Electrophysiological effects of 5-hydroxytryptamine on isolated human atrial myocytes, and the influence of chronic β-adrenoceptor blockade

Short title: Electrical effects of 5-HT in human atrial cells.

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Summary:

1 5-hydroxytryptamine (5-HT) has been postulated to play a pro-arrhythmic role in the human atria *via* stimulation of 5-HT_4 receptors.

2 The aims of this study were to examine the effects of 5-HT on the L-type Ca^{2+} current (I_{CaL}) action potential duration, the effective refractory period (ERP) and arrhythmic activity in human atrial cells, and to assess the effects of prior treatment with β-adrenoceptor antagonists.

3 Isolated myocytes, from the right atrial appendage of 27 consenting patients undergoing cardiac surgery who were in sinus rhythm, were studied using the whole cell perforated patch clamp technique at 37^{o}C.

4 5-HT (1 nM-10 μM) caused a concentration dependent increase in I_{CaL}, that was potentiated in cells from β-blocked (maximum response to 5-HT, E_{max}=299±12% increase above control) compared to non-β-blocked patients (E_{max}=220±6%, P<0.05), but with no change in either the potency (log EC_{50}: -7.09±0.07 *vs* -7.26±0.06) or Hill coefficient (n_H: 1.5±0.6 *vs* 1.5±0.3) of the 5-HT concentration-response curve.

5 5-HT (10 μM) produced a greater increase in the action potential duration at 50% repolarisation (APD_{50}) in cells from β-blocked patients (of 37±10 ms, ie: 589±197%) *vs* non-β-blocked patients (of 10±4 ms, ie: 157±54%; P<0.05). Both the APD_{90} and the ERP were unaffected by 5-HT.

6 Arrhythmic activity was observed in response to 5-HT in 5 of 17 cells (29%) studied from β-blocked, compared to 0 of 16 cells from the non-β-blocked patients (P<0.05).

7 In summary, the 5-HT-induced increase in calcium current was associated with a prolonged early plateau phase of repolarisation, but not late repolarisation or refractoriness, and the enhancement of these effects by chronic β-adrenoceptor blockade was associated with arrhythmic potential.

Keywords: human atrium; isolated myocytes; 5-HT_4 receptors; calcium current; action potential; refractory period; β-adrenergic antagonists; arrhythmias (mechanisms).

Abbreviations: 5-HT, 5-hydroxytryptamine; APD, action potential duration; β-blockers, β-adrenoceptors antagonists; EC_{50}, effective concentration causing 50% of maximal I_{CaL} response; E_{max}, maximal I_{CaL} response to 5-HT; ERP, effective refractory period; I_{CaL}, L-type Ca^{2+} current; n_H, Hill coefficient.

Introduction

5-hydroxytryptamine (5-HT) exerts a variety of effects in the heart, brain, adrenocortical cells, urinary bladder and alimentary canal *via* stimulation of the 5-HT_4 receptor sub-type (Hegde & Eglen, 1996). The 5-HT_4 receptor is functionally present in the human atrium (Blondel *et al.*, 1997) but not in the ventricle (Jahnel *et al.*, 1992), which has stimulated interest in its possible roles in the occurrence of atrial arrhythmia. It has been postulated that 5-HT is involved in the progression of atrial fibrillation (AF) in patients due to its release from aggregating platelets within the fibrillating atria (Kaumann, 1994). In addition, there has been concern about the potential for atrial arrhythmia generation when using 5-HT_4 receptor agonists as gastrokinetic agents (Medhurst & Kaumann, 1993; Tonini *et al.*, 1999). In human atrial isolated muscle, arrhythmic contractions were induced by 5-HT, and abolished by a selective 5-HT_4 receptor antagonist (Kaumann & Sanders, 1994). 5-HT has been shown to increase the magnitude of the L-type calcium current (I_{CaL}) in human atrial myocytes (Jahnel *et al.*, 1993; Ouadid *et al.*, 1992), which may contribute to intracellular calcium overload and arrhythmic activity. However, in a study of a pig model of atrial flutter/AF (Rahme *et al.*, 1999), the 5-HT_4 receptor antagonist, RS-100302, was demonstrated to be anti-arrhythmic, associated with prolongation of the atrial effective refractory period (ERP). This suggested that 5-HT may shorten the atrial ERP, which may predispose to AF by reducing the minimum pathlength required for re-entry. The importance of these potential arrhythmogenic effects of 5-HT, namely calcium overload and/or shortened refractoriness, have not yet been clarified in human atria.

One factor that may influence the arrhythmogenic potential of 5-HT is the prior treatment of the patient with β-adrenoceptor antagonists. The ability of 5-HT to cause rate-dependent arrhythmic contractions in strips of human atrial tissue was facilitated by chronic β-blockade (Kaumann & Sanders, 1994; Sanders *et al.*, 1995). In addition, prior β-blocker treatment increased the positive inotropic response of human atrial muscle to 5-HT (Sanders *et al.*, 1995; Wangemann *et al.*, 2003), but the influence of β-blockade on the effects of 5-HT on I_{CaL} is unknown. We have recently reported that chronic β-blockade is associated with prolongation of the basal action potential duration (APD) and ERP in human atrial myocytes, and that this effect

is not related to a change in I_{CaL} (Workman *et al.*, 2003). Such an increase in APD may result in increased contractile tension independent of I_{CaL} (Bers, 2002), and this may underlie the increased basal force of contraction in atrial strips from patients pre-treated with β_1-adrenoceptor antagonists (Wangemann *et al.*, 2003). However, the ionic mechanism by which chronic β-blockade enhances the effects of 5-HT on human atria, and in particular whether I_{CaL} is involved, has not been proven.

The aims of this work were: 1) to study how the increase in I_{CaL} by 5-HT may affect the action potential morphology, the cellular refractoriness and the occurrence of arrhythmic activity, and 2) to investigate the influence of chronic β-blocker therapy on the actions of 5-HT on I_{CaL}, APD, ERP and arrhythmic mechanism in human atrial single myocytes.

Methods

Tissue and cell isolation

The procedures for the removal of human tissue were approved by the institutional research ethics committee of Glasgow Royal Infirmary, and each patient's informed consent was obtained. The investigation conforms to the principles outlined in the Declaration of Helsinki (World Medical Association, 1997). Specimens of the right atrial appendage were obtained from patients undergoing cardiac surgery. The excised tissue (weight: 0.45±0.06 g) was placed in Tyrode's solution and transported to the laboratory for processing within 5 min of removal. Atrial cells were isolated by enzymatic dissociation and mechanical disaggregation, using a modification of the chunk method, described in detail by Workman *et al.* (2001).

Electrical recording techniques

Action potentials and ion currents were recorded using the whole cell patch clamp technique, with an Axopatch-1D amplifier (Axon Instruments). Cells were superfused at 37^{o}C at 1.5 ml min^{-1} (RC-24E fast exchange perfusion chamber, Warner) with a physiological salt 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. The perforated patch clamp technique, with nystatin (184 μM), was used to prevent cell dialysis, prolonging recording and minimising current "run-down". Microelectrodes were constructed from thin walled, filamented borosilicate glass (Clark Electromedical) using a micropipette puller (Narishige PP-83), and heat polished to resistances of 3-9 MΩ. To record calcium currents, electrodes were filled with a caesium-based solution (to eliminate outward K^+ currents) 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 potassium-based solution was used, containing (mM): KCl (30.0), HEPES (5.0), $MgCl_2$ (1.0), K methanesulfonic acid (100.0), NaCl (5.0). Using these solutions, a liquid junction potential of +5.0±0.2 mV (n=6) was measured (bath relative to pipette) and was compensated for prior to seal formation (Neher, 1992). Only single, elongated myocytes were selected for electrical recording. Following seal formation, a gradual reduction in the series resistance due to nystatin

pore formation was observed, which stabilised (after ~10 min) at 11.1±0.5 MΩ (n=84 cells). The mean cell capacity was 77±3 pF. Capacitative transients were subtracted electronically from the recordings. The voltage drop across the series resistance was routinely compensated for electronically, by 68-80%. The software program WinWCP (J. Dempster, Strathclyde University) was used both to stimulate and record electrical activity. All currents were normalized to the cell's capacity. Current and voltage signals were low pass filtered at 5 kHz and digitised (Digidata 1200 A-D converter, Axon Instruments) prior to storage on magnetic and compact discs.

Experimental protocols

I_{CaL} was recorded under voltage clamp conditions. The voltage-dependency of this current was measured from a holding potential of -40 mV with depolarising pulses of 100 ms duration (0.33 Hz), increasing in steps of 10 mV, up to +40 mV. The time course of change in I_{CaL} due to drugs was examined with repetitive voltage pulses, from -40 to +10 mV.

Actions potentials were stimulated at 75 beats min^{-1} (bpm) using 5 ms current pulses of 1.2x threshold strength, after current clamping resting cells at -75 to -80 mV, and keeping the holding current (<150 pA) constant thereafter. The stimulus threshold current amplitude was initially determined in each cell by stimulating repetitively with trains of three current pulses (5 ms duration), the 1st and 2nd being of equal, suprathreshold amplitude, and the 3rd pulse increasing progressively (from zero, in steps of 50 pA) until it produced a regenerative action potential. The stimulus strength was then kept constant throughout all recording protocols in each cell. The cell's ERP was measured using a standard S_1-S_2 stimulation protocol, with an 8-pulse conditioning train delivered at 75 bpm, and with S_1 and S_2 pulses of equal magnitude. The S_1-S_2 interval was shortened in 10 ms steps, and the ERP 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}) and 90% (APD_{90}) of the upstroke amplitude. I_{CaL} and/or action potentials and the ERP were recorded before, and 90 s after, drug additions and again 180 s after removal of drugs.

Drugs

5-HT (Sigma, St. Louis, Mo., USA) was made up as a 10 mM stock solution in distilled water. The specific 5-HT_4 antagonist GR-113808 (Kaumann, 1993) was kindly donated by Johnson & Johnson Pharmaceutical Research & Development and was prepared as a 10 mM stock solution in dimethyl sulfoxide.

Data analysis and statistics

Details of each patient's clinical characteristics and drug treatments were obtained from the case notes. The cardiac rhythm and heart rate were assessed from the pre-operative electrocardiogram. Only those 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 5-HT on I_{CaL} were fitted iteratively (Prism software, Graphpad) with variable slope sigmoidal concentration-response curves, using the Hill equation: $Y=E_{min}+[E_{max}-E_{min}]/[1+(x/EC_{50})^{nH}]$, where Y=$I_{CaL}$ density (pA/pF, expressed as % above control), E_{min}=I_{CaL} at 0 mM 5-HT (set to 0%), E_{max}=maximum I_{CaL} response elicitable by 5-HT (% above control), x=[5-HT] (mM), EC_{50}=[5-HT] producing 50% of E_{max} (mM) and n_H=Hill coefficient (describing the steepness of the slope). The curves were fitted to mean I_{CaL} values, obtained at five concentrations of 5-HT, within the range of 1 nM-10 μM. Use of the perforated patch technique maximised the duration of each experiment but it was not possible to measure I_{CaL} at every concentration studied in each cell. Curve fit values were compared using a two-tailed unpaired Student's *t*-test. Measurements taken from the action potentials were the resting potential (V_m), overshoot, amplitude and maximum upstroke velocity (V_{max}), the APD_{50} and APD_{90}, and the ERP. 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 Chi-square test (χ^2) was used to assess the level of significance of differences in the incidences of arrhythmic activity between groups. $P<0.05$ was regarded as statistically significant.

Results

Patients' characteristics

The majority of patients were male, underwent coronary artery bypass graft surgery, suffered from angina, and had normal left ventricular function (Table 1). Fifty-nine percent of the patients were taking β-adrenoceptor blockers, and 59% calcium channel blockers. Of the patients taking β-blockers, 50% were also receiving calcium channel blockers. All patients undergoing β-blockade were treated for >1 month with cardiac selective β_1-adrenoceptor antagonists, namely atenolol (69%), bisoprolol (19%) or metoprolol (12%). No patient was administered sotalol (which has additional class III anti-arrhythmic activity) except for 1 patient who was changed from sotalol to atenolol 1 week prior to surgery. Patients received their routine cardiac drugs on the day of surgery. Of the patients treated with calcium channel blockers, 44% were receiving diltiazem, a cardiac-acting drug, and the remaining 56% were taking dihydropyridines, with mainly vascular actions, namely amlodipine (38%), felodipine (12%) and nifedipine (6%). The heart rate was significantly reduced in β-blocked (62±4 bpm, n=16) compared to non-β-blocked patients (75±3 bpm, P<0.05; n=11), but was not altered by calcium channel blocker therapy (68±6 *vs* 66±3 bpm, for treated and non-treated patients, respectively).

Effects of 5-HT and GR-113808 on the calcium current in human atrial cells

5-HT produced a substantial increase in the amplitude of I_{CaL}, as shown by the original recordings in Figure 1a, and by the I_{CaL} current density-voltage relationships in Figure 1b. 5-HT (10 μM) increased the mean magnitude of peak I_{CaL} (recorded at +10 mV) from -4.7±0.8 to -11.9±2.2 pA pF^{-1} (P<0.05, n=7 cells from 6 patients), ie: by approximately 150%. This increase in I_{CaL} occurred without any change in the voltage dependency of the current, and was shown to be reversible on washout of 5-HT (-3.7±1.4 pA pF^{-1}; n=3 cells, 3 patients) (Figure 1b).

The time course of the effect of 5-HT on I_{CaL} and its blockade by the specific 5-HT_4 receptor antagonist, GR-113808, can be seen in Figure 2. 5-HT (0.1 μM) caused a stable

increase in I_{CaL}, which was completely antagonised by 0.1 μM GR-113808. The antagonism of the 5-HT-induced increase in I_{CaL} by GR-113808 was seen in 3 cells from 3 patients. The effect of this antagonist was partially reversible upon its washout (Figure 2). GR-113808 (0.1 μM) had no direct effect on basal (unstimulated by 5-HT) I_{CaL} (2 cells from 2 patients).

The concentration-response relationship of the effect of 5-HT on I_{CaL}

The effect of 5-HT on I_{CaL} was concentration dependent. Figure 3a shows the concentration-response curve of the effect of 5-HT between 1 nM and 10 μM, fitted to the mean values obtained at each concentration. The log EC_{50} calculated from this curve was -7.15±0.05, and the Hill coefficient (n_H) was 1.37±0.28 (n=17-33 cells, 9-17 patients). The maximum response of I_{CaL} to 5-HT (E_{max}) was observed to occur at around 1 μM 5-HT, with a value of 267±7%.

In Figure 3b, the 5-HT concentration-response data has been sub-divided into those obtained from patients with and without prior treatment with β-blockers. The respective log EC_{50} (-7.09±0.07 and -7.26±0.06) and n_H (1.5±0.6 and 1.5±0.3) values were not significantly different between these patient groups. By contrast, 5-HT caused a significantly greater increase in E_{max} in β-blocked (at 299±12%, n=9-18 cells, 4-11 patients) than in non-β-blocked patients (at 220±6%, n=8-15 cells, 5-7 patients; P<0.05) (Figure 3b). There was no significant difference in basal I_{CaL} between non-β-blocked (-5.4±0.7 pA pF^{-1}, n=30) and β-blocked patients (-4.2±0.3 pA pF^{-1}, n=50).

Pre-treatment with calcium channel blockers did not significantly alter the concentration-response curves to 5-HT on I_{CaL}. Similarly, basal I_{CaL} was not significantly different between cells from patients who had, and those from patients who had not received calcium channel blockers, at -4.8±0.6 (n=32) and -4.5±0.4 pA pF^{-1} (n=48), respectively.

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

Figure 4a shows original action potentials and ERP measurement, from a single human atrial myocyte obtained from a patient who had not been treated with a β-blocker. 5-HT (10

μM) produced a small, but significant, prolongation in the APD_{50}, but with little effect on the plateau amplitude, late repolarisation (APD_{90}) or the ERP. Mean data confirmed these effects of 5-HT in the cells from non-treated patients (Figure 4b). 5-HT (10 μM) increased the APD_{50} from 6±1 to 16±5 ms (P<0.05), an effect which was fully reversible upon washout of 5-HT, but there was no significant or reversible effect of 5-HT on the APD_{90} (193±20 *vs* 208±24 ms, P>0.05) or ERP (178±23 *vs* 195±23 ms, P>0.05). Other action potential measurements, including V_{max} (191±14 V s^{-1}), overshoot (59±3 mV) and amplitude (139±2 mV), were unaffected by 5-HT.

Chronic β-blocker treatment was associated with a greater prolongation of the early plateau phase of repolarisation by 5-HT than non-treatment. Figure 5a shows action potential traces and ERP measurements in a single cell from a β-blocked patient. 5-HT (10 μM) produced an elevation in the action potential plateau, with a consequent lengthening in the duration of the APD_{50}. Figure 5b shows mean data confirming this effect in the cells from β-blocked patients, with a marked and reversible increase in APD_{50} by 5-HT, from 13±5 to 50±11 ms (P<0.05). The mean prolongation in APD_{50} was 37±10 ms (P<0.05), representing an increase of 589±197% (P<0.05). This compares with a prolongation in APD_{50} by 5-HT in the cells from the non-β-blocked patients of 10±4 ms (P<0.05), or 157±54% (P<0.05). Both the absolute and percentage increase in APD_{50} by 5-HT were significantly greater in the cells from the patients treated with β-blockers, than in those from the non-treated patients (P<0.05 for each). The 5-HT-induced increase in the APD_{50} was abolished by the specific $5\text{-}HT_4$ antagonist GR-113808 (3 cells from 2 patients, one with and one without prior β-blocker treatment), and this effect of the antagonist was partially reversible. In cells from β-blocked patients, 5-HT had no significant effect on the APD_{90} (245±24 *vs* 240±29 ms, P>0.05) or ERP (231±21 *vs* 225±20 ms, P>0.05), nor on the action potential V_{max}, overshoot or amplitude. Prior treatment of patients with calcium channel blockers had no significant effect on basal APD_{50}, APD_{90} or ERP, nor on the effects of 5-HT on these parameters. There were no significant differences in the capacity of cells obtained from patients treated and not treated with either β-blockers or calcium channel blockers.

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

Chronic β-blockade was associated with an increased incidence of 5-HT-induced atrial cellular arrhythmic activity. Figure 6 shows an example of such activity, with the induction of delayed afterdepolarisations by 10 μM 5-HT, and their abolition following the removal of 5-HT, in a cell from a patient treated with a β-blocker. Abnormal depolarisations, including delayed afterdepolarisations and early afterdepolarisations were observed in 5 of 17 (29%) of the cells obtained from the patients who underwent chronic β-blockade in which action potentials were measured. By contrast, abnormal depolarisations were observed not to occur in response to 5-HT, in any of the 16 cells from the non-β-blocked patients in which action potentials were measured ($P<0.05$, χ^2 test).

Discussion

The present work has demonstrated, for the first time to our knowledge, that the effects of 5-HT to increase the L-type Ca^{2+} current and to prolong the early plateau phase of action potential repolarisation in human atrial myocytes were each potentiated by prior treatment of patients with β-blockers, with evidence of arrhythmogenic activity. Since 5-HT had no effect on late repolarisation or refractoriness, it would not be expected to contribute to the shortening of re-entrant circuits which stabilises AF. It is more likely that 5-HT may contribute to arrhythmia genesis, by causing afterdepolarisations *via* mechanisms involving increased I_{CaL} and cellular calcium overload (Levy, 1989).

Atrial fibrillation is known to be associated with platelet activation (Kamath *et al.*, 2001), and it has been proposed that the consequent release of 5-HT may be involved in the progression of the arrhythmia (Kaumann, 1994). The concentration of free 5-HT in human whole blood has been reported to range from nanomolar, rising to nearly micromolar, levels after activation of platelets (Joseph *et al.*, 1991). The present results confirm that within this concentration range 5-HT has important electrophysiological effects on human atrial myocytes. The novel finding of a prolongation by 5-HT of the early plateau phase of atrial action potential repolarisation, associated with plateau elevation, with no effect on late repolarisation or the ERP, is likely due to the observed increase in I_{CaL}. Whilst an involvement of the transient outward K^+ current, I_{TO}, which also contributes to early repolarisation, cannot be excluded, no reports of effects of 5-HT on I_{TO} were found. Furthermore, a predominant contribution from I_{CaL} is supported by a report that blockade of I_{CaL} by nifedipine, a calcium channel blocker, markedly depressed the action potential plateau in human atrial cells, with a relatively small effect on late repolarisation and none on the ERP (Workman *et al.*, 2001). The increase in I_{CaL} may contribute to the intracellular calcium overload that occurs in AF, which is thought to contribute to subsequent electrophysiological changes (Nattel, 1999). The reported reduction in 5-HT_4 receptor mRNA in chronically fibrillating human atria may represent an adaptive response in order to reduce calcium entry (Grammer *et al.*, 2001).

There has been only one previous, non-quantitative, study of the effect of 5-HT on human atrial action potentials, and 5-HT had no effect on action potential morphology but increased

tension in muscle strips, and increased I_{CaL} in myocytes (Jahnel *et al.*, 1993). In the present study, the majority of cells exhibited characteristic atrial type 3 action potentials (Dawodu *et al.*, 1996), ie: with a pronounced phase 1, and a plateau below the level of 50% repolarisation, making APD_{50} more susceptible to changes in plateau amplitude, and hence inward currents. There are no previous reports of the effects of 5-HT on the ERP in the human atrium. In a pig model of AF, the 5-HT_4 receptor antagonist, RS-100302, prolonged the ERP and was anti-arrhythmic (Rahme *et al.*, 1999). If that effect was a consequence of 5-HT_4 receptor blockade *in-vivo* it suggested that 5-HT should reduce the ERP, which, by reducing the minimum pathlength required for re-entry, would promote AF (Moe, 1962). Whilst this could represent a species-specific effect, or an additional direct action of the drug, it is also recognised that myocytes isolated by the "chunk" method may lack the delayed rectifier potassium current (Yue *et al.*, 1996). Thus, even if 5-HT increased this current *in-vivo*, shortening of late repolarisation and/or the ERP may not be observed in isolated cells. However, the report of an absence of effect of 5-HT on the action potential in intact human atrial muscle (Jahnel *et al.*, 1993) makes this less likely, since ERP in human atrial myocytes is closely related to APD_{90} (Workman *et al.*, 2001).

The concentration dependent increase in I_{CaL}, mediated via the 5-HT_4 receptor sub-type in the present report, with an EC_{50} of about 70 nM, is consistent with that reported for I_{CaL} in human atrial myocytes (Ouadid *et al.*, 1992) but is also of a similar potency to that reported for the positive inotropic effect of 5-HT in human atrial preparations (Jahnel *et al.*, 1992). The present, novel observation of potentiation by β-blockade of the 5-HT effect on I_{CaL} supports the likelihood that the reported positive inotropic action of 5-HT, which was also increased by β-blockade, is directly related to the increase in I_{CaL}. Our finding of an increased efficacy, and no change in the EC_{50}, of the effect of 5-HT on I_{CaL} with β-blockade is in agreement with the increased maximal inotropic effect of 5-HT in human atrial strips, without change in potency, reported by Sanders *et al.* (1995). This differs from data on cell shortening in the same study (increased potency, no increase in maximal response), but is supported by a recent report of sensitisation by β-blockade of inotropic responses to 5-HT in human atrial strips (Wangemann *et al.*, 2003). The reason for the discrepancy within the study of Sanders *et al.* (1995), and with

our results is unknown, but it suggests that the potentiation of the effect of 5-HT on I_{CaL} is correlated better with the maximal inotropic response than with myocyte shortening.

Chronic β-blockade has recently been shown to increase basal APD_{90} and ERP in human atrial myocytes, consistent with the trends in the present data, and this "pharmacological remodelling" was independent of an effect on I_{CaL} (Workman *et al.*, 2003). By contrast, the present effects of β-blockade, on responses to 5-HT, appear more likely to be related to changes in the phosphorylation state of the calcium channel. This may be due to intracellular cross-talk between G_s-coupled receptor populations, since chronic β_1-adrenoceptor blockade enhances inotropic effects mediated by β_2-adrenoceptors, histamine H_2 and 5-HT_4 stimulation (Wangemann *et al.*, 2003). It has been shown that the levels of mRNA encoding human atrial 5-HT_4 receptors were unaffected by chronic β-blockade (Grammer *et al.*, 2001) and the affinity of the β_2-adrenoceptor was also unaffected (Hall *et al.*, 1990). Thus, the increased efficacy of 5-HT with β-blockade that we, and others, have found is likely due to an enhancement of the intracellular biochemical cascade which links the receptor to calcium channels, rather than to changes in 5-HT_4 receptor density or affinity. An alteration in the activity of G-proteins may be involved (Grimm *et al.*, 1998; Wang *et al.*, 1999) and whilst expression of atrial G_s and G_i was unaffected by chronic β-blockade (Jia *et al.*, 1995), the activity of G_s was enhanced (Wang *et al.*, 1999). Furthermore, 5-HT-induced increases in adenylyl cyclase activity (Wangemann *et al.*, 2003) and intracellular cAMP (Jahnel *et al.*, 1993), leading to channel phosphorylation and increased I_{CaL} availability, are potentiated in atrial tissue from β-blocked patients.

In conclusion, these data indicate that, in the human atrium, the 5-HT-induced increase in calcium current is associated with a prolonged early plateau phase of repolarisation, but not late repolarisation or refractoriness, and that the enhancement of these effects by chronic β-adrenoceptor blockade is associated with arrhythmic potential. They provide further support for the hypothesis that 5-HT released by aggregating platelets during AF, or 5-HT_4 receptor agonists as therapeutic agents, may contribute to the origin and maintenance of atrial arrhythmia.

Acknowledgements

We wish to thank the cardiac surgical staff of Glasgow Royal Infirmary for kindly providing access to human tissues, and Julie Russell for excellent technical support and for maintaining the patient database. This work was supported by Johnson & Johnson Pharmaceutical Research and Development.

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Table 1

Patients' pre-operative clinical characteristics

	n	**%**
Patients	27	
Male/female	21/6	78/22
Age	66±2	
Surgery:		
CABG	22	82
CABG+AVR	5	18
Drugs:		
Ca^{2+} channel blocker	16	59
ß-blocker	16	59
ACE inhibitor	14	52
Nitrate	18	67
Diuretic	6	22
Lipid lowering	22	81
Digoxin	0	0
Warfarin	0	0
Symptoms:		
Angina	25	93
Palpitations	5	19
Hypertension	15	56
Hyperlipidaemia	21	78
Previous History:		
MI	10	37
Diabetes	2	7
LV Function:		
-normal	21	78
-mild-moderate	5	18
-severe	1	4

Table and Figure legends

Table 1. Patients' 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 at surgery.

Figure 1.

Effect of 5-HT on I_{CaL} current-voltage relationship in human atrial myocytes.

Fig. 1a. An example of original calcium current (I_{CaL}) traces obtained from a human atrial cell (in this case from a β-blocked patient), during depolarising voltage clamp pulses (100 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 5-HT at 10 μM (closed circle).

Fig. 1b. Current-voltage relationships of I_{CaL} expressed in terms of current density, pA pF^{-1} (n=7 cells, from 6 patients, treated and not treated with β-blockers). Values are means, with error bars denoting SEM, for control (open circles) 5-HT at 10 μM (closed circles) and after 3 min washout of 5-HT (open squares, n=3 cells, 3 patients). Asterisks indicate P<0.05 between control and 5-HT values at each voltage step (paired t-test).

Figure 2.

Effect of the specific 5-HT_4 antagonist GR-113808 on I_{CaL} stimulated by 5-HT in human atrial myocytes.

An example of the time course of change in peak I_{CaL} density (measured in this case in a cell from a β-blocked patient) plotted at 5 s resolution, in response to 0.001 to 0.1 μM 5-HT (open boxes), followed by the application of GR-113808 at 0.1 μM (solid box), and the subsequent washout of the antagonist and then of 5-HT. Inset traces show original currents recorded at the time points labelled.

Figure 3.

Concentration-dependent effects of 5-HT on I_{CaL} in human atrial myocytes.

Fig. 3a. Concentration-response relationship for 5-HT (0.001-10 μM) on peak I_{CaL} in human atrial myocytes. Values are means ±SEM (n=17-33 cells, 9-17 patients). The increase in I_{CaL} is expressed as a percentage of the control value before the addition of 5-HT. Mean data points were fitted by a variable slope sigmoidal curve using the Hill equation (see Methods).

Fig. 3b. Comparison of concentration-response curves for 5-HT on I_{CaL} and (inset) of maximal I_{CaL} response to 5-HT, E_{max}, between cells from patients not treated with β-blockers (open symbols; n=8-15 cells, 5-7 patients) and those from patients treated with β-blockers (closed symbols; n=9-18 cells, 4-11 patients). Asterisk denotes P<0.05 *vs* non-β-blocked group.

Figure 4.

Effect of 5-HT on action potentials and refractoriness in single human atrial cells from patients not treated with β-blockers.

Fig. 4a. Representative examples of original action potential recordings obtained from a single human atrial myocyte, from a patient not treated with a β-blocker, before (upper panel) and in the presence of 10 μM 5-HT (lower panel). Cells were paced at 75 bpm. Dotted lines in bold show the level of 50% of the action potential amplitude. The majority of cells displayed type 3 action potentials, ie: with pronounced phase 1 and a plateau amplitude below the 50% level. The effective refractory period (ERP), indicated by solid bars, was calculated as the longest S_1-S_2 interval failing to elicit an S_2 response of amplitude >80% of the preceding S_1 action potential. The S_2 response used to measure this interval is labelled with an arrow.

Fig. 4b. Mean (±SEM) action potential duration (ms) measured at 50 and 90% repolarisation (APD_{50} and APD_{90}, respectively; n=16 cells, 8 patients) and ERP (n=12 cells, 8 patients) in cells from patients not treated with β-blockers, in the absence (open bars), in the presence (closed bars) and after removal of 10 μM 5-HT (striped bars; n=11 cells, 8 patients for APD and n=9 cells, 7 patients for ERP). Asterisk denotes P<0.05 between control and 5-HT values (paired t-test).

Figure 5

Effect of 5-HT on action potential duration and refractoriness in human atrial cells from patients treated with β-blockers.

Fig. 5a. Representative examples of action potentials recorded from a single human atrial myocyte from a patient treated with a β-blocker, before (upper panel) and in the presence of 5-HT at 10 μM (lower panel). Cells were paced at 75 bpm. Dotted lines in bold show the level of 50% of the action potential amplitude. The effective refractory period (ERP), indicated by solid bars, was defined and calculated as in the legend of Fig. 4a.

Fig. 5b. Mean (±SEM) action potential duration (APD_{50} and APD_{90}; n=17 cells, 9 patients) and ERP (n=12 cells, 9 patients) in cells from patients treated with β-blockers, in the absence (open bars), in the presence (closed bars) and after removal of 10 μM 5-HT (striped bars; n=7 cells, 5 patients for APD and n=3 cells, 3 patients for ERP). The asterisk denotes $P<0.05$ between control and 5-HT values (paired t-test).

Figure 6.

Effect of 5-HT to promote abnormal depolarisations in cells from β-blocked patients.

An example of original recordings of action potentials obtained from a single human atrial myocyte (75 bpm pacing) from a patient treated with a β-blocker, before (top panel), in the presence of 5-HT at 10 μM (middle panel), and then following washout of 5-HT (bottom panel). The arrow indicates the presence of abnormal depolarisations, which occurred only in the presence of 5-HT.

Figure 1

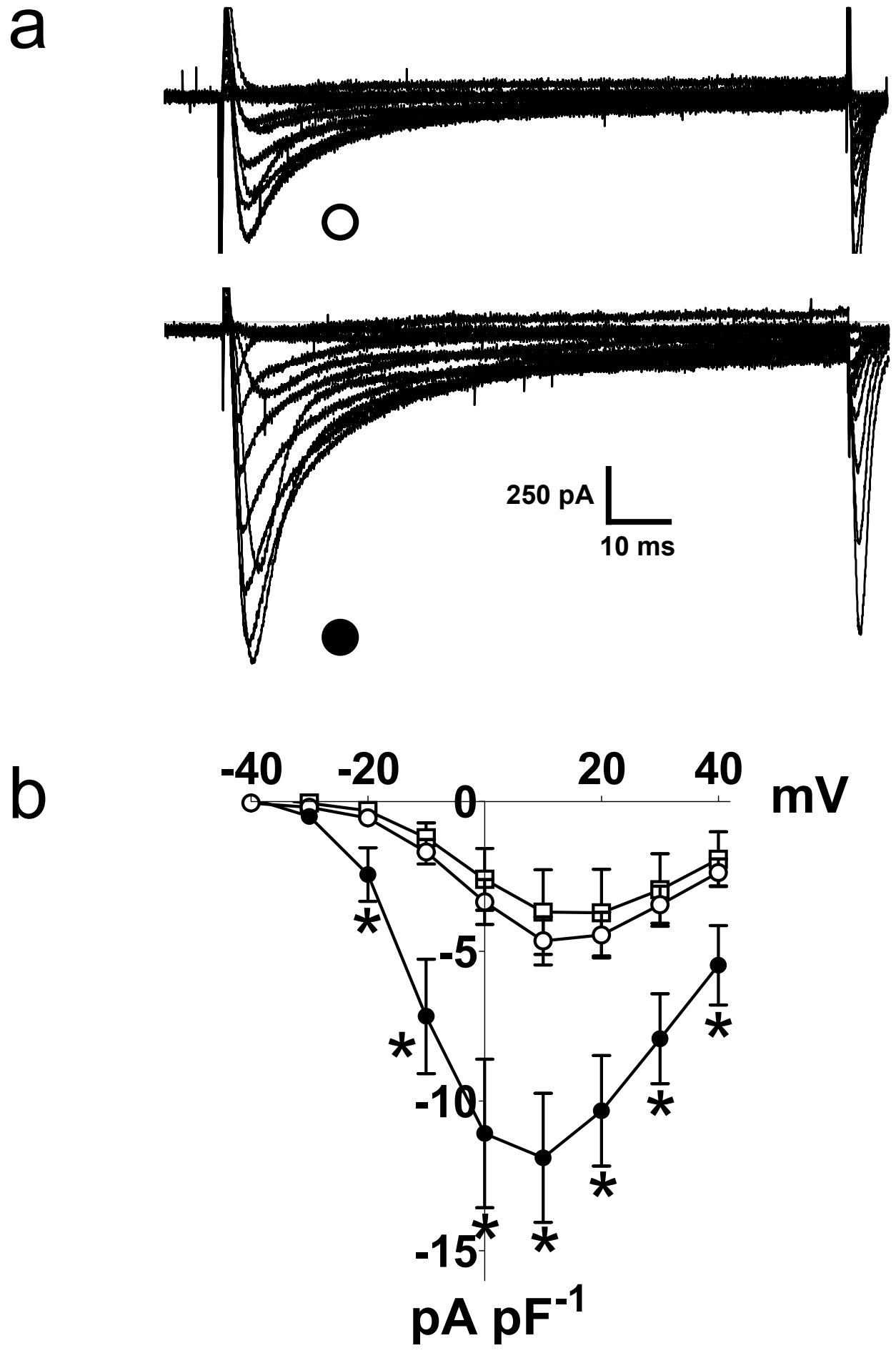
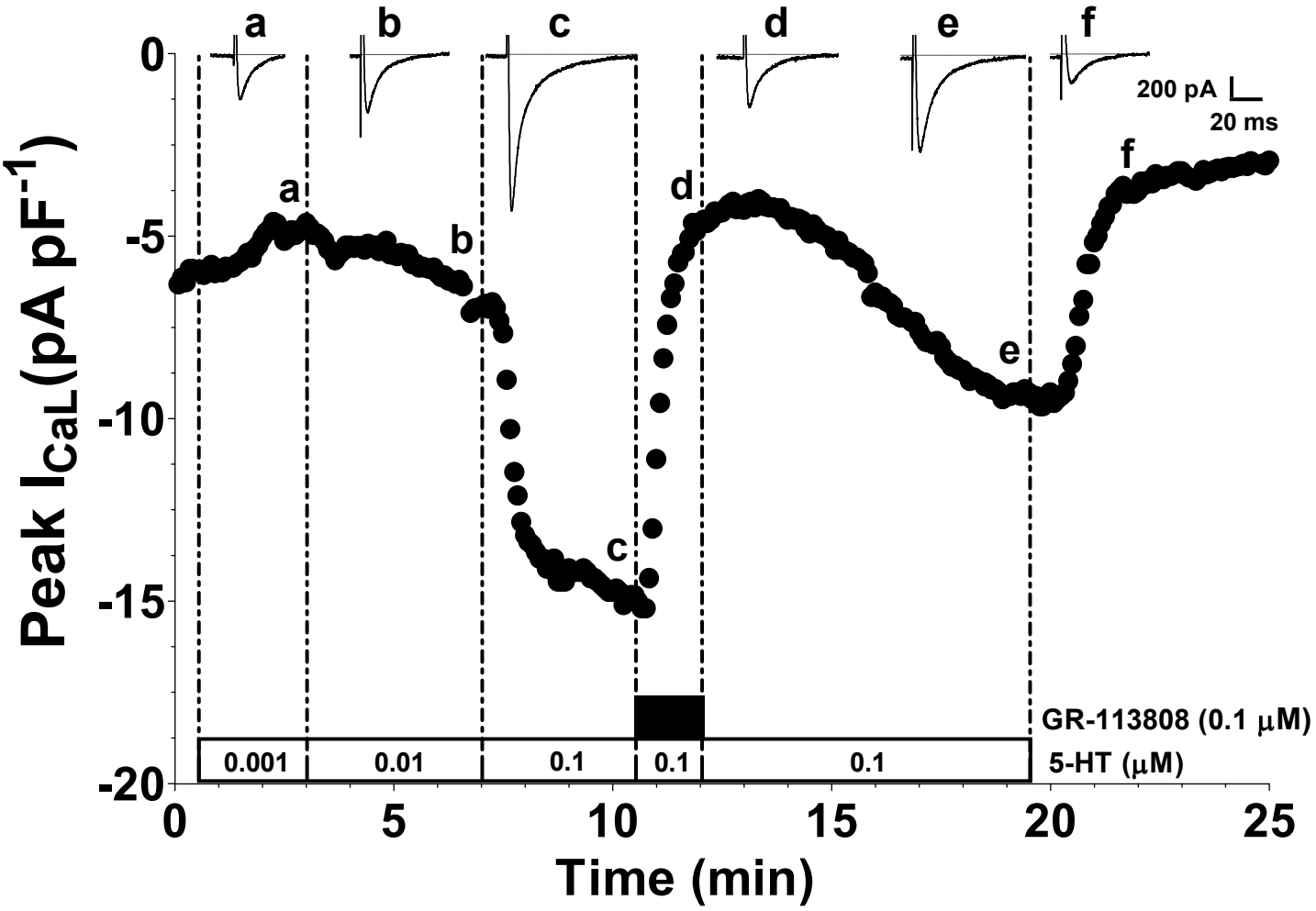


Figure 2


a
b
c
d
e
f
200 pA
20 ms
Peak I_{CaL}(pA pF^{-1})
0
-5
-10
-15
-20
0
5
10
15
20
25
Time (min)
GR-113808 (0.1 μM)
5-HT (μM)
0.001
0.01
0.1
0.1
0.1

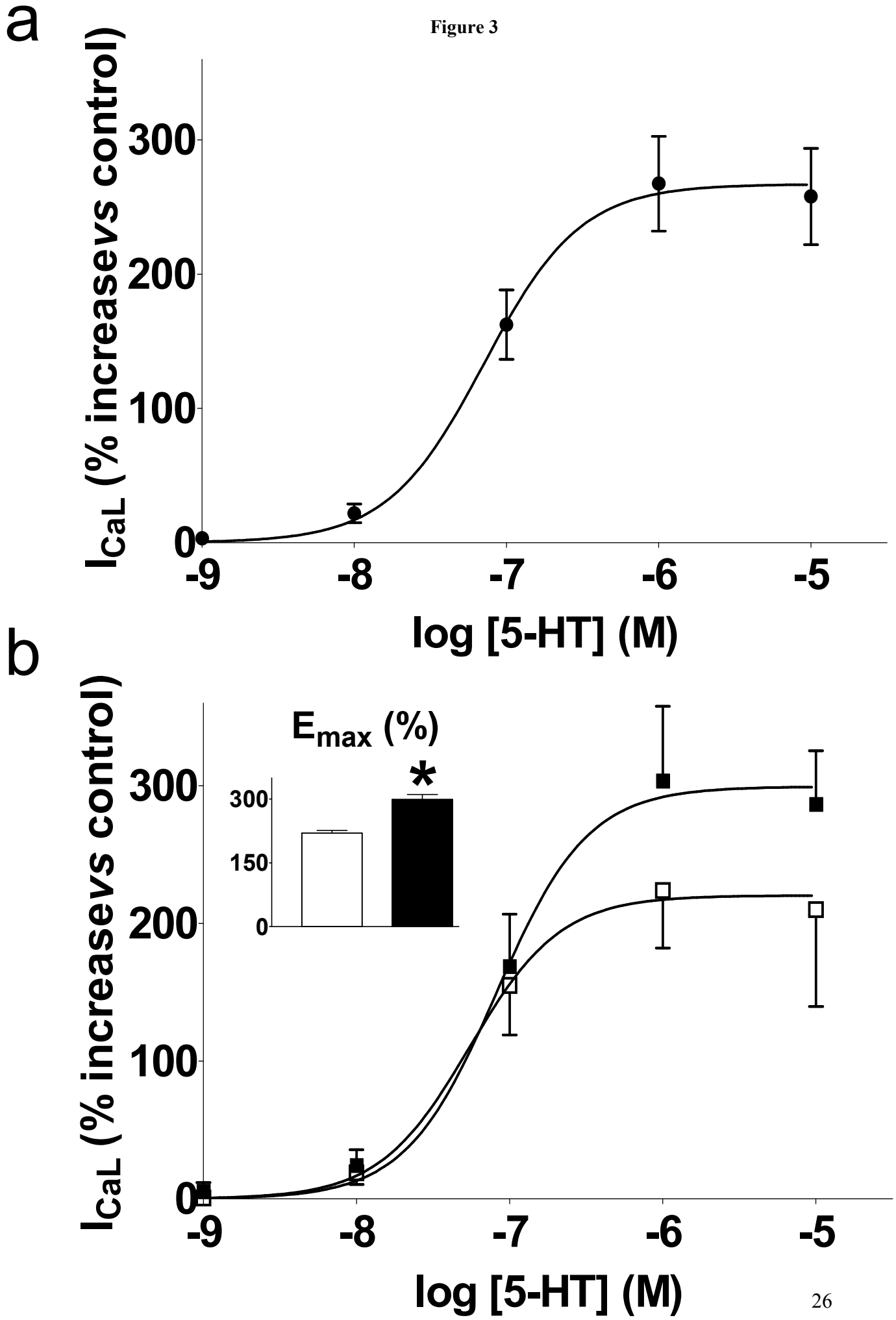

a
I_{CaL} (% increasevs control)
300
200
100
0
-9
-8
-7
-6
-5
log [5-HT] (M)
b
I_{CaL} (% increasevs control)
E_{max} (%)
*
300
150
0
300
200
100
0
-9
-8
-7
-6
-5
log [5-HT] (M)

Figure 4

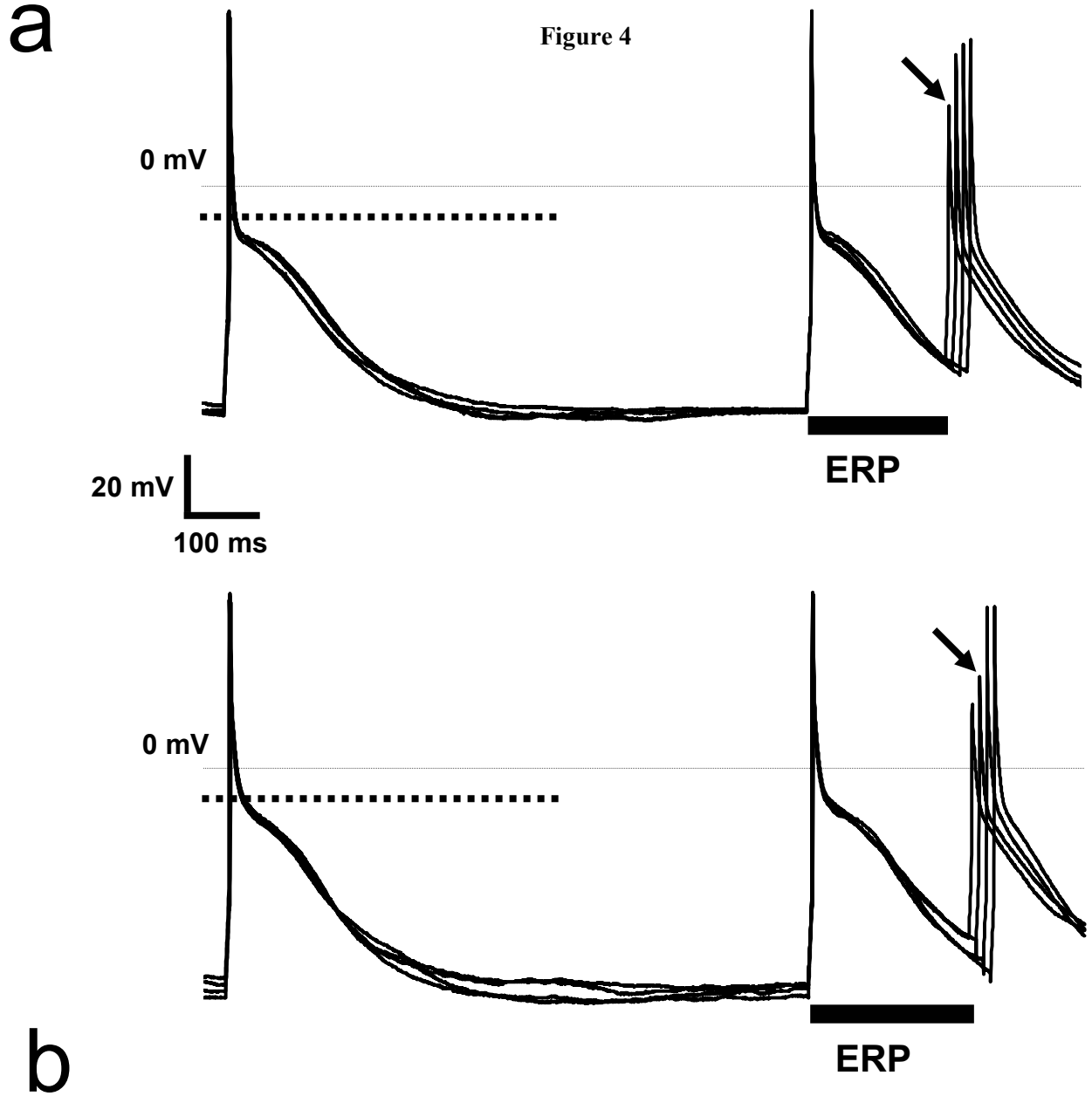

a
0 mV
ERP
20 mV
100 ms
0 mV
ERP
b
Duration (ms)
300
200
100
0
*
APD50
APD90
ERP

Figure 5

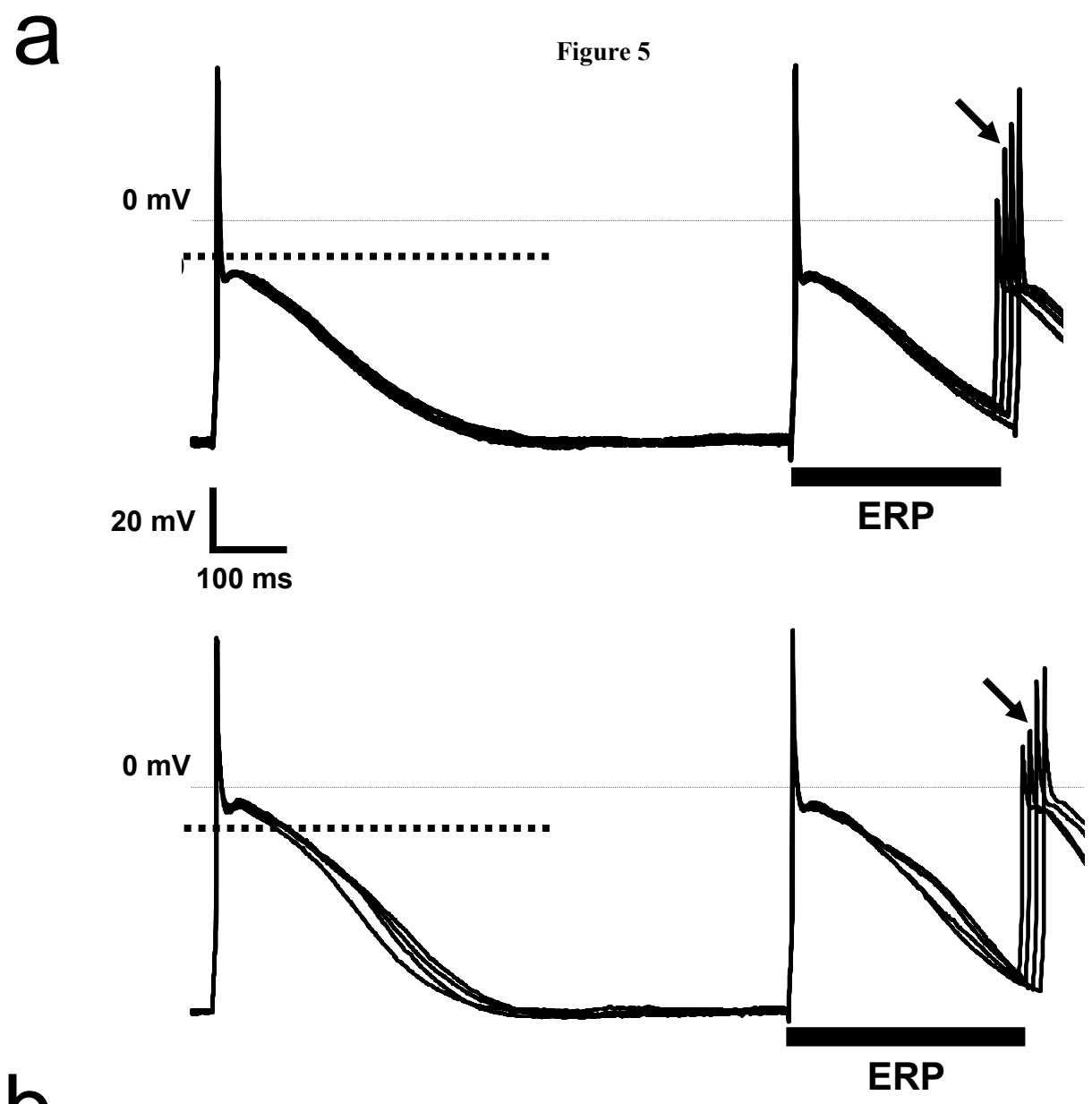

a
0 mV
20 mV
100 ms
ERP
0 mV
ERP
b
Duration (ms)
300
200
100
0
*
APD50
APD90
ERP

Figure 6