were removed and following aortic cannulation were perfused at a constant flow rate (5–6 ml g^{-1} min^{-1}) at 37°C in oxygenated (95% O_2/5% CO_2) solution containing (mM): NaCl 119, $NaHCO_3$ 25, KCl 4, KH_2PO_4 1.2, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 10 and Na-pyruvate 2. The atria were excised and the atrio-ventricular node crushed. Platinum electrodes were inserted into the high ventricular septum and the ventricles paced at 100 min^{-1}, using a 2 ms square wave stimulus at twice threshold voltage. Left ventricular developed pressure was measured *via* a pressure transducer (Spectramed P23XL; Spectramed, Inc., Oxnard, California) connected to a latex balloon in the left ventricle.

Monophasic action potentials were recorded using specially constructed suction electrodes. Two 1 mm diameter Ag-$AgCl_2$ electrodes were sealed into a perspex suction head with an inter-electrode distance of 2 mm (Bethell *et al*., 1998). The electrodes were apposed alternatively or simultaneously to the right and left ventricles with contact maintained using negative pressure suction. Action potentials were amplified (Gould universal amplifier model 13-4615-58, Gould, Essex, U.K.) and digitised using a CED 1401-plus analogue-to-digital converter operated using Spike 2 software (both from Cambridge Electronic Design, Cambridge, U.K.). Monophasic action potential durations at 90, 70, 50 and 10% repolarisation (mAP_{90}, mAP_{70}, mAP_{50}, mAP_{10}) were calculated using a custom-written Spike 2 analysis routine. Ventricular effective refractory periods were obtained following an 8 beat 600 ms drive train with extrastimuli delivered with an initial coupling interval of 160 ms, and subsequent 1 ms decrements until failure of impulse propagation occurred.

Hearts were perfused with prazosin (1 μM) to block α-adrenoceptors. (−)-CGP 12177 was perfused in the presence of (−)-propranolol (200 nM) to elicit responses through the putative β_4-adrenoceptor (Kaumann & Molenaar, 1996). To

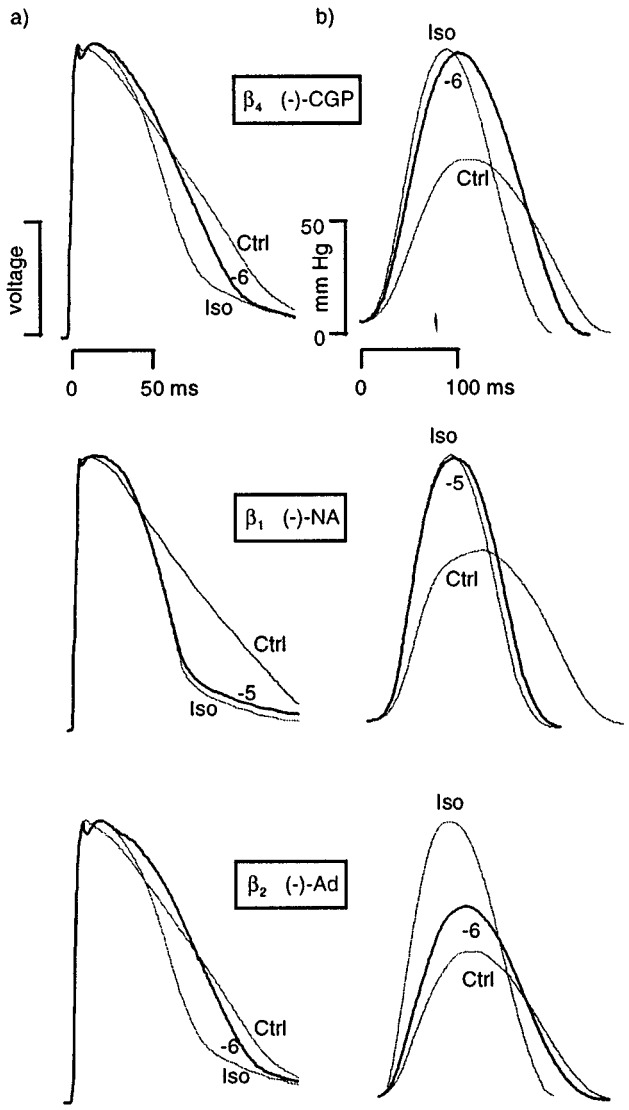

Figure 1 (a) and (b) Change in right ventricular monophasic action potential morphology and left ventricular developed pressure respectively, during perfusion with 10^{-6} M (−)-CGP12177, (−)-CGP [−6], 10^{-5} M (−)-noradrenaline, (−)-NA [−5] and 10^{-6}M (−)-adrenaline, (−)-Ad [−6], compared with control [Ctrl] and 10^{-4}M (−)-isoprenaline [Iso].

assess β_1-adrenoceptor-mediated effects (−)-noradrenaline was perfused in the presence of the selective antagonist ICI 118551 (50 nM) to block β_2-adrenoceptors. β_2-adrenoceptor responses were obtained with (−)-adrenaline in the presence of CGP 20712A (300 nM) to block β_1-adrenoceptors (Kaumann & Sanders, 1993). All antagonists were perfused for 30 min before addition of each agonist to the perfusate.

−Log EC_{50} values were obtained from individual experiments, and the data analysed using ANOVA. All results are expressed as mean ± s.e. mean.

Drugs BRL 37344 ((**RR**+**SS**) {4- [2- [[2-(3-chlorophenyl)-2-hydroxy-ethylamino] propyl] phenoxy] acetic acid} and (-)-CGP 12177 were gifts of SmithKline Beecham (Harlow, Essex, U.K.). CL 316243 (disodium (**R,R**) -5- [2- [[2-(3-chlorophenyl)-2-hydroxyethyl] -amino] propyl] 1,3-benzodioxole-2,2-dicarboxylate) was a gift of Wyeth-Ayerst Research (Princeton, New Jersey, U.S.A.). SR 59230A (3-(2-ethylphenoxy)-1-[(1**S**)-1,2,3,4-tetrahydronapht-1-ylamino]-2**S**-2-propanol) was a gift of Dr. L. Manara, Sanofi (Milan, Italy). CGP 20712A (2 - hydroxy - 5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluormethyl) 1 H-imidazole-2-yl) -phenoxy) propyl) amino) ethoxy) - benzamide monomethane sulphonate) was a gift of Ciba-Geigy (Basel, Switzerland). ICI 118551 (erythro-DL-1 (7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) was a gift of Zeneca (Wilmslow, Cheshire, U.K.). (−)-Noradrenaline (+)-bitartrate, (−)-adrenaline hydrochloride, (−)-propranolol, nadolol, (−)-isoprenaline (+)-bitartrate and prazosin were purchased from Sigma.

Results and Discussion (−)-CGP 12177, (−)-noradrenaline and (−)-adrenaline decreased monophasic action potential duration at 90%, 70% and 50% repolarisation ($mAP_{90,70,50}$), but increased duration at 10% repolarisation (mAP_{10}) (Figures 1 and 2). Although the increases in mAP_{10} were small, they were concentration dependent and occurred with each agonist. The three agonists also increased left ventricular developed pressure and hastened relaxation (Figure 1). The potency rank order for action potential shortening was (−)-CGP 12177 ⩾ (−)-adrenaline > (−)-noradrenaline, and for the increase in ventricular pressure and hastening of relaxation (−)-CGP 12177 > (−)-noradrenaline > (−)-adrenaline (Table 1). Only (−)-noradrenaline significantly shortened the ventricular effective refractory period (−27 ± 3 ms; $n=5$ ferrets) with a $-\log EC_{50}$ of 6.8 ± 0.2 and an intrinsic activity of 1.0 ± 0.1 when compared to 10^{-4} M (−)-isoprenaline ($n=5$ (−)-CGP12177, $n=3$ (−)-adrenaline). (−)-CGP 12177 and (−)-noradrenaline but not (−)-adrenaline caused extrasystoles. The effects of (−)-CGP 12177 were unaffected by the β_3-adrenoceptor-selective antagonist SR 59230A (1 μM) making β_3-adrenoceptor involvement unlikely. BRL 37344 (1 μM) ($n=5$) and CL 316243 (1 μM) ($n=6$) did not modify action potential duration nor ventricular pressure significantly.

The reported effects of β_1- and β_2-adrenoceptor stimulation on action potential duration are model-dependent (Liang *et al*., 1985; Xiao & Lakatta, 1993), with our results consistent with previous findings in multicellular preparations (Liang *et al*., 1985). The decrease in $mAP_{90,70,50}$ through activation of putative β_4-, β_1- and β_2-adrenoceptors seen here is consistent with augmented K^+ channel conductance resulting in an increased rate of phase 3 repolarisation, as has previously been shown to occur in isolated myocytes during adrenergic stimulation (reviewed in Gadsby, 1990). The prolongation of action potential plateau duration, as measured by mAP_{10}, is compatible with an increase in Ca^{2+} current, and is in line with the known activation of L-type Ca^{2+} channels by noradrenaline through presumptive β_1-adrenoceptor stimulation (Gadsby, 1990) and zinterol through β_2-adrenoceptors (Skeberdis *et al*., 1997). The increase in mAP_{10} duration by (−)CGP 12177 is therefore likely to be secondary to Ca^{2+} channel activation via the putative β_4-adrenoceptor.

Despite the substantial reduction in $mAP_{90,70,50}$ mediated by (−)-CGP 12177 however, only activation of β_1-adrenoceptors resulted in a significant reduction in the ventricular refractory period, suggesting differential coupling of β-adrenoceptor subtypes to individual ion channels. The underlying mechanisms for the triggering of arrhythmias induced *via* putative β_4-adrenoceptors and β_1-adrenoceptors may therefore be distinct.

The positive inotropic and lusitropic effects mediated by (−)-CGP 12177 are in agreement with those found in human ventricle (Kaumann & Molenaar, 1997). We detected no evidence of functional cardiac β_3-adrenoceptors since the β_3-

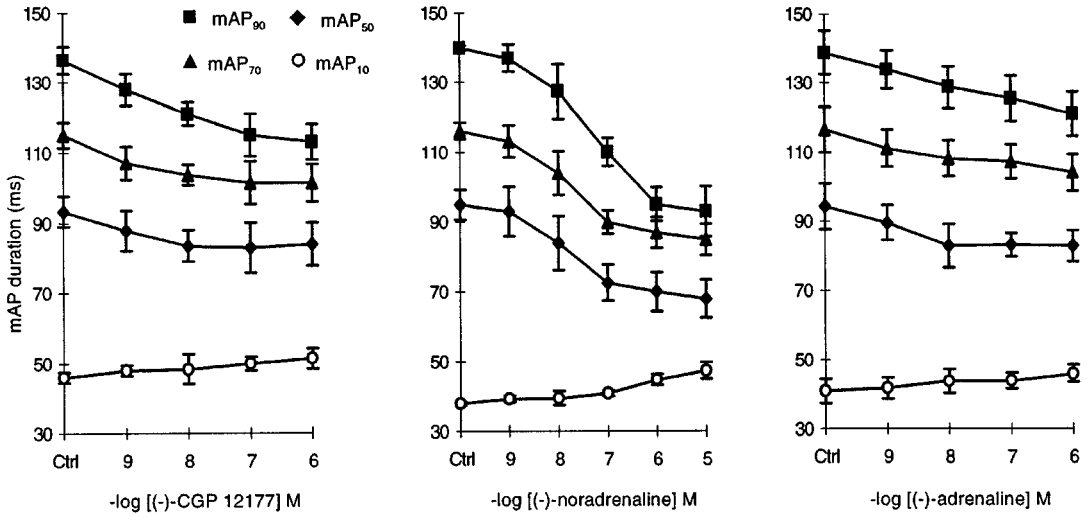

Figure 2 Right ventricular monophasic action potential absolute values at 90% [mAP_{90}], 70% [mAP_{70}], 50% [mAP_{50}] and 10% duration [mAP_{10}] during perfusion with incremental concentrations of (−)-CGP12177, (−)-noradrenaline and (−)-adrenaline respectively. One experiment with (−)-noradrenaline was excluded as repetitive arrhythmias prevented assessment of equilibrium effects.

Table 1 –log EC_{50} values for each agonist* with intrinsic activity compared to 10^{-4} M (–)-isoprenaline in brackets for monophasic action potentials at 90%, 70%, 50% and 10% duration, left ventricular developed pressure [LVDP], and left ventricular relaxation half time [$LVRt_{50}$]. LV = Left Ventricle, RV = Right Ventricle.

	β_4 (−)-CGP	β_1 (–)-NAd	β_2 (–)-Ad
LV mAP_{90}	8.0 [0.7]	7.3 [1.0]	7.8 [0.4]
mAP_{70}	8.1 [0.7]	7.2 [1.0]	7.8 [0.3]
mAP_{50}	8.2 [0.8]	7.1 [1.0]	8.1 [1.0]
mAP_{10}	7.7 [1.0]	6.0 [1.0]	8.3 [1.0]
RV mAP_{90}	8.2 [0.5]	7.5 [1.0]	8.0 [0.5]
mAP_{70}	8.3 [0.7]	7.4 [1.0]	8.1 [0.6]
mAP_{50}	7.7 [0.7]	7.8 [1.0]	8.4 [0.7]
mAP_{10}	7.8 [1.0]	6.6 [0.9]	7.3 [0.8]
LVDP	8.7 [0.9]	7.7 [1.0]	7.2 [0.5]
$LVRt_{50}$	8.0 [0.5]	7.7 [0.9]	7.3 [0.4]

*$n = 4-6$ ferrets for each agonist. s.e.mean of –log EC_{50} values all < 0.4; s.e.mean of intrinsic activity values all < 0.2.

adrenoceptor-selective agonists BRL 37344 and CL 316243 had no significant effect on left ventricular developed pressure or action potential duration. Although in human ventricular preparations it has been reported that catecholamines and β_3-adrenoceptor-selective agonists cause negative inotropic effects and shorten action potential duration through β_3-adrenoceptors (Gauthier *et al*., 1996) others have not been able to confirm cardiodepressant effects (Molenaar *et al*., 1997). The concentration of BRL 37344 used here (1 μM), is three orders of magnitude greater than the EC_{50} (1.4 nM) reported for ferret epithelial β_3-adrenoceptors (Webber & Stock, 1992). Thus, although ferrets possess β_3-adrenoceptors, they do not appear to couple to functional effects, or are not expressed in ferret ventricle.

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References

ARCH, J.R.S. & KAUMANN, A.J. (1993). β_3-Adrenoceptors and atypical β-adrenoceptors. *Med. Res. Rev.,* **48,** 663–729.

BETHELL, H.W.L., VANDENBERG, J.I., SMITH, G.A. & GRACE, A.A. (1998). Changes in ventricular repolarization during acidosis and low-flow ischemia. *Am. J. Physiol.,* **275,** H (in press).

GADSBY, D.C. (1990). Effects of β-adrenergic catecholamines on membrane currents in cardiac cells. In: *Cardiac electrophysiology: A textbook*. Eds. Rosen, M.R., Janse, M.J., & Wit, A.L. pp 857–876. New York: Futura.

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

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

KAUMANN, A.J. & FREESTONE, N. (1997). Atypical β-adrenoceptor activation by (−)-CGP 12177 increases cytosolic calcium in rat ventricular myocytes. *Pharmacologist,* **39,** 74 (abstract 293).

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. & MOLENAAR, P. (1997). Modulation of human cardiac function through 4 β-adrenoceptor populations. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **355,** 667–681.

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

KAUMANN, A.J. & SANDERS, L. (1993). Both β_1- and β_2-adrenoceptors mediate catecholamine-evoked arrhythmias in isolated human atrium. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **348,** 536–540.

LIANG, B.T., FRAME, L.H. & MOLINOFF, P.B. (1985). β_2-Adrenergic receptors contribute to catecholamine-stimulated shortening of action potential duration in dog atrial muscle. *Proc. Natl. Acad. Sci., USA,* **82,** 4521–4525.

MOLENAAR, P., SARSERO, D. & KAUMANN, A.J. (1997). Proposal for the interaction of non-conventional partial agonists and catecholamines with the 'putative β_4-adrenoceptor' in mammalian heart. *Clin. Exp. Pharmacol. Physiol.,* **24,** 647–656.

SARSERO, D., MOLENAAR, P. & KAUMANN, A.J. (1998). Validity of (−)-[^{3}H]-CGP 12177A as a radioligand for the 'putative β_4-adrenoceptor' in rat atrium. *Br. J. Pharmacol.,* **123,** 371–380.

SKEBERDIS, V.A., JUREVICIUS, J. & FISCHMEISTER, R. (1997). *Beta*-2 Adrenergic activation of L-Type Ca^{++} current in cardiac myocytes. *J. Pharmacol. Exp. Ther.,* **283,** 452–461.

WEBBER, S.E. & STOCK, M.J. (1992). Evidence for an atypical, or β_3-adrenoceptor in ferret tracheal epithelium. *Br. J. Pharmacol.,* **105,** 857–862.

XIAO, R.P. & LAKATTA, E.G. (1993). β_1-adrenoceptor stimulation and β_2-adrenoceptor stimulation differ in their effects on contraction, cytosolic Ca^{2+}, and Ca^{2+} current in single rat ventricular cells. *Circ. Res.,* **73,** 286–300.

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