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ORIGINAL ARTICLE

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Prucalopride is a partial agonist through human and porcine atrial 5-HT_4 receptors: comparison with recombinant human 5-HT_4 splice variants

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Abstract Prucalopride is a gastrointestinal prokinetic drug that acts through 5-HT_4 receptors, but its potential effects on cardiac atrial function are unknown. We investigated the effects of prucalopride on human right atrium, piglet left atrium, and piglet sinoatrial node. The effects of prucalopride on 5-HT_4 receptor splice variants a, b, g and i, known to be expressed in human atrium, were studied for comparison. Prucalopride was an inotropic partial agonist, compared with 5-HT, on paced human atrial trabeculae ($-\log EC_{50}M$=7.4) and porcine left atria ($-\log EC_{50}M$=7.2), with intrinsic activity of 0.77 and 0.63 respectively. Prucalopride (1 μM) surmountably antagonized the positive inotropic effects of 5-HT on human (pK_P=7.2) and porcine (pK_P=7.1) atrium. Prucalopride was also a chronotropic partial agonist ($-\log EC_{50}M$=7.4, intrinsic activity=0.72 with respect to 5-HT) on spontaneously beating piglet atria. The cardiostimulant effects of prucalopride were prevented by GR113808 (1 μM), consistent with mediation through 5-HT_4 receptors. Prucalopride bound to recombinant 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$, and 5-$HT_{4(i)}$ receptors, labeled by [^{3}H]GR113808, with pK_i values of 7.6, 7.5, 7.4, and 7.8 respectively. Prucalopride stimulated adenylyl cyclase as a partial agonist on 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, and 5-$HT_{4(i)}$ receptors with intrinsic activities of 0.82, 0.86, and 0.78 and $-\log EC_{50}$ values of 7.2, 7.3, and 7.2 respectively. At the 5-$HT_{4(g)}$ receptor prucalopride acted as a full agonist ($-\log EC_{50}M$=8.0) compared with 5-HT in the cell line tested, which was probably due to high receptor expression levels. We conclude that prucalopride is a cardiostimulatory partial agonist through human and porcine 5-HT_4 receptors. Since prucalopride acts similarly through 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$, and 5-$HT_{4(i)}$ receptors, any of these variants could be involved in the mediation of cardiostimulation.

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Introduction

Serotonin (5-Hydroxytryptamine, 5-HT) causes cardiostimulation through human atrial (Kaumann et al. 1990) and ventricular (Brattelid et al. 2004a) 5-HT_4 receptors. 5-HT elicits tachycardia in man (Le Mesurier et al. 1959). Through 5-HT_4 receptors 5-HT increases human atrial contractile force (Kaumann et al. 1990, 1991a) and can produce arrhythmias in isolated human atrium (Kaumann and Sanders 1994), human atrial myocytes (Sanders et al. 1995), and human ventricle (Brattelid et al. 2004a). The proarrhythmic effects of 5-HT may have a role in the production and maintenance of atrial fibrillation (Kaumann 1994). Although the gastrokinetic drug cisapride is only a weak inotropic partial agonist on human atrial 5-HT_4 receptors (Kaumann et al. 1991a) it causes slight tachycardia in man (Bateman 1986).

Prucalopride is a 5-HT_4 receptor-selective agonist (Briejer et al. 2001; Pindon et al. 2002) with enterokinetic properties (Prins et al. 2000) and appears to be effective for the treatment of constipation in elderly patients (Coremans et al. 2003). Information about the interaction of prucalopride with human atrial 5-HT_4 receptors is lacking. The effects of prucalopride were therefore investigated on human and porcine atrium, as well as on spontaneously beating right atria from new-born piglets, an experimental model of human atrial 5-HT_4 receptors (Kaumann 1990). The effects of prucalopride were compared with those of 5-HT. Prucalopride was found to be a partial agonist. To compare agonist potency and blocking potency, the antagonism by prucalopride of the effects of 5-HT was also investigated.

At least nine splice variants of the 5-HT_4 receptor are known (Langlois and Fischmeister 2003; Bockaert et al. 2004; Brattelid et al. 2004b). Because the 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$, and 5-$HT_{4(i)}$ splice variants are expressed in human atrium (Bach et al. 2001; Medhurst et al. 2001; Brattelid et al. 2004b), the agonist effects and affinity of prucalopride and 5-HT were studied for these splice variants for comparison with the cardiac effects.

Materials and methods

Human right atrium Right atrial appendages, freshly obtained from patients undergoing coronary artery bypass operation, were set up and paced at 1 Hz at 37°C as described previously (Kaumann et al. 1990). Sixteen patients were men (64±2 years of age, range 51–76) and one patient a 76-year-old woman. All patients were treated with β blockers, usually atenolol or metoprolol. None of the patients had heart failure. Experiments were carried out in the presence of (−)-propranolol 200 nM. A single cumulative concentration-effect curve to prucalopride was determined between 1 nM and 10 μM, followed by 5-HT (200–600 μM) and (−)-isoprenaline (200–600 μM). The experiment was terminated by raising the $CaCl_2$ concentration from 2.25 mM to 6.7 mM. An identical protocol was used in tissues preincubated with 1 μM of the 5-HT_4 receptor-selective antagonist GR113808 for 90 min (Kaumann 1993). The blocking potency against serotonin of prucalopride was investigated by determining a single concentration-effect curve to 5-HT (in the presence of cocaine 6 μM and (−)-propranolol 200 nM) in the absence or presence of 1 μM prucalopride and the K_P for prucalopride estimated using the method described by Marano and Kaumann (1976). Positive inotropic effects of the partial agonists were analyzed as described previously (Kaumann et al. 1991a).

Piglet right atrium Spontaneously beating right atria from new-born piglets (males, 1–2 days of age) were used as described (Kaumann 1990). Experiments were carried out in the presence of (−)-propranolol (200 nM). Cumulative concentration-effect curves for prucalopride were carried out in 1/2 log concentration steps from 1 nM to 10 μM. To determine intrinsic activity, 5-HT (200–600 μM) was administered in the presence of 10 μM prucalopride and the experiment terminated with (−)-isoprenaline (600 μM). To assess the 5-HT_4 nature of the agonist effects, experiments with identical protocol were carried out on separate atria in the presence of GR113808 (1 μM), a 5-HT_4 receptor-selective antagonist (Medhurst and Kaumann 1992), preincubated for 90 min. The positive chronotropic effects of prucalopride were analyzed as described previously (Kaumann 1990).

Piglet left atrium Left atria obtained from new-born piglets were divided into two halves and paced at 1 Hz at 37°C and incubated with (−)-propranolol (200 nM), cocaine (6 μM), and isobutyl-methyl-xanthine (20 μM; Kaumann et al. 1991b). On one half of the left atrium a cumulative concentration-effect curve to prucalopride (1 nM−10 μM) was determined, followed by a cumulative concentration-effect curve to 5-HT on both halves. The experiment was continued by the administration of (−)-isoprenaline (200–600 μM) and terminated by raising the $CaCl_2$ concentration from 2.25 mM to 6.7 mM. The antagonism of the positive inotropic effects of 5-HT by prucalopride (10 μM) was analyzed using the method described by Marano and Kaumann (1976) and dissociation equilibrium constants K_P for prucalopride were calculated.

Binding of prucalopride to recombinant 5-HT_4 splice variants Membranes were prepared from stable HEK293 cell lines expressing one of the 5-HT_4 receptor splice variants (either the a, b, g or i) as described previously (Krobert et al. 2001). All radioligand binding assays were conducted on thawed membranes. Radioligand binding assays were incubated for 1 h at 24°C with 0.24–0.7 nM [^{3}H]GR113808 alone or in the presence of increasing concentrations (95 pM–100 μM) of prucalopride or 5-HT and pK_i was calculated as described previously (Krobert et al. 2001) using a K_d value of 0.047 nM for [^{3}H]GR113808 (Bach et al. 2001).

Adenylyl cyclase stimulation through recombinant 5-HT_4 splice variants Adenylyl cyclase (AC) activity was measured by determining conversion of [α-^{32}P]ATP to [^{32}P]cAMP in membranes as described previously (Krobert et al. 2001). In brief, AC activities were measured on 10-μl aliquots in a final volume of 50 μl in the presence of 0.1 mM [α-^{32}P]ATP ($1–2\times10^6$ cpm/assay), 4 mM $MgCl_2$, 20 μM GTP, 1 mM EDTA, 1 mM [^{3}H]cAMP (approximately 10,000 cpm/assay), 1 μM 3-isobutyl-1-methyl xanthine (IBMX; Sigma), a nucleoside triphosphate regenerating system consisting of 20 mM creatine phosphate (Sigma), 0.2 mg/ml creatine phosphokinase (Sigma), and 40 U/ml myokinase (Sigma), and additives described in the text and figures. Incubation was for 20 min at 32°C. Cyclic AMP that formed was quantified by the double column chromatography system described by Salomon et al. (1974), as modified by Bockaert et al. (1976). Prucalopride- and 5-HT-stimulated AC activity (in the absence and presence of 200 nM prucalopride) was analyzed by non-linear regression using Microsoft Excel with the Solver add-in. The data were fitted to the equation

$$Y = a + (b - a)x/(c + x),$$

where a is adenylyl cyclase activity in the absence of the agonist, b is maximal adenylyl cyclase activity stimulated by the agonist, c is EC_{50}, and x is the concentration of agonist.

Protein measurements The protein concentration of the membrane preparations was measured using the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA) using bovine serum albumin (BSA) as standard.

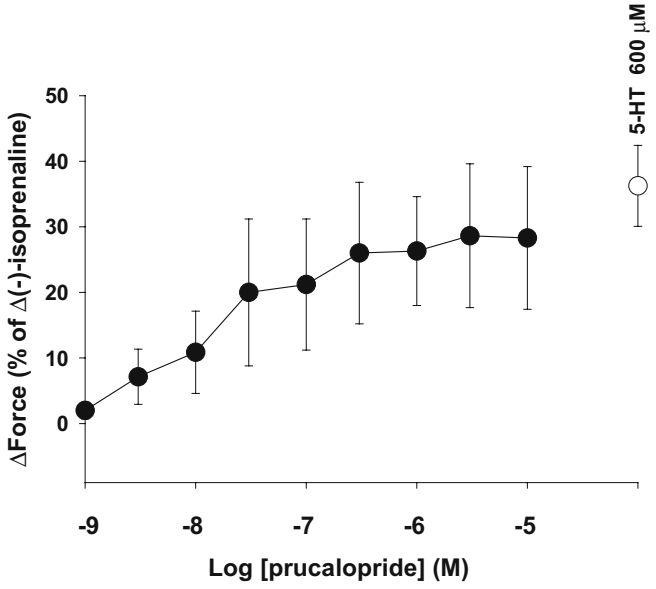

Fig. 1 Positive inotropic effects of prucalopride on human atrial trabeculae. Basal force, force under 200 μM (−)-isoprenaline and force under 6.7 mM $CaCl_2$ were 5.3±1.2, 12.7±2.6, and 13.3±2.5 mN respectively. Data were from 7–10 patients

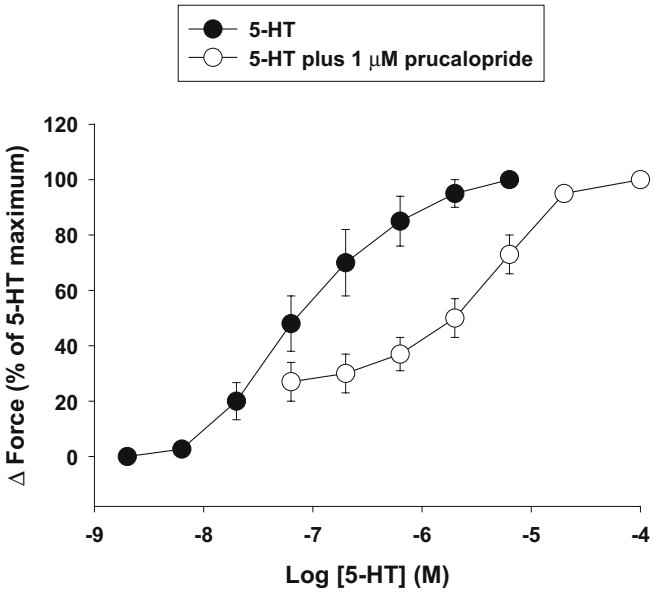

Fig. 2 Antagonism of the positive inotropic effects of 5-HT by prucalopride on human atrium. Basal forces before 5-HT (*closed circles*) and prucalopride (*open circles*) were 4.2±1.4 and 4.7±1.8 mN respectively. Maximal forces under 5-HT alone and 5-HT in the presence of prucalopride were 7.4±1.1 and 8.0±1.3 mN respectively. Data were from 8 human trabeculae obtained from 3 patients

Drugs Prucalopride was a gift of Johnson & Johnson Pharmaceutical Research & Development (Beerse, Belgium). 5-Hydroxytryptamine hydrochloride (5-HT, serotonin) was from Sigma. GR113808 (1-methyl-1H-indole-3-carboxylic acid, [1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl] methyl ester) maleate was from Tocris (Bristol, UK).

Radiochemicals [^{3}H]GR113808 (84 Ci/mmol), [α-^{32}P] ATP (400 Ci/mmol) and [^{3}H]cAMP (30–50 Ci/mmol) were from Amersham.

Statistics All results are given as mean ± SEM of *n* experiments where *n* refers to the number of piglets, patients or biochemical assays, unless stated otherwise. Statistical comparisons were made using one-way ANOVA and Bonferroni correction.

Results

Human right atrium

Concentration-dependent increases in contractile force were observed with prucalopride with $-\log EC_{50}M$=7.2 and intrinsic activity 0.77±0.09 with respect to 5-HT (Fig. 1, Table 1). The positive inotropic effects of prucalopride (*n*=4) were completely prevented by 1 μM GR113808 (results not shown), consistent with mediation through 5-HT_4 receptors.

In other experiments, 1 μM prucalopride produced positive inotropic responses (that tended to fade partially) and shifted the concentration effect curve of 5-HT to the right compared with a curve in the absence of serotonin in other tissues from the same patients (Fig. 2). The intrinsic activity of prucalopride with respect to 5-HT before fading

Table 1 Comparison of agonist potency ($-\log EC_{50}$, M) with blocking potency ($-\log K_P$, M) vs. 5-HT of prucalopride on human right atrium and piglet left atrium

Species	$-\log EC_{50}$, M	*n*	$-\log K_p$, M	*n*
Man	7.43±0.25	7	7.17±0.21	3
Piglet	7.17±0.07	4	7.09±0.21	4

n refers to number of patients or piglets. To estimate K_P, 8 human atrial trabeculae and 12 piglet half left atria were used

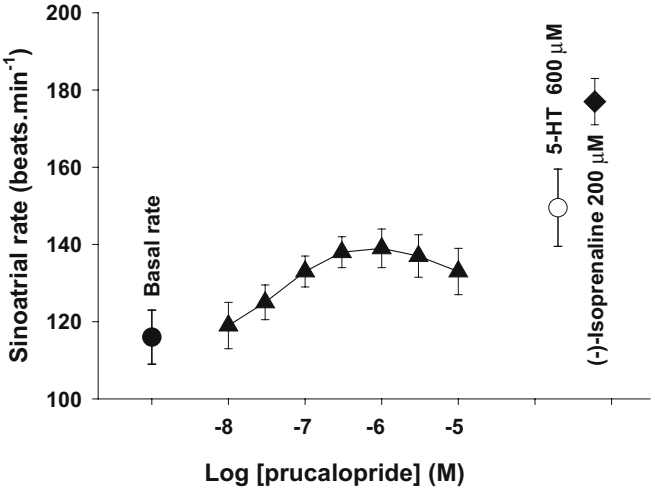

Fig. 3 Positive chronotropic effects of prucalopride on spontaneously beating right atria. Data were from 5 piglets

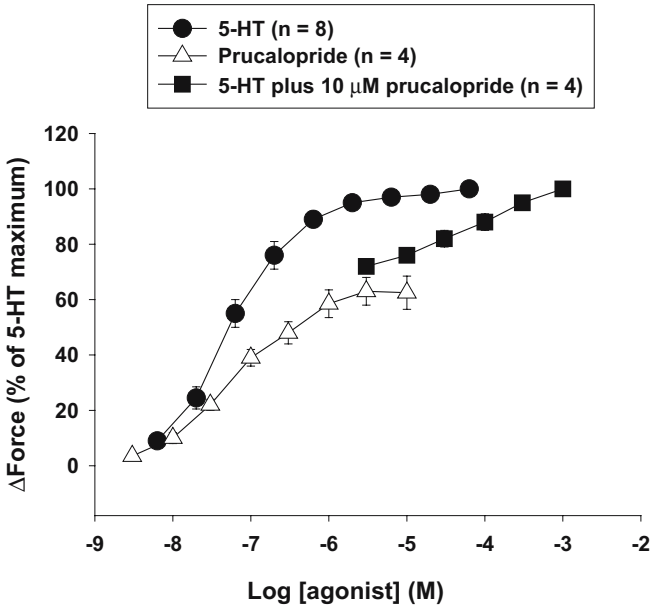

Fig. 4 Antagonism of the positive inotropic effects of 5-HT by prucalopride (10 μM) on piglet left atrium. Basal forces before 5-HT (*closed circles*) and prucalopride (*open triangles*) were 8±1.2 and 7.7±0.9 mN respectively. Maximal forces under 5-HT alone and 5-HT in the presence of prucalopride were 12.4±1.3 and 11.9±1.7 mN respectively. *n* refers to the number of hemiatria

was 0.55±0.08. The estimated dissociation equilibrium constant K_P (−log, M), estimated from these experiments, was not significantly different from the EC_{50} (−log, M) of prucalopride, estimated from separate experiments (Table 1). Thus, prucalopride appears to function as a classical partial agonist for 5-HT_4 receptors on human right atrial myocardium.

Spontaneously beating atrium of new-born piglet

Concentration-dependent increases in sinoatrial beating rate were observed with prucalopride with $-\log EC_{50}M$= 7.35±0.25 and intrinsic activity 0.72±0.11 with respect to 5-HT (Fig. 3). The positive chronotropic effects of prucalopride (*n*=4) were prevented by 1 μM GR113808 (results not shown), consistent with mediation through 5-HT_4 receptors.

Left atrium of new-born piglet

Prucalopride caused concentration-dependent increases in contractile force up to 10 μM, a concentration that caused a shift of the concentration-effect curve of 5-HT to the right compared with curves for 5-HT in the absence of prucalopride (Fig. 4). The intrinsic activity of prucalopride with respect to 5-HT was 0.65±0.05. The inotropic potency (−log EC_{50}) and blocking potency of prucalopride (−log

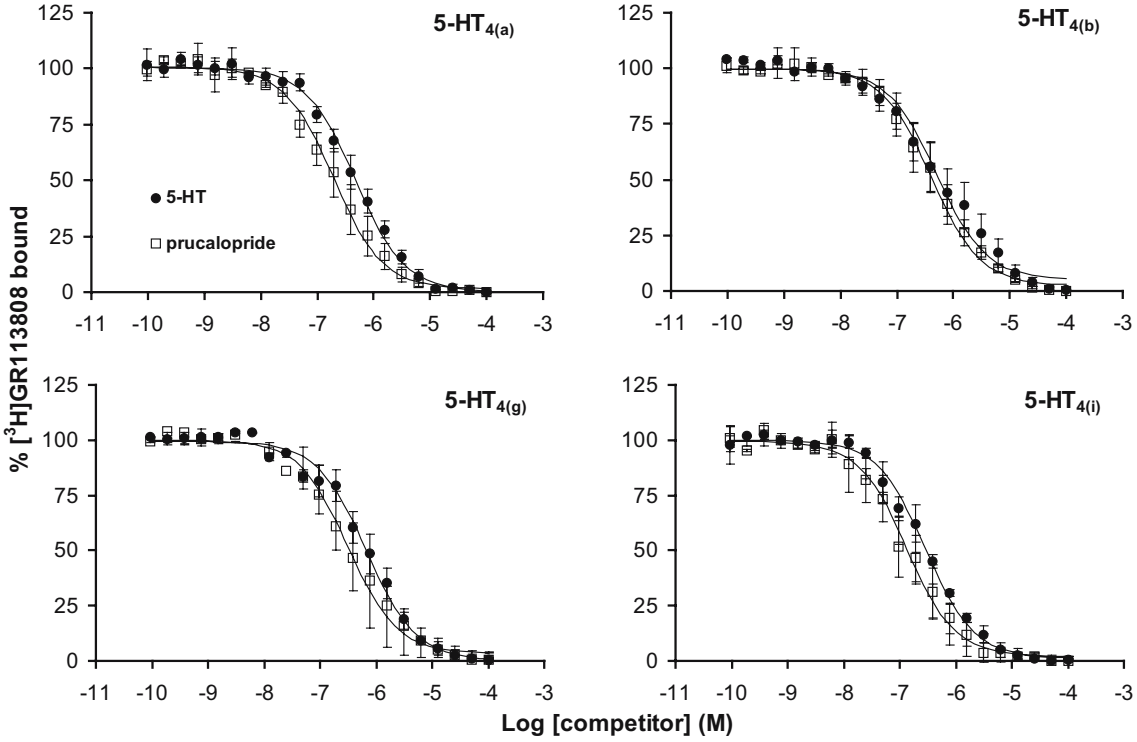

Fig. 5 Prucalopride and 5-HT displacement of [^{3}H]GR113808 binding to membranes from HEK293 cells stably expressing human 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$ or 5-$HT_{4(i)}$ splice variants separately, at densities of 1.28±0.15, 1.54±0.14, 2.63±0.96, and 0.88±0.31 pmol/mg respectively. Data shown are the mean ± SEM from 3–5 assays

Table 2 Comparison of the agonist potency (adenylyl cyclase stimulation) and the binding affinity of prucalopride and 5-HT for recombinant 5-HT_4 receptor splice variants

Splice variant	Prucalopride				5-Hydroxytryptamine		
	$-\log EC_{50}$, M	*n*	pK_i	*n*	$-\log EC_{50}$	$-\log EC_{50P}$	pK_i
5-$HT_{4(a)}$	7.23±0.04	3	7.60±0.26	3	7.15±0.06	6.61±0.05	7.28±0.05
5-$HT_{4(b)}$	7.34±0.05	3	7.45±0.51	5	7.05±0.01	6.80±0.03	7.24±0.71
5-$HT_{4(g)}$	7.97±0.15*	3	7.37±0.57	3	7.94±0.02*	6.88±0.12	7.11±0.31
5-$HT_{4(i)}$	7.18±0.01	3	7.79±0.36	3	7.15±0.05	6.79±0.1	7.42±0.11

pK_i=data from binding assays (Fig. 5); *n* values apply to both agonists
*$P<0.05$ with respect to other splice variants
$-\log EC_{50P}=-\log EC_{50}$ for 5-HT in the presence of prucalopride

K_P) were not significantly different (Table 1), suggesting that prucalopride behaves as a classical partial agonist.

Binding constants of prucalopride on recombinant human 5-HT_4 splice variants

The ability of prucalopride and 5-HT to compete with [^{3}H]GR113808 for binding to each of the four 5-HT_4 splice variants was examined in HEK293 cells stably expressing these receptors. Prucalopride was marginally more potent than 5-HT to displace [^{3}H]GR113808 on all four 5-HT_4 splice variants (Fig. 5, Table 2).

Agonist and blocking effects of prucalopride on recombinant human 5-HT_4 splice variants

The agonist and antagonist ability of prucalopride at the human 5-HT_4 receptor splice variants was investigated in stably transfected HEK293 cell lines expressing one of the 5-HT_4 receptor splice variants at relatively low levels, with

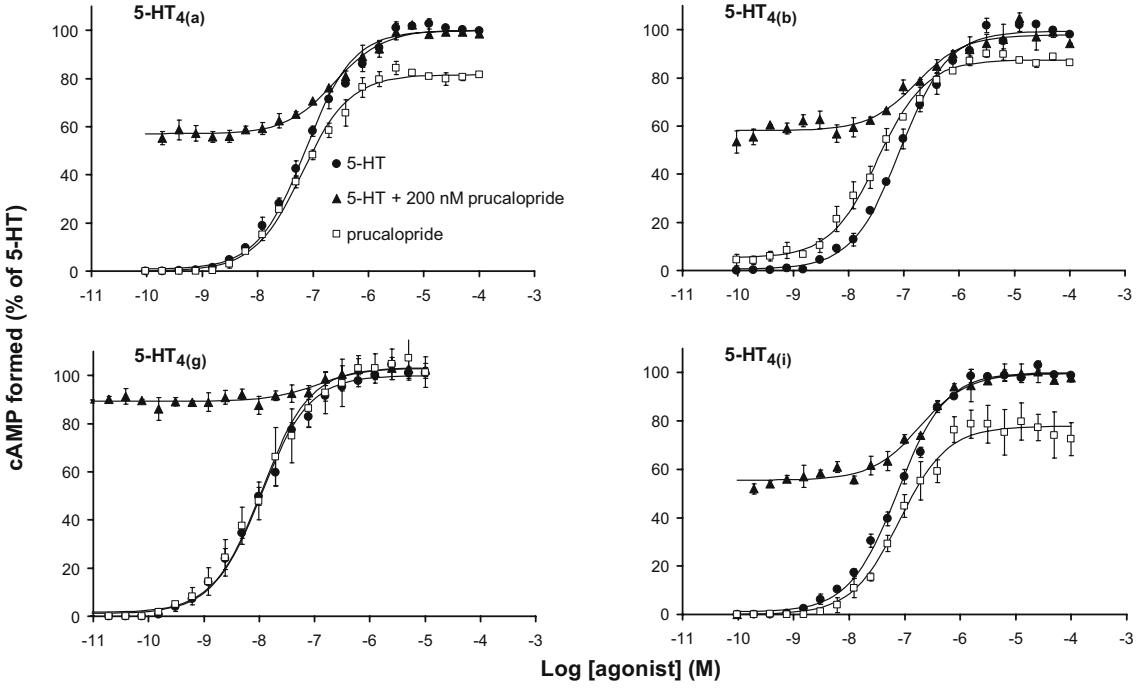

Fig. 6 Activation of adenylyl cyclase by prucalopride and 5-HT through human 5-$HT_{4(a)}$ (*upper left*), 5-$HT_{4(b)}$ (*upper right*), 5-$HT_{4(g)}$ (*lower left*) or 5-$HT_{4(i)}$ (*lower right*) receptors, expressed at densities of 5-$HT_{4(a)}$ 0.72±0.06, 5-$HT_{4(b)}$ 0.37±0.04, 5-$HT_{4(g)}$ 2.48±0.31, 5-$HT_{4(i)}$ 1.16±0.05 pmol/mg respectively. Membranes (10 µl aliquots) of HEK293 cell lines stably expressing either receptor were incubated in the presence of increasing concentrations of prucalopride (*open squares*), 5-HT (*circles*) or 5-HT in the presence of 200 nM prucalopride (*triangles*). All data shown are the mean ± SEM from three assays and AC activity is presented as a percentage of maximal 5-HT-stimulated AC activity. The basal and maximal 5-HT-stimulated AC activity in fmol/mg protein/min given respectively were: 5-$HT_{4(a)}$ 43±13 and 182±44; 5-$HT_{4(b)}$ 24±4 and 179±5; 5-$HT_{4(g)}$ 120±19 and 400±21; 5-$HT_{4(i)}$ 54±15 and 148±41

the exception of the 5-$HT_{4(g)}$ variant (Fig. 6). Prucalopride caused concentration-dependent increases in cAMP formation up to 10 μM in all four splice variants and, indicative of a partial agonist, the intrinsic activity of prucalopride with respect to serotonin was 0.82 at the 5-$HT_{4(a)}$, 0.86 at the 5-$HT_{4(b)}$, and 0.78 at the 5-$HT_{4(i)}$ splice variant. However, the intrinsic activity of prucalopride at the 5-$HT_{4(g)}$ variant was equal to 5-HT, most likely reflecting spare receptors due to the high level of receptor expression (Bruheim et al. 2003). Similar to that reported above in human and piglet atrium, 200 nM prucalopride caused a shift of the concentration-effect curve of serotonin to the right compared with curves for serotonin in the absence of prucalopride (Fig. 6). The $-\log EC_{50}$ of 5-HT in the presence of 200 nM prucalopride is presented in Table 2. The blocking potency of prucalopride ($-\log K_P$,M), estimated from EC_{50} ratios of 5-HT in the presence and absence of prucalopride, was 7.08±0.21, 6.59±0.06, 7.71±0.13, and 6.78±0.33 for 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$, and 5-$HT_{4(i)}$ receptors respectively.

Discussion

Our results show that prucalopride is a cardiostimulatory partial agonist through 5-HT_4 receptors in human and porcine atria. The affinity of prucalopride, estimated for human atrial 5-HT_4 receptors, was similar to the affinity of prucalopride for recombinant human 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$, and 5-$HT_{4(i)}$ receptors.

Prucalopride-evoked cardiostimulation

As initially proposed (Kaumann et al. 1990, 1991a), 5-HT increases the L-type Ca^{2+} current through 5-HT_4 receptors in human atrial myocytes, presumably through channel phosphorylation by cyclic AMP-dependent protein kinase (Ouadid et al. 1992). The positive inotropic effects of prucalopride on human atrium are probably due to an increase in L-type Ca^{2+} current, observed very recently with prucalopride on human atrial myocytes (Pau et al. 2005). Prucalopride was also a partial agonist, increasing L-type Ca^{2+} current maximally by 42% compared with the maximal effect of 5-HT (Pau et al. 2005). The positive inotropic effects of prucalopride can therefore be attributed to Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum through ryanodine channels, leading to enhanced contractility.

Unlike 5-HT, which can induce atrial arrhythmias (Kaumann and Sanders 1994), no arrhythmic contractions were observed with prucalopride. Proarrythmic early depolarizations were observed with 5-HT on human atrial myocytes (Pau et al. 2003), but not with prucalopride (Pau et al. 2005), consistent with our present observations. The proarrhythmic effects of 5-HT have been suggested to be related to Ca^{2+} overload (Kaumann 1994), consistent with the results of Pau et al. (2003) in human atrial myocytes. In contrast, the lack of proarrhythmic activity of prucalopride was attributed by Pau et al. (2005) to the smaller increase in L-type Ca^{2+} current compared with 5-HT. It would thus appear unlikely that prucalopride could elicit atrial arrhythmias in patients with constipation. Because of the antagonism of the effects of 5-HT (Figs. 2, 4) prucalopride would actually be expected to block 5-HT_4 receptors, thereby preventing possible arrhythmic effects caused by endogenously released 5-HT.

5-HT can cause tachycardia in man (Le Mesurier et al. 1959), as mimicked through piglet sinoatrial 5-HT_4 receptors (Kaumann 1990). Prucalopride was also a chronotropic partial agonist through piglet sinoatrial 5-HT_4 receptors. Prucalopride was a 6-fold and 8-fold more potent chronotropic agonist than the gastrokinetic agents renzapride and cisapride respectively (Kaumann 1990). The relevance to man of piglet sinoatrial tachycardia caused by gastrokinetic compounds is uncertain. Cisapride causes a small increase in heart rate in man (Bateman 1986), but data for prucalopride are not available.

Non-discriminating interaction of prucalopride with 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$, and 5-$HT_{4(i)}$ receptors

The splice variants a, b, g, and i of the 5-HT_4 receptor are expressed in human atrium (Bach et al. 2001; Medhurst et al. 2001; Brattelid et al. 2004b). The binding affinities of the four recombinant 5-HT_4 splice variants for prucalopride were similar and also resembled the affinity estimates of prucalopride for human atrial 5-HT_4 receptors. We have previously reported that recombinant 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors have near identical pharmacology and that either variant could mediate the effects of 5-HT on human atrium (Bach et al. 2001). Here we have shown that under our conditions the pharmacology of prucalopride and 5-HT with recombinant 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, and 5-$HT_{4(i)}$ receptors is quite similar. Only through 5-$HT_{4(g)}$ receptors are both 5-HT and prucalopride significantly more potent agonists than through the three other variants (Table 2), and prucalopride is a full agonist. The difference is probably due to the higher density of transfected 5-$HT_{4(g)}$ receptors used, resulting in greater amplification of the receptor signals induced by the agonists (i.e., spare receptors, Bruheim et al. 2003), than that of the other variants. In support, Pindon et al. (2002) reported 10-fold greater agonist potencies (cAMP) of prucalopride on both recombinant 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors than those measured in our assays in clones with the high receptor densities of 3.1 and 7 pmol/mg protein respectively. The agonist potency of prucalopride reported by Pindon et al. (2002) at 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors was similar to our values at 5-$HT_{4(g)}$. A similar dependence of agonist potencies on receptor levels was previously reported for 5-HT at 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors (Bach et al. 2001). The binding affinity of prucalopride was previously estimated for 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors transfected into HEK293 cells and found to be 10-fold and 4-fold higher (Briejer et al. 2001) than in our binding assays. The reason for this difference is

not clear, but is presumably due to different experimental conditions in each laboratory.

Conclusions

Prucalopride is a partial agonist for porcine and human atrial 5-HT_4 receptors. The affinity of prucalopride, estimated for atrial 5-HT_4 receptors, is similar to the binding affinity of prucalopride for the four human recombinant splice variants 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(g)}$, and 5-$HT_{4(i)}$. 5-HT and prucalopride also have similar agonist effects through the four splice variants. Because these four splice variants are expressed in human atrium, the participation of each variant in the pharmacology of 5-HT and prucalopride cannot be ruled out.

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