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Electrophysiological Effects of Prucalopride, a Novel Enterokinetic Agent, on Isolated Atrial Myocytes from Patients Treated with β-Adrenoceptor Antagonists

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ABSTRACT

Prucalopride is a selective 5-hydroxytryptamine type 4 (5-HT_4) receptor agonist developed for the treatment of gastrointestinal disorders. The endogenous agonist 5-HT acting via 5-HT_4 receptors increases the L-type Ca^{2+} current (I_{CaL}) with potentially proarrhythmic consequences (Pau et al., 2003). The aims of this study were to investigate the effects of prucalopride on I_{CaL}, action potentials, refractory period, and arrhythmic activity in human atrial myocytes, and to compare these with the effects of 5-HT, using the whole-cell perforated patch-clamp technique. Prucalopride (10^{-9} to 10^{-4} M) produced a concentration-dependent increase in I_{CaL} amplitude, with a maximum response at 10 μM, from −5.3 ± 0.6 to −10.9 ± 1.5 pA/pF ($p < 0.05$; $n = 22$ cells, 10 patients), without affecting its voltage-dependence. Subsequent application of 10 μM 5-HT further increased I_{CaL} to −17.7 ± 2.8 pA/pF ($p < 0.05$; $n = 16$ cells, 9 patients). The increase in I_{CaL} by prucalopride, 98 ± 15%, was significantly smaller than that by 5-HT, 233 ± 26% ($p < 0.05$). Prucalopride (10 μM) significantly increased the action potential duration at 50% repolarization (APD_{50}) from 12 ± 2 to 17 ± 3 ms ($p < 0.05$; $n = 22$ cells, 9 patients). Following washout of prucalopride, 5-HT (10 μM) increased APD_{50}, to a greater extent, from 14 ± 3 to 32 ± 7 ms ($p < 0.05$; $n = 11$ cells; 8 patients). The APD_{75}, APD_{90}, and effective refractory period were unaffected by prucalopride or 5-HT. Furthermore, 5-HT induced abnormal depolarizations in 27% of the cells studied, whereas prucalopride induced none ($p < 0.05$). In conclusion, in human atrial cells, prucalopride, at concentrations markedly above those used therapeutically, acted as partial agonist on I_{CaL} and APD_{50}, with no effect on late repolarization or refractory period, and was devoid of arrhythmic activity.

Activation of 5-hydroxytryptamine type 4 (5-HT_4) receptors may have multiple functional roles in the human body, including inotropic effects in the atrium, prokinetic activity in the gastrointestinal tract, and involvement in memory and learning processes in the brain (Eglen et al., 1995; Hegde and Eglen, 1996). Moreover, 5-HT_4 receptors have been implicated in various pathological conditions including atrial arrhythmias (Kaumann, 1994), neurodegenerative diseases (Wong et al., 1996), and gastrointestinal motility disorders (Talley, 1992). Consequently, 5-HT_4 receptors have been a target for the development of novel 5-HT_4 agonists (e.g., to treat functional bowel disorders (Talley, 2001) and 5-HT_4 antagonists (e.g., to treat atrial fibrillation; Kaumann, 1994).

In human atrium, 5-HT_4 receptors are the only subtype present (Kaumann et al., 1990; Blondel et al., 1997), and activation with 5-HT increases the amplitude of the L-type Ca^{2+} current (I_{CaL}) via the cyclic AMP-dependent cascade (Ouadid et al., 1992). Proarrhythmic activity of 5-HT has been demonstrated previously in human atrial muscle (Kaumann and Sanders, 1994) and in human atrial myocytes (Pau et al., 2003), probably associated with the increased I_{CaL}. Moreover, it has been postulated that the release of 5-HT from platelets may be involved in the origin and maintenance of atrial fibrillation (Kaumann, 1994). In a pig model of atrial fibrillation, a 5-HT_4 antagonist, RS-100302, has been reported to be antiarrhythmic by prolonging the effective refractory period (Rahme et al., 1999), suggesting that endogenously released 5-HT may shorten the effective refractory period, thereby sustaining re-entry circuits. For these reasons, there has been concern that 5-HT_4 receptor agonists when used as gastrokinetic agents in humans may generate

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ABBREVIATIONS: 5-HT_4, 5-hydroxytryptamine type 4; RS-100302, *N*-(2-{4-[3-(8-amino-7-chloro-2,3-dihydrobenzo[1,4]dioxin-5-yl)-3-oxo-propyl]-piperdin-1-yl}-ethyl)-methanesulfonamide; HERG, human *ether-a-go-go*-related gene; APD, action potential duration; ERP, effective refractory period; bpm, beat(s) per minute; GR-113808, [1-[2-(methylsulfonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate.

atrial arrhythmias via intracellular Ca^{2+} overload and/or a reduced refractoriness (Tonini et al., 1999; Yusuf et al., 2003).

Prucalopride, a novel 5-HT_4 receptor agonist with a benzofurancarboxamide structure, is currently under investigation for the treatment of idiopathic chronic constipation (Coremans et al., 2003). It has been demonstrated to be a potent and specific 5-HT_4 receptor agonist in studies on human native and recombinant 5-HT_4 receptor subtypes (Prins et al., 2000; Briejer et al., 2001; Pindon et al., 2002). There has been only one previous study on the effects of prucalopride on cardiac ion currents, specifically the rapid component of the recombinant delayed rectifier K^+ current (I_{Kr}) encoded by the cloned human *ether-a-go-go*-related gene (HERG) (Potet et al., 2001). This study showed that micromolar concentrations of prucalopride significantly blocked I_{Kr}. Although I_{Kr} is present in the human ventricle (Li et al., 1996), in the human atrium it is not clear whether I_{Kr} may play a substantial role in action potential repolarization (Wang et al., 1994; Lee and Lee, 1998; Bertaso et al., 2002). The electrophysiological effects of prucalopride have not yet been studied in human atrium.

The aims of this study were to assess in human atrial myocytes the electrophysiological effects of prucalopride on I_{CaL}, action potential duration (APD), cellular effective refractory period (ERP), and arrhythmic activity; and to compare the effects of prucalopride with those of the endogenous agonist 5-HT.

Materials and Methods

Tissue and Cell Isolation. Procedures for obtaining human tissue were approved by the institutional ethics committee of Glasgow Royal Infirmary and conform to the Declaration of Helsinki (World Medical Association, 1997). Samples of the right atrial appendage were obtained from consenting patients undergoing cardiac surgery. All patients had received prior therapy with β-adrenoceptor antagonists. Atrial cells were isolated enzymatically using a method described in detail by Workman et al. (2001).

Electrical Recording Techniques. The whole-cell perforated patch-clamp technique was used to record action potentials and calcium currents, as described in detail by Pau et al. (2003). Briefly, cells were superfused at 37°C with a physiological solution containing: 130.0 mM NaCl, 4.0 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10.0 mM glucose, and 10.0 mM HEPES, pH 7.4. To record calcium currents, electrodes were filled with a solution containing: 30.0 mM CsCl, 5.0 mM HEPES, 1.0 mM $MgCl_2$, 100.0 mM Cs methanesulfonic acid, and 5.0 mM NaCl. To record action potentials, an internal solution containing 30.0 mM KCl, 5.0 mM HEPES, 1.0 mM $MgCl_2$, 100.0 mM K methanesulfonic acid, and 5.0 mM NaCl was used. The series resistance was observed to stabilize between 5 and 15 min at 9.9 ± 0.3 MΩ (n = 96 cells), with a mean cell capacity of 78 ± 2 pF. Capacitative transients were compensated electronically from the recordings, and the voltage drop across the series resistance was also compensated (68–80%). The software program WinWCP (J. Dempster, University of Strathclyde, Glasgow, UK) was used both to stimulate and record electrical activity. All currents were normalized to the cell's capacity.

Experimental Protocols. Voltage clamp was used to record I_{CaL}. The voltage dependence of this current was measured from a holding potential of −40 mV, increasing in steps of 10 mV, up to +40 mV, with pulses of 250-ms duration (0.33 Hz). The time-dependent effect of drugs on peak I_{CaL} was measured with pulses of 250-ms duration, from −40 to + 10 mV (0.2 Hz).

Current clamp was used to record actions potentials and the cellular effective refractory period at the physiological rate of 75 beats/min (bpm) using 5-ms stimulating pulses of 1.2× threshold strength. The ERP was measured using a standard S_1-S_2 stimulation protocol and was defined as the longest S_1-S_2 interval that failed to elicit an S_2 action potential of amplitude >80% of the preceding S_1 action potential. The APD was calculated as the interval between the action potential upstroke and repolarization to the level of 50% (APD_{50}), 75% (APD_{75}), and 90% (APD_{90}) of the upstroke amplitude. I_{CaL}, action potentials, and the ERP were recorded before, and 90 s after, drug additions and again 180 s after removal of drugs.

Drugs. Prucalopride (R093877; Briejer et al., 2001) and the specific 5-HT_4 antagonist GR-113808 (Kaumann, 1993) were donated by Johnson and Johnson Pharmaceutical Research and Development (Beerse, Belgium) and were dissolved in dimethyl sulfoxide with a stock solution of 10 mM and subsequently diluted in physiological solutions. 5-HT (Sigma-Aldrich, St. Louis, MO) was made up as a 10 mM stock solution in distilled water.

Data Analysis and Statistics. Clinical characteristics and drug treatments of each patient were obtained from the case notes. Only patients in sinus rhythm at the time of surgery were included. Cells were excluded from analysis if either the APD_{50} or peak I_{CaL} decreased irreversibly during the protocol. Concentration-response data for the effect of prucalopride on I_{CaL} were fitted iteratively (Prism 3.0; GraphPad Software Inc., San Diego, CA) using a variable slope sigmoidal concentration-response curve (Hill equation). The curves were fitted to mean I_{CaL} values, obtained at six concentrations of prucalopride between 1 nM and 100 μM. The concentration-response curve for 5-HT was obtained previously in our lab (Pau et al., 2003). Curve-fit values were compared using a two-tailed unpaired Student's t test. Time-dependent inactivation of I_{CaL} was fitted by a biexponential function using the WCP software program and was defined by the following equation: $I_{CaL}(t) = A_1.\exp(-t/\tau_1) + A_2.\exp(-t/\tau_2) + C$, where A_1, A_2, τ_1, and τ_2 are the amplitudes and decay time constants of the respective exponential components, and C is the steady-state amplitude. Data are expressed as mean ± S.E.M., with n being equal to the number of cells studied. Mean values were compared using two-tailed paired or unpaired Student's t tests, as appropriate. A Fisher's exact test was used to assess the level of significance of differences in the incidences of arrhythmic activity between drugs. $p < 0.05$ was regarded as statistically significant.

Results

Patients' Clinical Characteristics. All patients were undergoing coronary artery bypass graft surgery, were taking β-adrenoceptor antagonists, suffered from angina, and none had severe left ventricular dysfunction (Table 1). Eighty-two percent of the patients were also treated with an angiotensin-converting enzyme inhibitor and 36% of the patients with a calcium channel blocker. β-Adrenoceptor antagonists had been administered for more than 10 days prior to surgery. No patient was administered sotalol (a β-blocker with additional class III antiarrhythmic activity). Patients received their routine cardiac drugs on the day of surgery. Mean heart rate was 58 ± 2 bpm (n = 11).

Effects of Prucalopride, 5-HT, and GR-113808 on I_{CaL} in Human Atrial Cells. Prucalopride produced a significant increase in the amplitude of I_{CaL}, as shown in Fig. 1 by the I_{CaL} current density-voltage relationships. An example of original I_{CaL} recordings is also shown (Fig. 1, inset). Prucalopride (10 μM) increased the mean magnitude of peak I_{CaL} (recorded at +10 mV) from −5.7 ± 0.7 pA/pF to −10.2 ± 1.6 pA/pF ($p < 0.05$, n = 10 cells, 7 patients). This increase in peak I_{CaL} occurred without any change in the voltage dependence of the current and was reversible on washout of prucalopride (−5.7 ± 1.1 pA/pF; n = 7 cells, 5 patients).

TABLE 1
Patients' preoperative clinical characteristics
Values are numbers of patients (n and percentage of total, respectively) with selected clinical characteristics, except for age (mean ± S.E.M.). All patients were in sinus rhythm on the day of surgery.

	n	%
Patients	11	
Male/female	7/4	64/36
Age	62 ± 3	
Surgery		
CABG	10	91
CABG + AVR	1	9
Drugs		
β-Adrenoceptor antagonist	11	100
Ca^{2+} channel blocker	4	36
ACE inhibitor	9	82
Nitrate	6	55
Diuretic	4	36
Lipid-lowering	11	100
Symptoms		
Angina	11	100
Hypertension	4	36
Hyperlipidaemia	10	91
Previous History		
MI	7	64
Diabetes	2	18
LV Function		
Normal	6	55
Mild–moderate	5	45
Severe	0	0

CABG, coronary artery bypass graft surgery; AVR, aortic valve replacement; ACE, angiotensin-converting enzyme; MI, myocardial infarction; LV, left ventricular.

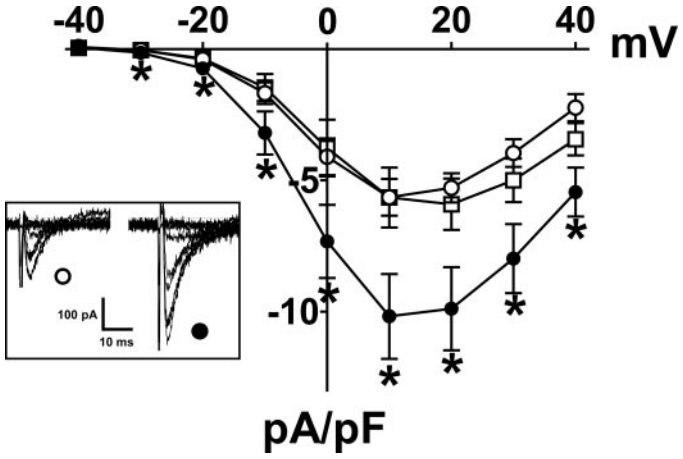

Fig. 1. Effect of prucalopride on I_{CaL} current-voltage relationship in human atrial myocytes. Current-voltage relationships of I_{CaL} expressed in terms of current density, pA/pF (n = 10 cells, 7 patients). Values are means with error bars denoting S.E.M. for control (open circles), prucalopride at 10 μM (closed circles), and after 3-min washout of prucalopride (open squares, n = 7 cells, 5 patients). *, $p < 0.05$ between control and prucalopride values at each voltage step (paired Student's t test). Inset, example of original calcium current (I_{CaL}) traces obtained from a human atrial cell during depolarizing voltage-clamp pulses (250 ms, 0.33 Hz) from −40 to +40 mV, in 10-mV incremental steps, from a holding potential of −40 mV, under control conditions (open circle), and in the presence of prucalopride at 10 μM (closed circle), is shown.

The time course of change in I_{CaL} by prucalopride and its blockade by the specific 5-HT_4 receptor antagonist, GR-113808, can be seen in Fig. 2. Prucalopride at 10 μM caused a stable increase in I_{CaL}, which was completely antagonized by GR-113808 at 1 μM (n = 5 cells, 3 patients). This increase, from a control value of −5.9 ± 1.2 to −13.1 ± 3.8 pA/pF, was abolished by GR-113808 to −5.4 ± 1.2 pA/pF

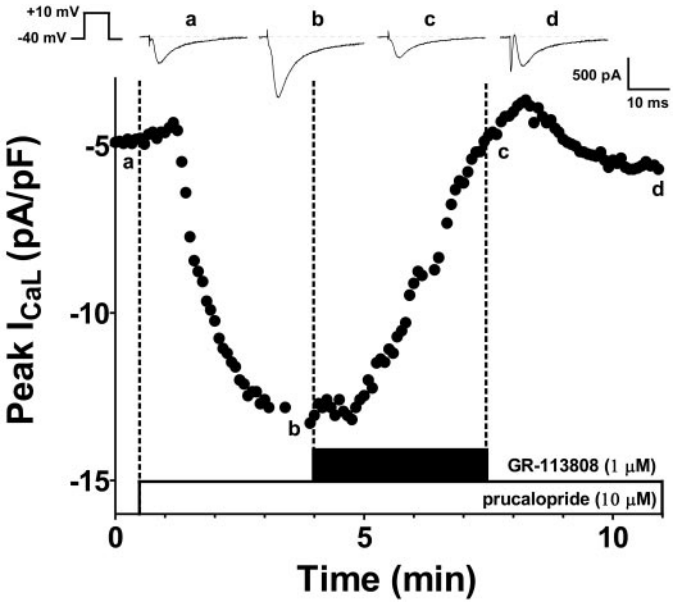

Fig. 2. Effect of the specific 5-HT_4 antagonist GR-113808 on I_{CaL} stimulated by prucalopride in a human atrial myocyte. An example of the time course of change in peak I_{CaL} density (pA/pF) plotted at 5-s resolution, in response to 10 μM prucalopride (open box), followed by the application of GR-113808 at 1 μM (solid box), and the subsequent washout of the antagonist. Inset traces (a–d) show original currents recorded at the time points labeled.

($p < 0.05$). The effect of this antagonist was partially reversible upon its washout (to 6.5 ± 1.1 pA/pF).

Figure 3A shows that the increase in I_{CaL} with prucalopride (10 μM) was less than that produced by 5-HT (10 μM) in the same cell. This was confirmed by mean data. Prucalopride increased I_{CaL} from −5.5 ± 0.8 to −10.5 ± 1.7 pA/pF ($p < 0.05$, n = 16 cells, 9 patients). Subsequent superfusion of 5-HT alone, in the same cells, increased I_{CaL} further, to −17.7 ± 2.8 pA/pF ($p < 0.05$). The percentage increase in I_{CaL} produced by 5-HT (233 ± 26% compared with control) was significantly greater than that produced by prucalopride (89 ± 15%; $p < 0.05$) (Fig. 3B). The time-dependent inactivation of I_{CaL} was unchanged by the application of prucalopride (10 μM) or 5-HT (10 μM). The fast (τ_1) and the slow (τ_2) inactivation time constants of basal I_{CaL} were 4.4 ± 0.3 and 47.7 ± 8.1 ms, respectively, and were not significantly different in the presence of prucalopride (τ_1 = 6.0 ± 1.8 ms and τ_2 = 41.9 ± 6.2 ms) or 5-HT (τ_1 = 5.6 ± 1.8 ms and τ_2 = 40.0 ± 5.1 ms). The respective fractions of the fast (A_1 = 72.5 ± 1.9%) and slow (A_2 = 27.5 ± 1.9%) components of the I_{CaL} inactivation amplitude remained unchanged by the application of either prucalopride or 5-HT.

The Concentration-Response Relationship of the Effect of Prucalopride on I_{CaL}. The concentration-response curve of the effect of prucalopride on peak I_{CaL} obtained in atrial cells from patients with prior β-adrenoceptor antagonists treatment is shown in Fig. 4. This figure also shows the superimposed concentration-response curve of the effect of 5-HT on peak I_{CaL} obtained previously in our lab in atrial cells from a similar group of patients (Pau et al., 2003). Prucalopride (from 1 nM to 100 μM) elicited a concentration-dependent increase in the amplitude of peak I_{CaL} (measured at +10 mV), with a maximum response (E_{max}) equal to an increase of 93 ± 14% above control (n = 7–22 cells, 2–9

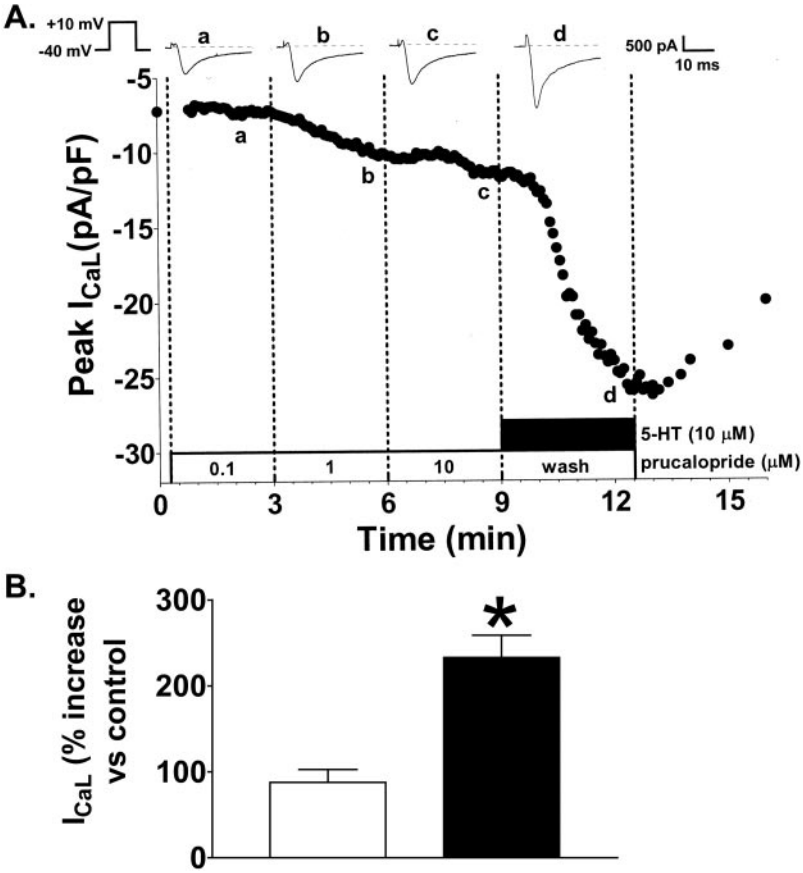

Fig. 3. Effect of prucalopride followed by 5-HT on I_{CaL} in human atrial myocytes. A, effect of a range of concentrations (0.1–10 μM) of prucalopride, followed by a maximal concentration of 5-HT (10 μM) given cumulatively on I_{CaL}. The effect of washout of 5-HT is also shown. Inset traces (a–d) show original currents recorded at the time points labeled. B, mean data (±S.E.M.) for the effect of prucalopride (10 μM; open bars) and 5-HT (10 μM; solid bars) on I_{CaL} (n = 16 cells, 9 patients). Values are expressed as a percentage increase above control. *, $p < 0.05$ between prucalopride and 5-HT-induced increases in I_{CaL} (paired Student's t test).

patients). This was significantly less than that produced by the maximum response of 5-HT (E_{max} = 299 ± 12% above control; $p < 0.05$). However, there were no significant differences between prucalopride and 5-HT for either the log EC_{50} (−6.66 ± 0.44 versus −7.09 ± 0.07, respectively) or the Hill coefficient, n_H (0.76 ± 0.46 versus 1.46 ± 0.56, respectively).

Effect of Prucalopride and 5-HT on Action Potentials and the Refractory Period. Fig. 5 shows representative traces of action potentials and measurements of ERP illustrating the effects of 10 μM prucalopride (Fig. 5B) or 5-HT (Fig. 5C), compared with control conditions (Fig. 5A). Prucalopride (10 μM) caused a small but significant prolongation in the APD_{50} from 12 ± 2 to 17 ± 3 ms ($p < 0.05$; n = 22 cells, 9 patients), representing an increase of 6 ± 2 ms ($p < 0.05$). This effect was fully reversible upon washout of prucalopride (12 ± 3 ms; n = 13 cells, 8 patients). There was no significant or reversible effect of prucalopride on the APD_{75} (141 ± 12 versus 146 ± 11 ms), APD_{90} (237 ± 18 versus 236 ± 18 ms), or ERP (224 ± 25 versus 232 ± 28 ms).

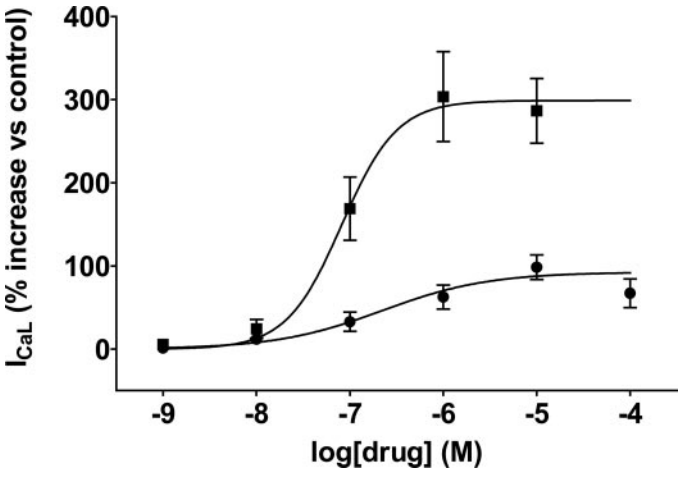

Fig. 4. Concentration-dependent effects of prucalopride and 5-HT on I_{CaL} in human atrial myocytes. Comparison of the concentration-response relationship for prucalopride (10^{-9} to 10^{-4}; solid circles; n = 7–22 cells, 2–9 patients) and 5-HT (10^{-9} to 10^{-5}; solid squares; n = 9–18 cells, 4–11 patients; from Pau et al., 2003) on peak I_{CaL}. Values are means ± S.E.M. The increase in I_{CaL} is expressed as a percentage of the control value before the addition of prucalopride and 5-HT.

In 11 of the 22 cells (from 8 patients) studied with prucalopride, we investigated the effects of 5-HT at 10 μM following 3-min washout of prucalopride at 10 μM. As shown in Fig. 6A, the prolongation of the APD_{50} produced by 5-HT, from a control value of 14 ± 3 to 32 ± 7 ms ($p < 0.05$), was greater than the prolongation produced by prucalopride (12 ± 2 to 19 ± 5 ms; $p < 0.05$), in these cells. The mean duration by which the APD_{50} was increased was also greater with 5-HT (18 ± 5 ms) compared with that with prucalopride (7 ± 3 ms; $p < 0.05$). The effect of 5-HT was fully reversible after 3-min washout (15 ± 4 ms; n = 7 cells, 6 patients). 5-HT did not significantly affect the APD_{75}, APD_{90} or the ERP, consistent with our previous report (Pau et al., 2003). Figure 6B shows that the calcium channel blocker nifedipine at 10 μM prevented the prucalopride and the 5-HT-induced increases in APD_{50} in the 11 (5 patients) and 4 (2 patients) cells studied, respectively. This concentration of nifedipine had been shown to markedly reduce I_{CaL} from −4.8 ± 0.8 to −0.2 ± 0.1 pA/pF ($p < 0.05$; n = 5 cells, 4 patients).

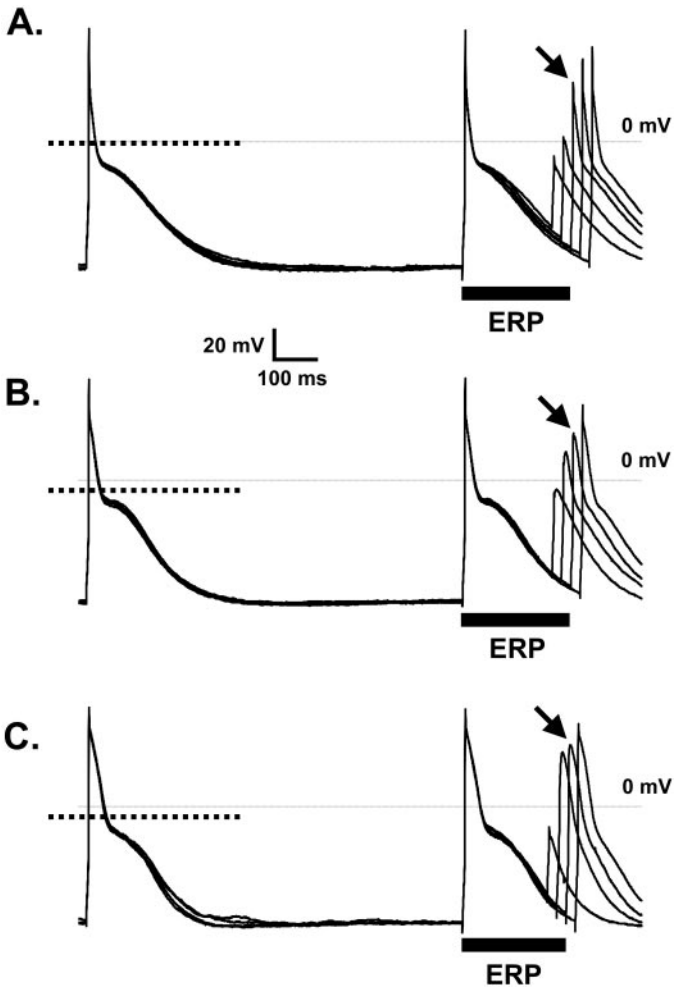

Fig. 5. Effect of prucalopride and 5-HT on action potentials and refractoriness in a single human atrial myocyte. Representative examples of original action potential recordings before (A), in the presence of 10 μM prucalopride (B), and after 3-min washout of prucalopride, 10 μM 5-HT (C). Cells were paced at 75 bpm. Dotted lines in bold show the level of 50% of the action potential amplitude. Solid bars, ERP. The S_2 response used to measure this interval is labeled with an arrow.

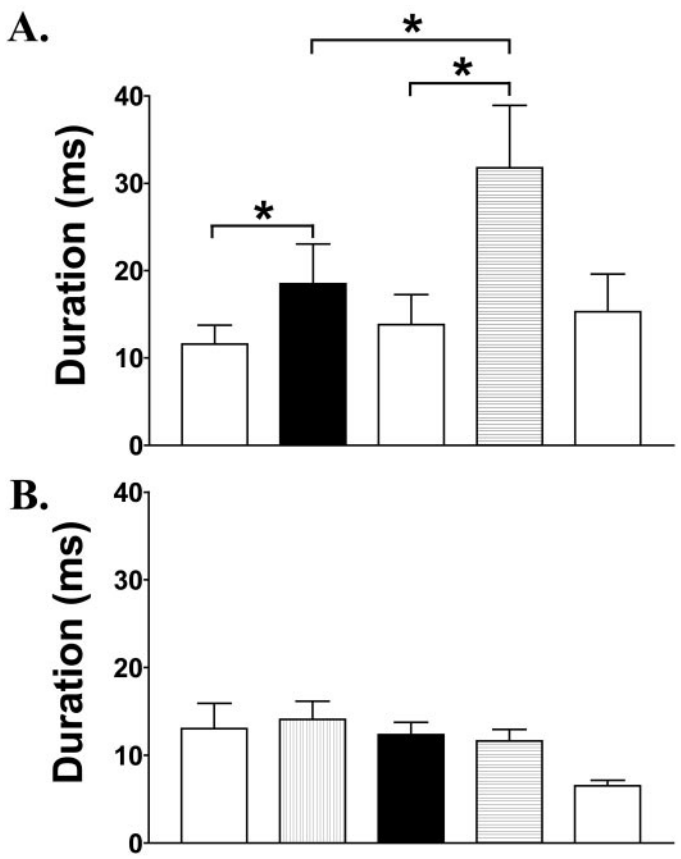

Fig. 6. Effect of prucalopride, 5-HT, and nifedipine on APD_{50} in human atrial cells. A, mean (±S.E.M.) action potential duration (milliseconds) measured at 50% repolarization (APD_{50}; $n = 11$ cells, 8 patients) in the absence (open bars) and presence (closed bars) of 10 μM prucalopride or 10 μM 5-HT (horizonal striped bars). *, $p < 0.05$ versus control or drug as indicated by the dotted line (paired Student's t test). B, mean action potential data for APD_{50}, in the absence (open bars) and presence (vertical striped bars; $n = 11$ cells, 5 patients) of 10 μM nifedipine, 10 μM nifedipine + 10 μM prucalopride (closed bars), and 10 μM nifedipine + 10 μM 5-HT (horizontal striped bars; $n = 4$ cells, 2 patients).

Effects of Prucalopride and 5-HT on Arrhythmic Activity in Human Atrial Cells. Abnormal depolarizations were not observed in any of the 22 cells to which 10 μM prucalopride was applied. In contrast, when 10 μM 5-HT was subsequently applied (to 11 of these), abnormal depolarizations occurred in 3 (27%) of the cells studied ($p < 0.05$). Figure 7 shows an example of abnormal depolarizations caused by 5-HT, but not by prucalopride, and their abolition by GR-113808 at 1 μM. In another cell, from a different patient, 5-HT induced abnormal depolarizations in the absence, but not in the presence, of nifedipine at 10 μM (not shown).

Discussion

This study has demonstrated, for the first time to our knowledge, that prucalopride in human atrial cells acted as a partial rather than full agonist on the L-type calcium current and action potential early repolarization via 5-HT_4 receptors. Similarly to 5-HT, prucalopride lacked effects on the late phase of the atrial action potential repolarization and the effective refractory period. Unlike 5-HT, prucalopride did not induce abnormal depolarizations.

Prucalopride has been shown previously to be a full agonist in several areas of the human body including the gastrointestinal (Prins et al., 2000) and central nervous (Robert et al., 2001) systems. Studies using either native or recombinant 5-HT_4 receptors have shown that prucalopride had similar, or even greater, efficacy than the endogenous agonist 5-HT (Prins et al., 2000; Pindon et al., 2002; Lezoualc'h and Robert, 2003). The EC_{50} value for prucalopride in human atrial cells in the present study was higher than that obtained with human recombinant 5-HT_{4a} and 5-HT_{4b} receptor isoforms (Briejer et al., 2001; Pindon et al., 2002), which indicated a lower potency of the agonist in human atrial cells. In this study, we included only patients who had been previously treated with β-adrenoceptors antagonists. Such prior therapy did not affect the mRNA expression of 5-HT_4 receptors and L-type Ca^{2+} channels subunits α_{1c}, α_2/δ_1, β_{1a}, β_{1b}, and β_{1c} in the human atrium (Grammer et al., 2001). However, we have previously shown in human atrial cells that prior β-blockade enhanced the maximal response of I_{CaL} to 5-HT but with no effect on the EC_{50} or the Hill coefficient of the concentration-response curve for 5-HT (Pau et al., 2003).

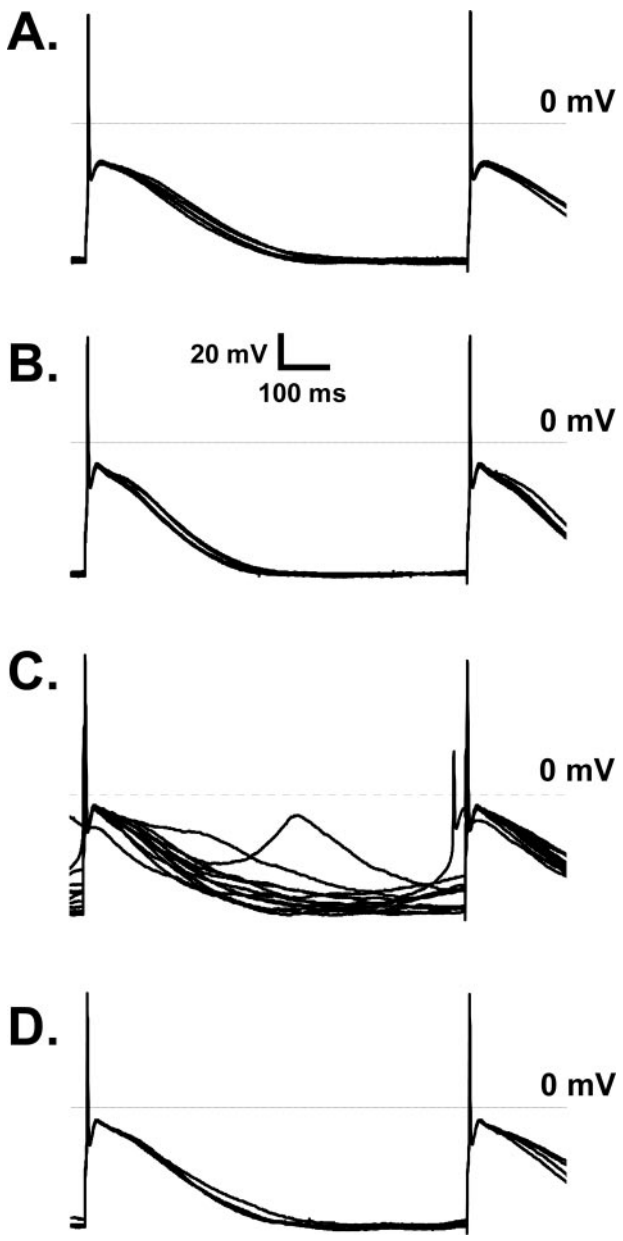

Fig. 7. Effect of prucalopride and 5-HT on arrhythmic activity in human atrial cells. Effect of 5-HT, but not of prucalopride, to promote abnormal depolarizations in a human atrial myocyte and its blockade by the specific 5-HT_4 antagonist GR-113808. An example of original recordings of action potentials obtained from a single human atrial myocyte (paced at 75 bpm), before (A), in the presence of 10 μM prucalopride (B), 10 μM 5-HT (C) (following 3-min washout of prucalopride), and then blockade of 5-HT-induced abnormal depolarizations by 1 μM GR-113808 (D).

Hence, our finding of lower potency remains relevant to patients who have not been receiving β-adrenoceptor antagonists.

To date, nine 5-HT_4 receptor subtypes (5-$HT_{4a\text{-}i/hb/n}$) have been identified and localized in the human body (Langlois and Fischmeister, 2003; Brattelid et al., 2004), but only some of these (5-$HT_{4a\text{-}c}$, 5-HT_{4g}, 5-HT_{4i}, and 5-HT_{4n}) have been shown to be present in the human atrium (Bach et al., 2001; Medhurst et al., 2001; Vilaró et al., 2002; Brattelid et al., 2004). In addition, the pharmacological properties of the 5-HT_4 isoforms have been associated with different receptor levels of expression (Bach et al., 2001; Medhurst et al., 2001; Vilaró et al., 2002; Brattelid et al., 2004). Thus, it is possible that prucalopride acts as partial agonist with low potency in human atrial cells because of the expression of specific 5-HT_4 isoforms and/or because of the different levels of expression of these isoforms present in the human atrium, compared with other tissues. In addition, 5-HT and prucalopride, acting via 5-HT_4 receptors, may differently affect the signal transduction cascade, including the phosphorylation of different isoforms of the L-type Ca^{2+} channel (Pindon et al., 2002). These questions may be resolved following the development of more selective agonists, antagonists, and antibodies acting on specific 5-HT_4 receptor subtypes or by coexpressing the L-type Ca^{2+} channels with the recombinant 5-HT_4 receptors isoforms in a 5-HT receptor-free cell model.

Prucalopride significantly prolonged the early repolarization phase of the action potential without affecting the late phase or the refractory period, likely due to the observed increase in I_{CaL}. This was confirmed by the experiments using the calcium channel blocker, nifedipine, which abolished the effects of prucalopride and 5-HT on the action potential. In addition, prucalopride had a less marked action to prolong APD_{50} than did 5-HT (at maximally effective concentrations), consistent with the partial activity observed on I_{CaL}. However, we cannot exclude effects on other currents of importance during repolarization in the human atrium, such as the transient outward K^+ current, I_{TO}, and the ultra-rapid component of the delayed rectifier K^+ current, I_{Kur}.

The fact that a 5-HT_4 antagonist, RS-100302, prolonged the ERP in an in vivo pig model of atrial fibrillation (Rahme et al., 1999) suggested that 5-HT_4 agonists may shorten the ERP and, therefore, be pro-arrhythmic by reducing the minimum path length required for re-entry. However, this is not supported by our experiments in isolated human atrial cells, in which neither prucalopride nor 5-HT abbreviated the late phase of repolarization or the refractory period. It is noteworthy that in canine atrial cells isolated using the chunk method, the repolarizing, delayed rectifier K^+ current was recorded in significantly fewer cells than in those isolated by enzyme perfusion (Yue et al., 1996). Also, it has been reported that, in the human atrium, this current is limited to only a subpopulation of cells (Wang et al., 1994) or even absent (Lee and Lee, 1998; Bertaso et al., 2002). These two factors may explain the lack of effect of prucalopride and 5-HT to shorten late repolarization and refractory period in human atrial myocytes. Alternatively, in the pig, the 5-HT_4 antagonist, RS-100302, may have had a direct effect, independently of blocking 5-HT receptors to prolong ERP.

In the present study, we investigated the effects on I_{CaL} of a range of concentrations of prucalopride, from 1 nM to 100 μM, to obtain a full concentration-response relationship of the drug. For comparison, the effective therapeutic range of prucalopride in humans has been found to be between 1 and 10 nM with a mean plasma concentration of around 2 ng/ml ($\sim$5 nM) after a 4-week treatment period and with a half-life of prucalopride around 24 h (Emmanuel et al., 2002). Therefore, the concentration of 10 μM used here to investigate the effects of prucalopride on action potentials, refractoriness, and arrhythmic activity was 1000-fold higher than its normal therapeutic concentration in humans. Nevertheless, despite using atrial cells from patients with prior treatment with β-adrenoceptor antagonists, which has been shown to increase the incidence of 5-HT-induced arrhythmic contrac-

tions (Sanders et al., 1995) and abnormal depolarizations (Pau et al., 2003), we have shown that 10 μM prucalopride did not induce abnormal depolarizations in any of the atrial cells studied. In contrast, 10 μM 5-HT, after perfusion with 10 μM prucalopride, induced abnormal depolarizations in ~27% of the cells studied, which is also consistent with our previous observations in human atrial myocytes (Pau et al., 2003). It is noteworthy that in the study by Sanders et al. (1995), the partial 5-HT_4 agonist, renzapride, was not reported to produce arrhythmic contractions in human atrial cells, whereas 5-HT produced such contractions in human atrial cells and strips. In the present study, it is likely that prucalopride lacked arrhythmogenic activity because it acts as a partial rather than a full agonist and caused a significantly smaller increase in I_{CaL} and calcium loading than did 5-HT.

There is major concern about the potential for ventricular arrhythmias when using 5-HT_4 receptor agonists as gastrokinetic agents (Tonini et al., 1999; Yusuf et al., 2003). This is related to the ability of several drugs, including the enterokinetic cisapride, to block I_{Kr}, with associated prolongation of the repolarization phase of the ventricular action potential that underlies the acquired long QT syndrome (Viskin, 1999). The only report of effects of prucalopride on I_{Kr} encoded by cardiac HERG demonstrated that at micromolar concentrations, prucalopride significantly blocked HERG current expressed in a kidney cell line (Potet et al., 2001). However, at therapeutic concentrations, prucalopride had no effect on the QT interval in humans (De Schryver et al., 2002; Krogh et al., 2002), consistent with a lack of effect of prucalopride on human ventricular I_{Kr}. The present findings in human atrial myocytes of a lack of effect of prucalopride on late repolarization and refractoriness are also consistent with an absence of effect on atrial delayed rectifier K^+ current. An additional mechanism by which prucalopride might affect action potential duration would be by influencing the time course of inactivation of I_{CaL}, as well as its peak density, but we demonstrated here that prucalopride and 5-HT had no effects on I_{CaL} inactivation in human atrial cells.

In conclusion, these data indicate that, in the human atrium, the prucalopride-induced increase in calcium current was associated with prolongation of the early phase of action potential repolarization but not late repolarization, refractoriness, or arrhythmic activity. Moreover, prucalopride, when compared with 5-HT, behaved as a partial rather than full agonist in enhancing I_{CaL} and APD_{50} in human atrial myocytes. These results further support the hypothesis that the expression of specific 5-HT_4 receptor subtypes in human atrial cells may mediate different functional electrophysiological responses to different agonists, and this needs to be taken into account when using 5-HT_4 agonists in humans.

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References

Bach T, Syversveen T, Kvingedal AM, Krobert KA, Brattelid T, Kaumann AJ, and Levy FO (2001) $5HT_{4a}$ and 5-HT_{4b} receptors have nearly identical pharmacology and are both expressed in human atrium and ventricle. *Naunyn Schmiedeberg's Arch Pharmacol* **363:**146–160.

Bertaso F, Sharpe CC, Hendry BM, and James AF (2002) Expression of voltage-gated K^+ channels in human atrium. *Basic Res Cardiol* **97:**424–433.

Blondel O, Vandecasteele G, Gastineau M, Leclerc S, Dahmoune Y, Langlois M, and Fischmeister R (1997) Molecular and functional characterization of a 5-HT_4 receptor cloned from human atrium. *FEBS Lett* **412:**465–474.

Brattelid T, Kvingedal AM, Krobert KA, Andressen KW, Bach T, Hystad ME, Kaumann AJ, and Levy FO (2004) Cloning, pharmacological characterisation and tissue distribution of a novel 5-HT_4 receptor splice variant, 5-HT_{4i}. *Naunyn-Schmiedeberg's Arch Pharmacol* **369:**616–628.

Briejer MR, Bosmans JP, Van Daele P, Jurzak M, Heylen L, Leysen JE, Prins NH, and Schuurkes JA (2001) The in vitro pharmacological profile of prucalopride, a novel enterokinetic compound. *Eur J Pharmacol* **423:**71–83.

Coremans G, Kerstens R, De Pauw M, and Stevens M (2003) Prucalopride is effective in patients with severe chronic constipation in whom laxatives fail to provide adequate relief: results of a double-blind, placebo-controlled clinical trial. *Digestion* **67:**82–89.

De Schryver AM, Andriesse GI, Samsom M, Smout AJ, Gooszen HG, and Akkermans LM (2002) The effects of the specific $5HT_4$ receptor agonist, prucalopride, on colonic motility in healthy volunteers. *Aliment Pharmacol Ther* **16:**603–612.

Eglen RM, Wong EH, Dumuis A, and Bockaert J (1995) Central 5-HT_4 receptors. *Trends Pharmacol Sci* **16:**391–398.

Emmanuel AV, Roy AJ, Nicholls TJ, and Kamm MA (2002) Prucalopride, a systemic enterokinetic, for the treatment of constipation. *Aliment Pharmacol Ther* **16:**1347–1356.

Grammer JB, Zeng X, Bosch RF, and Kuhlkamp V (2001) Atrial L-type Ca^{2+}-channel, β-adrenoreceptor and 5-hydroxytryptamine type 4 receptor mRNAs in human atrial fibrillation. *Basic Res Cardiol* **96:**82–90.

Hegde SS and Eglen RM (1996) Peripheral 5-HT_4 receptors. *FASEB J* **10:**1398–1407.

Kaumann AJ (1993) Blockade of human atrial 5-HT_4 receptors by GR 113808. *Br J Pharmacol* **110:**1172–1174.

Kaumann AJ (1994) Do human atrial 5-HT_4 receptors mediate arrhythmias? *Trends Pharmacol Sci* **15:**451–455.

Kaumann AJ and Sanders L (1994) 5-Hydroxytryptamine causes rate-dependent arrhythmias through 5-HT_4 receptors in human atrium: facilitation by chronic β-adrenoceptor blockade. *Naunyn-Schmiedeberg's Arch Pharmacol* **349:**331–337.

Kaumann AJ, Sanders L, Brown AM, Murray KJ, and Brown MJ (1990) A 5-hydroxytryptamine receptor in human atrium. *Br J Pharmacol* **100:**879–885.

Krogh K, Jensen MB, Gandrup P, Laurberg S, Nilsson J, Kerstens R, and De Pauw M (2002) Efficacy and tolerability of prucalopride in patients with constipation due to spinal cord injury. *Scand J Gastroenterol* **37:**431–436.

Langlois M and Fischmeister R (2003) 5-HT_4 receptor ligands: applications and new prospects. *J Med Chem* **46:**319–344.

Lee KS and Lee EW (1998) Ionic mechanism of ibutilide in human atrium: evidence for a drug-induced Na^+ current through a nifedipine inhibited inward channel. *J Pharmacol Exp Ther* **286:**9–22.

Lezoualc'h F and Robert SJ (2003) The serotonin 5-HT_4 receptor and the amyloid precursor protein processing. *Exp Gerontol* **38:**159–166.

Li GR, Feng J, Yue L, Carrier M, and Nattel S (1996) Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes. *Circ Res* **78:**689–696.

Medhurst AD, Lezoualc'h F, Fischmeister R, Middlemiss DN, and Sanger GJ (2001) Quantitative mRNA analysis of five C-terminal splice variants of the human 5-HT_4 receptor in the central nervous system by TaqMan real time RT-PCR. *Brain Res Mol Brain Res* **90:**125–134.

Ouadid H, Seguin J, Dumuis A, Bockaert J, and Nargeot J (1992) Serotonin increases calcium current in human atrial myocytes via the newly described 5-hydroxytryptamine$_4$ receptors. *Mol Pharmacol* **41:**346–351.

Pau D, Workman AJ, Kane KA, and Rankin AC (2003) Electrophysiological effects of 5-hydroxytryptamine on isolated human atrial myocytes and the influence of chronic β-adrenoceptor blockade. *Br J Pharmacol* **140:**1434–1441.

Pindon A, van Hecke G, van Gompel P, Lesage AS, Leysen JE, and Jurzak M (2002) Differences in signal transduction of two 5-HT_4 receptor splice variants: compound specificity and dual coupling with $G_{\alpha s}$- and $G_{\alpha i/o}$-proteins. *Mol Pharmacol* **61:**85–96.

Potet F, Bouyssou T, Escande D, and Baro' I (2001) Gastrointestinal prokinetic drugs have different affinity for the human cardiac human ether-a'-gogo K^+ channel. *J Pharmacol Exp Ther* **299:**1007–1012.

Prins NH, Shankley NP, Welsh NJ, Briejer MR, Lefebvre RA, Akkermans LM, and Schuurkes JA (2000) An improved in vitro bioassay for the study of 5-HT_4 receptors in the human isolated large intestinal circular muscle. *Br J Pharmacol* **129:**1601–1608.

Rahme MM, Cotter B, Leistad E, Wadhwa MK, Mohabir R, Ford APDW, Eglen RM, and Feld GK (1999) Electrophysiological and antiarrhythmic effects of the atrial selective 5-HT_4 receptor antagonist RS-100302 in experimental atrial flutter and fibrillation. *Circulation* **100:**2010–2017.

Robert SJ, Zugaza JL, Fischmeister R, Gardier AM, and Lezoualc'h F (2001) The human serotonin 5-HT_4 receptor regulates secretion of non-amyloidogenic precursor protein. *J Biol Chem* **276:**44881–44888.

Sanders L, Lynham JA, Bond B, del Monte F, Harding SE, and Kaumann AJ (1995) Sensitization of human atrial 5-HT_4 receptors by chronic β-blocker treatment. *Circulation* **92:**2526–2539.

Talley NJ (1992) Review article: 5-hydroxytryptamine agonists and antagonists in the modulation of gastrointestinal motility and sensation: clinical implications. *Aliment Pharmacol Ther* **6:**273–289.

Talley NJ (2001) Serotoninergic neuroenteric modulators. *Lancet* **358:**2061–2068.

Tonini M, De Ponti F, Di Nucci A, and Crema F (1999) Review article: cardiac adverse effects of gastrointestinal prokinetics. *Aliment Pharmacol Ther* **13:**1585–1591.

Vilaro MT, Domenech T, Palacios JM, and Mengod G (2002) Cloning and characterization of a novel human 5-HT_4 receptor variant that lacks the alternatively spliced carboxy terminal exon: RT-PCR distribution in human brain and periphery of multiple 5-HT_4 receptor variants. *Neuropharmacology* **42:**60–73.

Viskin S (1999) Long QT syndromes and torsade de pointes. *Lancet* **354:**1625–1633.
Wang Z, Fermini B, and Nattel S (1994) Rapid and slow components of delayed rectifier current in human atrial myocytes. *Cardiovasc Res* **28:**1540–1546.
Wong EHF, Reynolds GP, Bonhaus DW, Hsu S, and Eglen RM (1996) Characterization of [^{3}H]GR 113808 binding to 5-HT_4 receptors in brain tissues from patients with neurodegenerative disorders. *Behav Brain Res* **73:**249–252.
Workman AJ, Kane KA, and Rankin AC (2001) The contribution of ionic currents to changes in refractoriness of human atrial myocytes associated with chronic atrial fibrillation. *Cardiovasc Res* **52:**226–235.
World Medical Association (1997) World Medical Association Declaration of Helsinki: recommendations guiding physicians in biomedical research involving human subjects. *Cardiovasc Res* **35:**2–3.
Yue L, Feng J, Li GR, and Nattel S (1996) Transient outward and delayed rectifier currents in canine atrium: properties and role of isolation methods. *Am J Physiol* **270:**H2157–H2168.
Yusuf S, Al Saady N, and Camm AJ (2003) 5-hydroxytryptamine and atrial fibrillation: how significant is this piece in the puzzle? *J Cardiovasc Electrophysiol* **14:**209–214.

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