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Electrophysiological effects of prucalopride, a novel enterokinetic agent, on isolated atrial myocytes from patients treated with beta-adrenoceptor antagonists

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Running title

a) Electrical effects of prucalopride in human atrial myocytes

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d) Abbreviations: 5-HT_4, 5-hydroxytryptamine type 4; APD, action potential duration; EC_{50}, effective concentration causing 50% of maximal I_{CaL} response; E_{max}, maximal I_{CaL} response to drug; ERP, effective refractory period; I_{CaL}, L-type calcium current.

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Abstract

Prucalopride is a selective 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 pro-arrhythmic 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}-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-dependency. 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% repolarisation (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 ERP were unaffected by prucalopride or 5-HT. Furthermore, 5-HT induced abnormal depolarisations 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 repolarisation or refractory period, and was devoid of arrhythmic activity.

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Introduction

Activation of 5-hydroxytryptamine type 4 receptors (5-HT_4) 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 sub-type 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). Pro-arrhythmic 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 atrial arrhythmias via intracellular Ca^{2+} overload and/or a reduced refractoriness (Tonini et al., 1999; Yusuf et al., 2003).

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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}. Whilst 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 repolarisation (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 (1) the electrophysiological effects of prucalopride on I_{CaL}, action potential duration (APD), cellular effective refractory period (ERP) and arrhythmic activity, and (2) to compare the effects of prucalopride with those of the endogenous agonist 5-HT.

Methods

Tissue and cell isolation

Procedures for obtaining human tissue were approved by the institutional ethics committee of Glasgow Royal Infirmary, and conforms 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^{o}C with a physiological solution containing (mM): NaCl (130.0), KCl (4.0), $CaCl_2$ (2.0), $MgCl_2$ (1.0), glucose (10.0), HEPES (10.0), pH 7.4. To record calcium currents, electrodes were filled with a solution containing (mM): CsCl (30.0), HEPES (5.0), $MgCl_2$ (1.0), Cs methanesulfonic acid (100.0), NaCl (5.0). To record action potentials, an internal solution containing (mM): KCl (30.0), HEPES (5.0), $MgCl_2$ (1.0), K methanesulfonic acid (100.0), NaCl (5.0) was used. The series resistance was observed to stabilise between 5-15 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, 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-dependency 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 per min (bpm) using 5 ms stimulating pulses of 1.2 x 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 which 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 repolarisation 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 & Johnson Pharmaceutical Research & Development (Beerse, Belgium) and were dissolved in dimethyl sulfoxide (DMSO) with a stock solution of 10 mM and subsequently diluted in physiological solutions. 5-HT (Sigma, St. Louis, Mo., USA) 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

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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, San Diego, CA, USA) using a variable slope sigmoidal concentration-response curve (Hill equation). The curves were fitted to mean I_{CaL} values, obtained at 6 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 bioexponential function using the WCP software programme 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 and τ_1, 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 ± standard error of the mean (SEM), 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.

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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 (ACE) 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 anti-arrhythmic 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 Figure 1 by the I_{CaL} current density-voltage relationships. An example of original I_{CaL} recordings is also shown (inset of Figure 1). 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 dependency of the current, and was reversible on washout of prucalopride (-5.7±1.1 pA/pF; n=7 cells, 5 patients).

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 Figure 2. Prucalopride at 10 μM caused a stable increase in I_{CaL}, which was completely antagonised 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

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pA/pF, was abolished by GR-113808 to -5.4±1.2 pA/pF ($p<0.05$). 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 to control) was significantly greater than that produced by prucalopride (89±15%; $p<0.05$) (Figure 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 ms 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 Figure 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 patients). This was significantly less than that produced by the maximum

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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 vs -7.09±0.07, respectively) or the Hill coefficient, n_H (0.76±0.46 vs 1.46±0.56, respectively).

Effect of prucalopride and 5-HT on action potentials and the refractory period

Figure 5 shows representative traces of action potentials and measurements of ERP illustrating the effects of 10 μM prucalopride (Figure 5B) or 5-HT (Figure 5C), compared to control conditions (Figure 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 vs 146±11 ms), APD_{90} (237±18 vs 236±18 ms) or ERP (224±25 vs 232±28 ms).

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 Figure 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 to 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 cells (2 patients) 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).

Effects of prucalopride and 5-HT on arrhythmic activity in human atrial cells

Abnormal depolarisations 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 depolarisations occurred in 3 (27%) of the cells studied ($p<0.05$). Figure 7 shows an example of abnormal depolarisations 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 depolarisations 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 repolarisation via 5-HT_4 receptors. Similarly to 5-HT, prucalopride lacked effects on the late phase of the atrial action potential repolarisation and the effective refractory period. Unlike 5-HT, prucalopride did not induce abnormal depolarisations.

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 systems (Robert et al., 2001). 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}, 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). Hence our finding of lower potency remains relevant to patients who have not been receiving β-adrenoceptors antagonists.

To date, nine 5-HT_4 receptor subtypes (5-$HT_{4a\text{-}i/hb/n}$) have been identified and localised in the human body (Langlois and Fischmeister, 2003; Brattelid et al., 2004), but only

some of these (5-HT_{4a-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; Vilaro' 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; Vilaro' 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 to 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 co-expressing 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 repolarisation 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 repolarisation 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 repolarisation or the refractory period. It is noteworthy that in canine atrial cells isolated using the "chunk" method, the repolarising, 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 & Lee, 1998; Bertaso et al., 2002). These two factors may explain the lack of effect of prucalopride and 5-HT to shorten late repolarisation 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, in order 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 (~5 nM) after 4 weeks 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 contractions (Sanders et al., 1995) and abnormal depolarisations (Pau et al., 2003), we have shown that 10 μM prucalopride, did not induce abnormal depolarisations in any of the atrial cells studied. In contrast, 10 μM 5-HT, after perfusion with 10 μM prucalopride, induced abnormal depolarisations in ~27% of the cells studied which is also consistent with our previous

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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, whilst 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 repolarisation 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 (Krogh et al., 2002; De Schryver 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 repolarisation 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 repolarisation, but not late repolarisation, refractoriness or arrhythmic

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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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Footnotes

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Legends for figures

Figure 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, from 7 patients). Values are means, with error bars denoting SEM, for control (open circles), prucalopride at 10 μM (closed circles) and after 3 min washout of prucalopride (open squares, n=7 cells, 5 patients). Asterisks indicate $p<0.05$ between control and prucalopride values at each voltage step (paired Student's t-test). As an inset, an example of original calcium current (I_{CaL}) traces obtained from a human atrial cell during depolarising voltage clamp pulses (250 ms, 0.33 Hz) from -40 mV 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.

Figure 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 labelled.

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Figure 3.

Effect of prucalopride followed by 5-HT on I_{CaL} in human atrial myocytes.

3A. The 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} is shown. The effect of washout of 5-HT is also shown. Inset traces (a-d) show original currents recorded at the time points labelled.

3B. Mean data (± SEM) for the effect of prucalopride (10 μM; open bars) and 5-HT (10 μM; solid bars) on I_{CaL} are shown (n=16 cells, 9 patients). Values are expressed as a percentage increase above control. The asterisk indicates $p<0.05$ between prucalopride and 5-HT-induced increases in I_{CaL} (paired Student's t-test).

Figure 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}-10^{-4}; solid circles; n=7-22 cells, 2 to 9 patients) and 5-HT (10^{-9}-10^{-5}; solid squares; n=9-18 cells, 4 to 11 patients; from Pau et al., 2003) on peak I_{CaL}. Values are means ±SEM. The increase in I_{CaL} is expressed as a percentage of the control value before the addition of prucalopride and 5-HT.

Figure 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. The cellular effective refractory period (ERP) is indicated by solid bars. The S_2 response used to measure this interval is labelled with an arrow.

Figure 6.

Effect of prucalopride, 5-HT and nifedipine on APD_{50} in human atrial cells.

6A. Mean (±SEM) action potential duration (ms) measured at 50% repolarisation (APD_{50}; n=11 cells, 8 patients), in the absence (open bars) and in the presence of 10 μM prucalopride (closed bars) or 10 μM 5-HT (vertical striped bars). Asterisks denotes $p<0.05$ versus control or drug as indicated by the dotted line (paired Student's t-test).

6B. Mean action potential data for APD_{50}, in the absence (open bars) and in the presence of 10 μM nifedipine (horizontal striped bars; n=11 cells, 5 patients), 10 μM nifedipine + 10 μM prucalopride (closed bars) and 10 μM nifedipine + 10 μM 5-HT (vertical striped bars; n=4 cells, 2 patients).

Figure 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 depolarisations in a human atrial myocyte and its blockade by the specific $5\text{-}HT_4$ 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 depolarisations by 1 μM GR-113808 (D).

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Table 1. Patients' pre-operative clinical characteristics.

Values are numbers of patients (*n* and % of total, respectively) with selected clinical characteristics, except for age (mean±SEM). CABG=coronary artery bypass graft surgery, AVR=aortic valve replacement, ACE=angiotensin converting enzyme, MI=myocardial infarction, LV=left ventricular. 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

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Figure 1

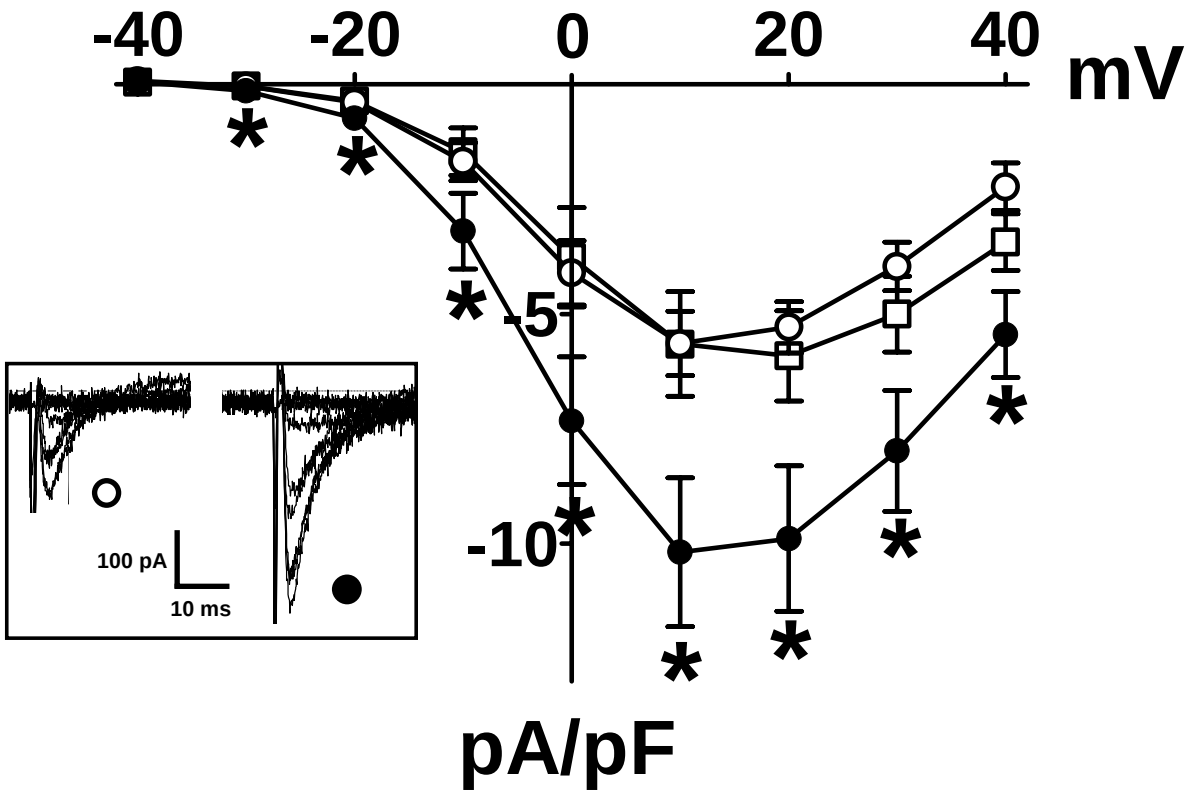

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Figure 2.

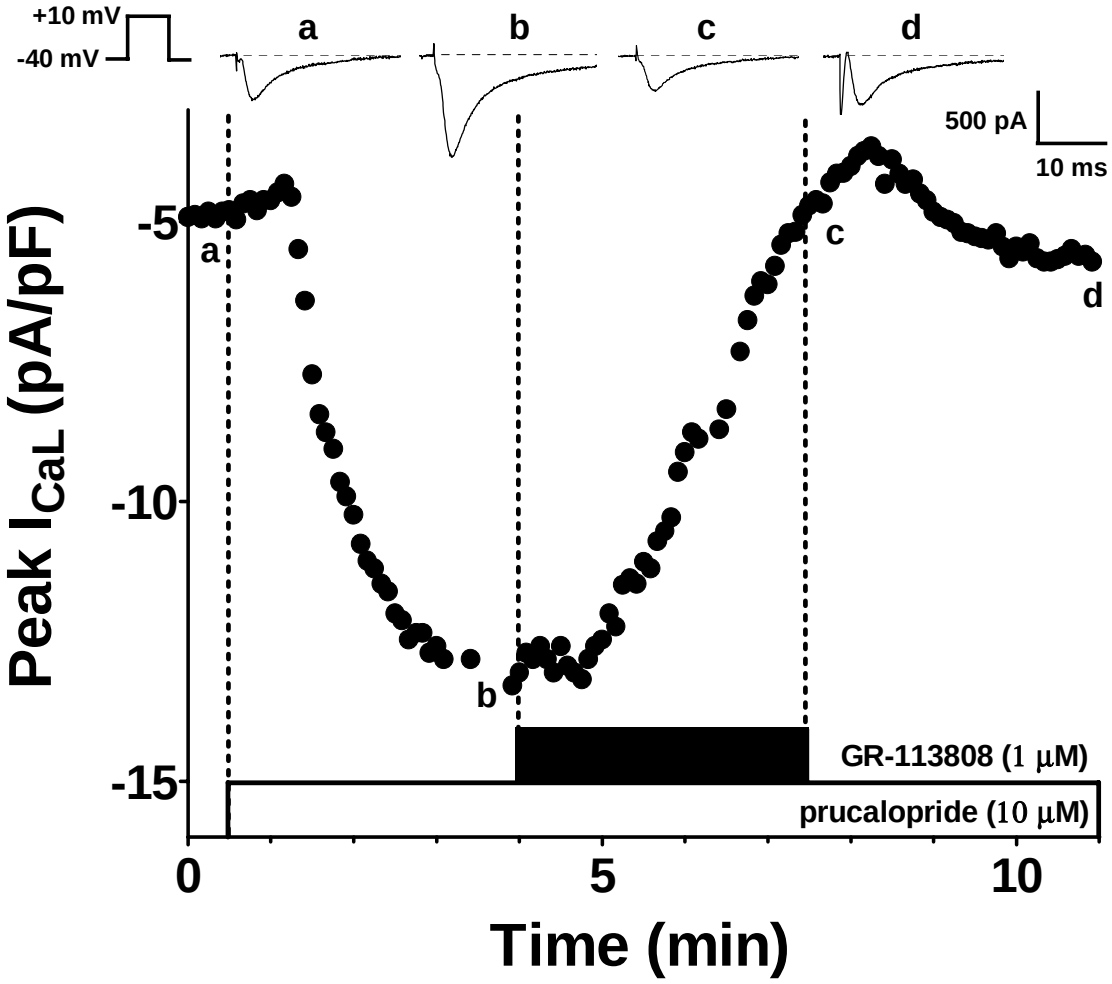

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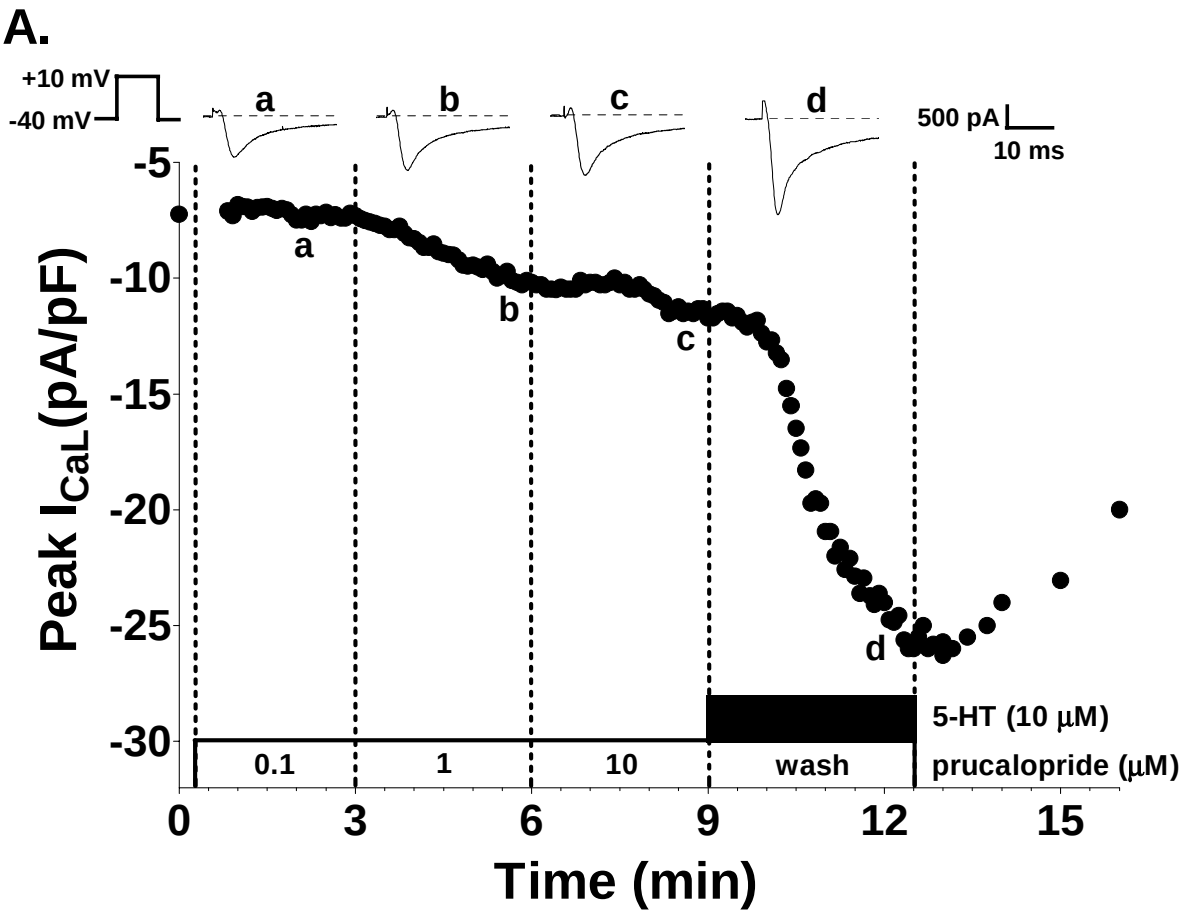
Figure 3.



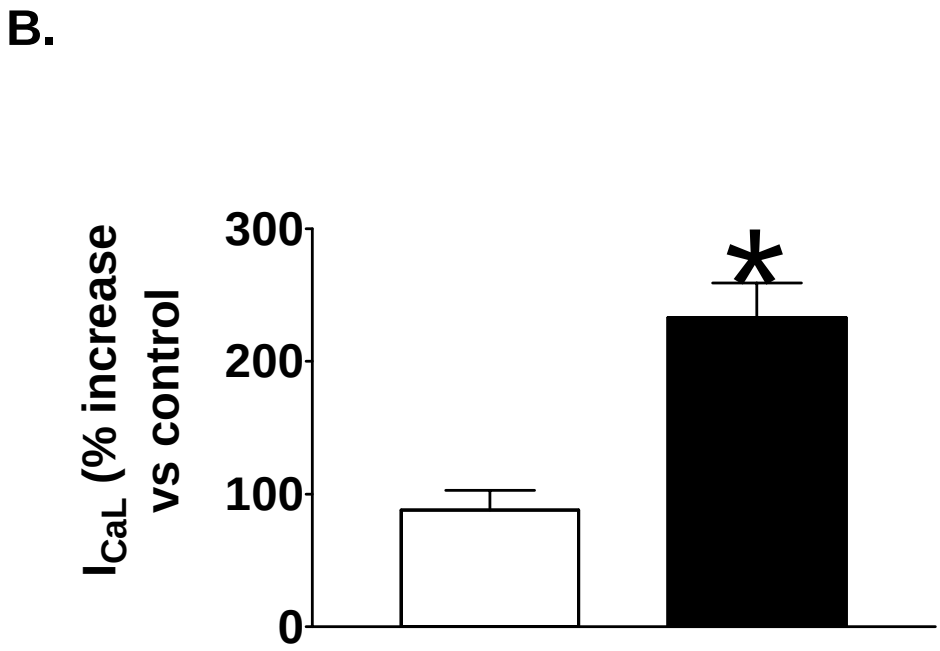


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Figure 4.

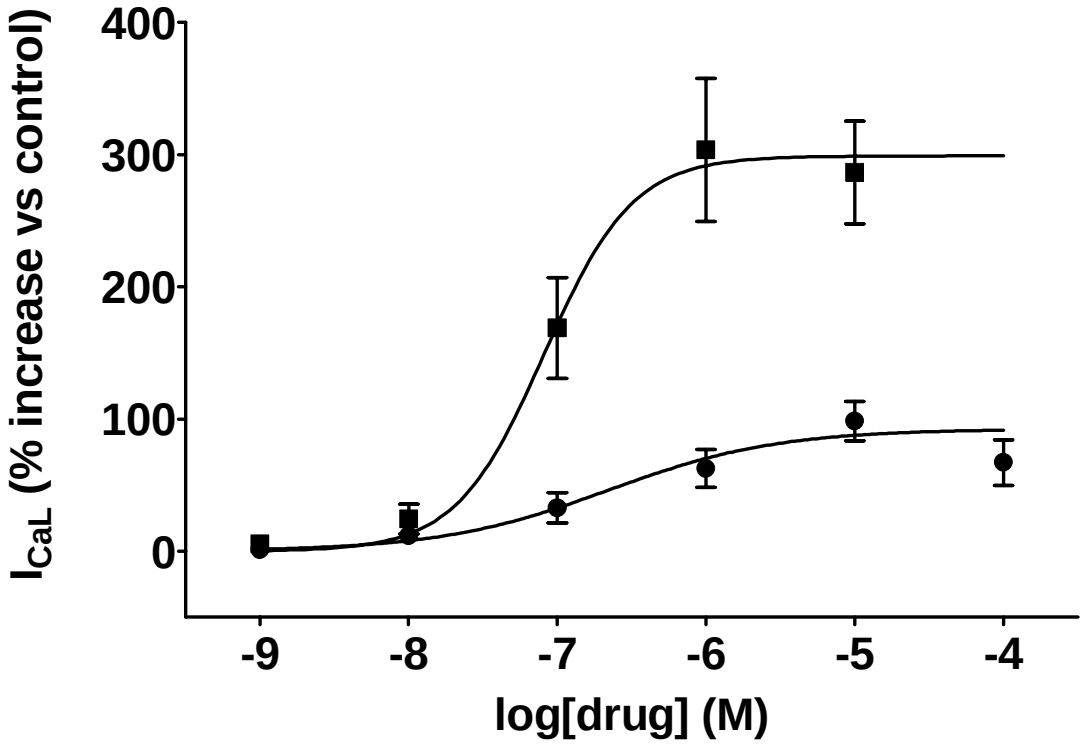
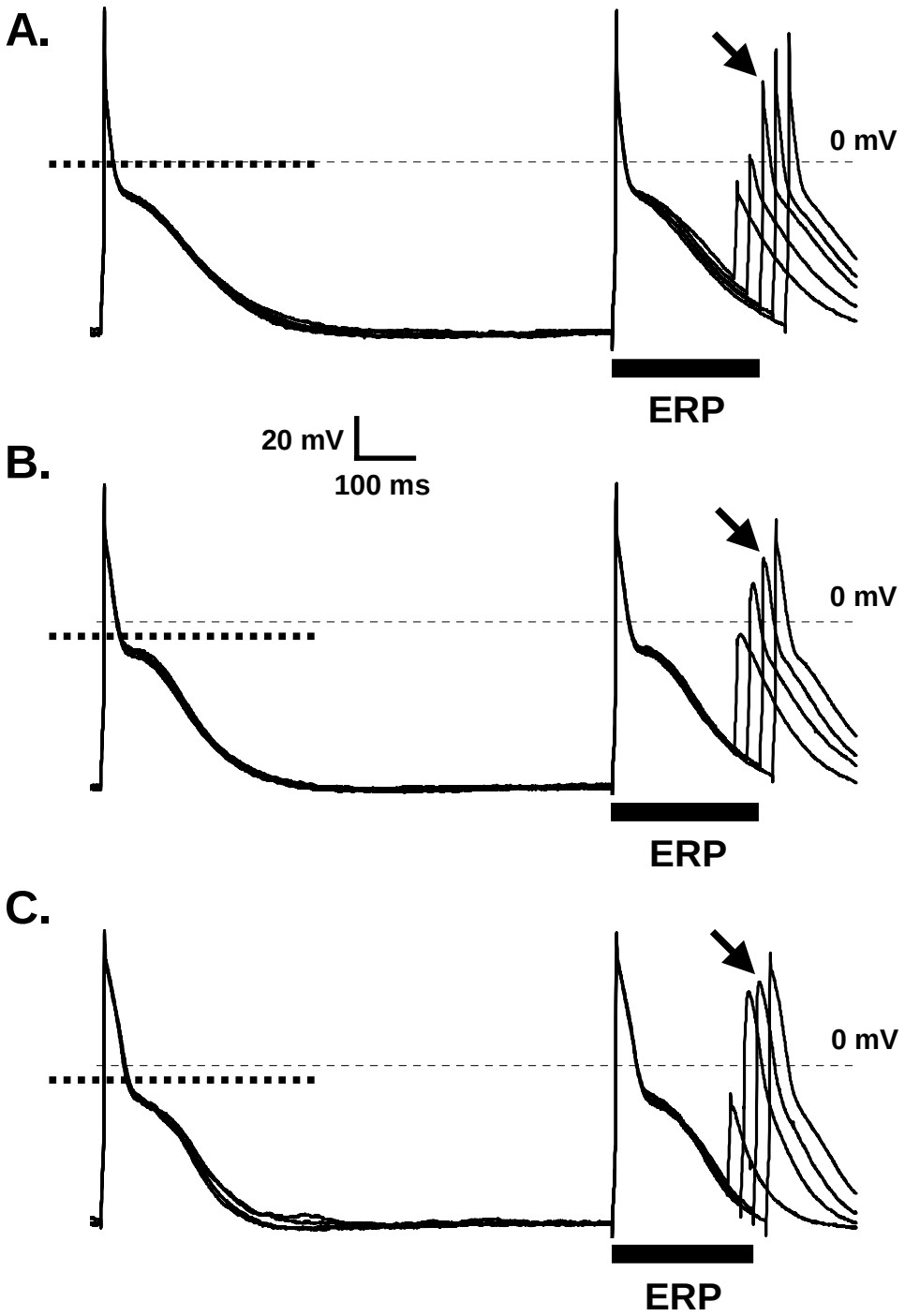


Figure 5



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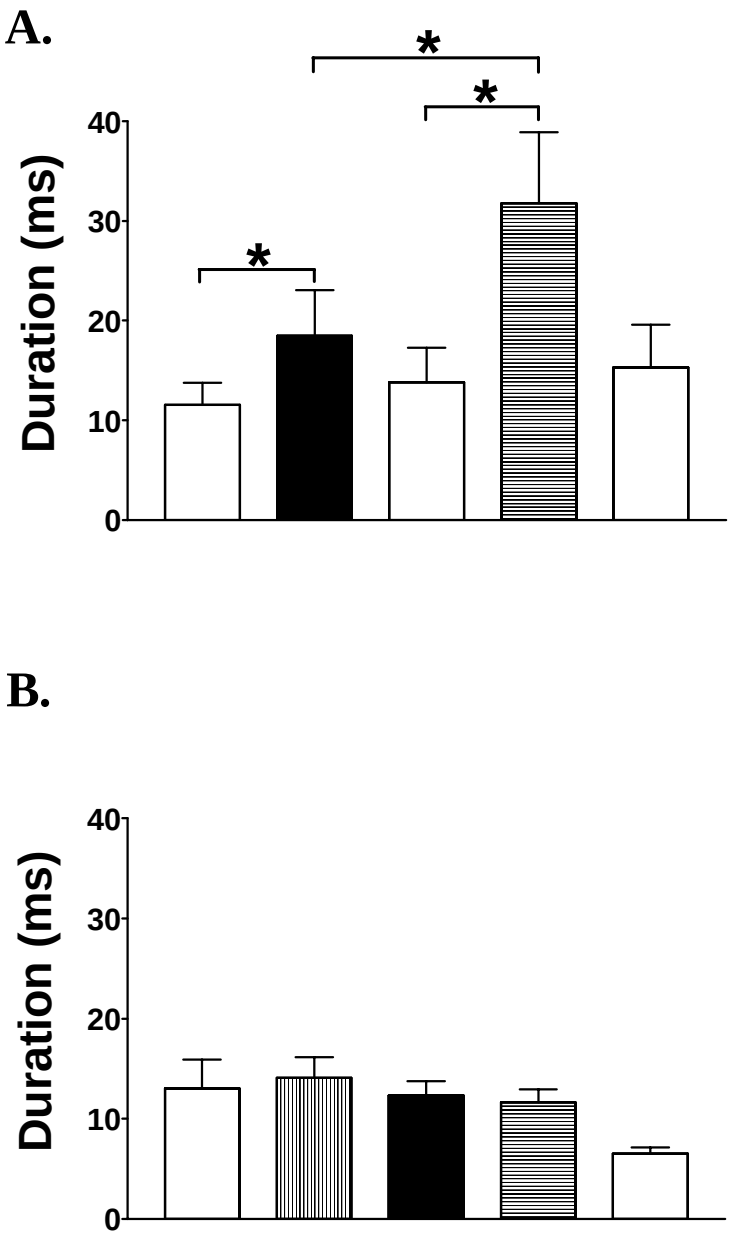

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Figure 7.