Electrophysiological and arrhythmogenic effects of 5-hydroxytryptamine on human atrial cells are reduced in atrial fibrillation

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Running Head: Electrical effects of 5-HT in atrial fibrillation

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Abstract:

5-hydroxytryptamine (5-HT) is pro-arrhythmic in atrial cells from patients in sinus rhythm (SR) via activation of 5-HT_4 receptors, but its effects in atrial cells from patients with atrial fibrillation (AF) are unknown. The whole-cell perforated patch-clamp technique was used to record L-type Ca^{2+} current (I_{CaL}), action potential duration (APD) and arrhythmic activity at 37ºC in enzymatically-isolated atrial cells obtained from patients undergoing cardiac surgery, in SR or with chronic AF. In the AF group, 5-HT (10 μM) produced an increase in I_{CaL} of 115±21% above control (n=10 cells, 6 patients) that was significantly smaller than that in the SR group (232±33%; $p<0.05$; n=27 cells, 12 patients). Subsequent co-application of isoproterenol (1 μM) caused a further increase in I_{CaL} in the AF group (by 256±94%) that was greater than that in the SR group (22±6%; $p<0.05$). The APD at 50% repolarisation (APD_{50}) was prolonged by 14±3 ms by 5-HT in the AF group (n=37 cells, 14 patients). This was less than that in the SR group (27±4 ms; $p<0.05$; n=58 cells, 24 patients). Arrhythmic activity in response to 5-HT was observed in 22% of cells in the SR group, but none was observed in the AF group ($p<0.05$). Atrial fibrillation was associated with reduced effects of 5-HT, but not of isoproterenol, on I_{CaL} in human atrial cells. This reduced effect on I_{CaL} was associated with a reduced APD_{50} and arrhythmic activity with 5-HT. Thus, the potentially arrhythmogenic influence of 5-HT may be suppressed in AF-remodelled human atrium.

Keywords: patch-clamp; myocytes; atrial fibrillation; L-type calcium current; action potentials; 5-HT; isoproterenol.

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in the developed word and its prevalence will increase with the ageing of the population [1]. The occurrence of AF may be related to structural heart disease, but in many cases there is not a clear aetiology. One of the factors that has been postulated to be responsible for the origin and maintenance of AF is the release of 5-hydroxytryptamine (5-HT, serotonin) from aggregating platelets in human atria [2,3]. 5-HT is a naturally occurring monoamine, widely distributed in the animal kingdom, which exerts complex effects throughout the body, including the gastrointestinal and cardiovascular systems [4,5]. 5-HT has been shown to act, via 5-HT_4 receptors [6], as a positive inotropic, chronotropic and lusitropic agent in the human atrium [7]. 5-HT increases the L-type Ca^{2+} current, I_{CaL}, using the same biochemical pathway as isoproterenol (ISO), a β-adrenoceptor agonist, through activation of cyclic AMP [8]. The 5-HT-induced increase in peak I_{CaL} density has been associated with a prolongation of the early plateau phase of the action potential duration (APD_{50}) and with arrhythmic activity in atrial myocytes from patients in sinus rhythm (SR) [9]. However, it is not known if the ability of 5-HT to increase I_{CaL}, APD_{50} and induce arrhythmic activity is preserved in atrial myocytes from patients with chronic AF. Another factor that may influence the arrhythmogenic potential of 5-HT is chronic β-adrenoceptor antagonist treatment of patients in SR prior to surgery [9-11]. The ability of 5-HT to increase I_{CaL}, APD_{50} and to cause arrhythmic activity in human atrial myocytes is increased by chronic β-blockade in myocytes from patients in SR [9], but the influence of chronic β-blockade on the electrophysiological effects of 5-HT in myocytes from patients with AF is unknown. There has been only one previous study of 5-HT and AF, which showed a reduction of mRNA expression of 5-HT_4 receptors, but not of β-adrenoceptors, in the fibrillating human atrium [12].

The aims of this study were to investigate the effects of 5-HT with and without ISO on I_{CaL}, action potential duration (APD), cellular effective refractory period (cERP) and cellular

arrhythmic depolarisations (cADs) in atrial myocytes from patients with chronic AF, and to compare these with the effects in atrial myocytes from patients in SR. We also assessed the influence of chronic treatment of patients with a β-adrenoceptor antagonist on the electrophysiological actions of 5-HT in atrial myocytes from patients with and without AF.

Methods

Tissue and cell isolation

Procedures for obtaining human tissue were approved by the local ethical committee and conform to the institutional guidelines. This investigation conforms to the principles outlined in the Declaration of Helsinki [13]. Samples of the right atrial appendage were obtained from 81 consenting patients undergoing cardiac surgery; 66 in SR and 15 in chronic AF. Cardiac rhythm was determined from the pre-operative electrocardiogram, and duration of AF was confirmed from the patients' medical records. Atrial myocytes were isolated enzymatically as described previously [9].

Electrical recording techniques

The whole-cell perforated patch-clamp technique was used to record I_{CaL}, APD, cERP and cADs as described before [9]. Cells were superfused at 37^{o}C with a physiological solution containing (mM): NaCl (130), KCl (4), $CaCl_2$ (2), $MgCl_2$ (1), glucose (10), HEPES (10), pH 7.4. To record I_{CaL}, a pipette solution containing (mM): CsCl (30), HEPES (5), $MgCl_2$ (1), Cs methanesulfonic acid (100), NaCl (5), nystatin (0.18) was used. To record action potentials, a pipette solution containing (mM): KCl (30), HEPES (5), $MgCl_2$ (1), K methanesulfonic acid (100), NaCl (5), nystatin (0.18) was used. 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. The series resistance in cells from patients with and without AF was 9.5±0.3 MΩ (n=99 cells) and 10.4±0.2 MΩ (n=352 cells), with a mean cell capacity of 92±2 pF and 78±1 pF ($p<0.05$), respectively. 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 to stimulate and record electrical activity.

Experimental protocols

The voltage-dependency of I_{CaL} was measured from a holding potential of -40 mV, with pulses of 250 ms duration at 0.33 Hz increasing in steps of 10 mV, up to +40 mV. The time-dependent effect of drugs was measured on peak I_{CaL} with depolarising pulses from -40 to +10 mV (0.2 Hz). Actions potentials were stimulated at the physiological rate of 75 beats/min (bpm) using 5 ms current pulses of 1.2x threshold strength, after current clamping resting cells at -80 mV, and keeping the holding current (<150 pA) constant thereafter throughout all recording protocols in each cell. The holding current density (pA/pF) was found not to be significantly different between cells from patients in SR and AF, with or without prior treatment with a β-adrenoceptor antagonist. The cellular 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 cERP 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 cERP were recorded before, and 90 s after, drug additions and again 180 s after removal of drugs. The spontaneous development of cellular arrhythmic depolarisations was recorded during the measurement of the cERP protocol. Cellular ADs were defined as any transient depolarisation during phase 3 or a transient depolarisation of >3mV during phase 4.

Data analysis and statistics

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 peak I_{CaL} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration-response curve. Time-dependent inactivation of peak I_{CaL} was fitted by a bioexponential function [21]. All data have been analysed using cell means, except when stated that patient means were used. Mean ±standard error of the mean (SEM) values were compared using paired or unpaired Student's t-test, with $p<0.05$ regarded as statistically significant. A Fisher's exact test was used to assess the incidences of arrhythmic activity between groups of cells. To permit sub-analysis within the SR group, data have been used from 27 patients in SR who were included in a previous publication [9].

Drugs

5-HT and ISO (Sigma) were prepared as 10 mM stock solution in distilled water. Nifedipine (Sigma) and GR-113808 (a gift from Johnson & Johnson Pharmaceutical Research & Development) were dissolved in dimethyl sulfoxide at 10 mM and subsequently diluted in physiological solutions.

Results

Patients' clinical characteristics

Clinical characteristics of patients in SR or with chronic AF (>6 months) are shown in Table 1. Those patients with prior treatment with β-adrenoceptor antagonists were treated for at least 1 week. In the SR group, the majority received a cardiac selective β_1-adrenoceptor antagonist, namely atenolol (77% of patients), bisoprolol (17%), metoprolol (4%); 1 patient received the cardiac non-selective β-adrenoceptor antagonist, propranolol (2%). Those patients with AF were treated with atenolol (57%), bisoprolol (29%) or with the cardiac non-selective β-adrenoceptor antagonist, carvedilol (14%). Patients received their routine drugs on the day of surgery. Mean ventricular rate was 66±2 bpm in 66 patients in SR versus 69±5 bpm in 15 patients with AF ($p>0.05$). The ventricular rate was reduced in β-blocked (60±2 bpm, n=47) compared to non-β-blocked patients (76±3 bpm, $p<0.05$; n=19) from the SR group of patients. In patients with AF, the ventricular rate was similar in β-blocked (72±7 bpm, n=8) compared to non-β-blocked patients (66±7 bpm, $p>0.05$; n=7).

Effects of 5-HT on I_{CaL} and influence of chronic AF

The density of basal peak I_{CaL} was smaller in cells from patients with AF (-3.0±0.2 pA/pF; n=50 cells, 14 patients) than in cells from those in SR (-5.4±0.3 pA/pF, $p<0.05$; 178 cells, 58 patients). Within the AF group of patients, no significant difference was found in basal I_{CaL} between patients who had (-2.9±0.2 pA/pF; n=9) and who had not (-3.2±0.3 pa/pF; n=5) undergone mitral valve replacement (MVR) surgery. Examples of original I_{CaL} recordings from a cell from a patient in SR or with AF are shown in Figure 1 in the absence (upper panel) or in the presence (lower panel) of 5-HT (10 μM). The 5-HT-induced increase in the mean magnitude

of I_{CaL} in cells from patients with and without AF is shown in Figure 1 (inset). This increase in I_{CaL} occurred without any change in its voltage dependency, and was reversible on washout of 5-HT.

The concentration-response curves of the effect of 5-HT on peak I_{CaL} density (pA/pF) obtained in cells from patients with and without AF, are shown in Figure 2A. 5-HT (0.001-10 μM) elicited a concentration-dependent increase in the amplitude of peak I_{CaL}, with a maximum response (E_{max}) equal to an increase of 187±2% above control in cells from patients with AF. This was significantly less than that produced by 5-HT in cells from patients in SR (E_{max}=260±7%; $p<0.05$; Figure 2B). In addition, the concentration of 5-HT producing half-maximal response (expressed as $logEC_{50}$) was significantly higher in cells from patients with AF (-6.35±0.02) than in cells from those in SR (-6.84±0.17; $p<0.05$), indicating a reduced potency of 5-HT (Figure 2C). The Hill coefficient, a measure of cooperativity of the agonist with the receptor, was 0.68±0.02 in cells from patients with AF, not significantly different than in cells from those in SR (0.66±0.14). A higher concentration of 5-HT (100 μM) caused a significantly smaller I_{CaL} response in comparison to the 10 μM 5-HT in all cells studied, with and without AF (not shown). The $5\text{-}HT_4$ antagonist GR-113808 at 1 μM blocked the 5-HT-induced increase of peak I_{CaL} in cells from patients in SR (n=4 cells, 2 patients) or with AF (n=4 cells, 2 patients). Basal peak I_{CaL} was abolished by the Ca^{2+} channel blocker nifedipine at 10 μM in cells from patients in SR (3 cells, 2 patients) or with AF (3 cells, 2 patients).

Effects of 5-HT on I_{CaL} and influence of chronic β-blockade

Chronic β-blockade did not significantly affect basal peak I_{CaL} in cells from patients in SR (-5.5±0.3 vs -5.0±0.5 pA/pF; n=137 cells from 41 β-blocked patients and n=41 cells from 17 non β-blocked patients, respectively) or with AF (-3.2±0.3 vs -2.9±0.3 pA/pF; n=24 cells from 7 β-blocked patients and n=26 cells from 7 non β-blocked patients). The influence of chronic treatment of patients with a β-adrenoceptor antagonist on the concentration-response curves of the effect of 5-HT on peak I_{CaL} obtained in cells from patients with and without AF is shown in Figure 3A. The E_{max} of the increase in I_{CaL} by 5-HT was greater in cells from β-blocked patients compared with non β-blocked patients in the AF group (205±4% vs 158±1%; $p<0.05$) and SR groups (282±14% vs 216±4%; $p<0.05$; Figure 3B), respectively. Neither the potency (Figure 3C) nor the n_H of the 5-HT concentration-response curve were affected by chronic treatment of patients with a β-adrenoceptor antagonist with and without AF. Similar increases in E_{max} by 5-HT were obtained when the comparisons were made of patient means, from β-blocked and non β-blocked patients in AF (234±5% vs 171±2%; $p<0.05$) or SR (316±17% vs 231±4%; $p<0.05$), respectively.

Effects of 5-HT, with and without ISO, on I_{CaL}, and influence of chronic AF

In subsets of cells from patients with or without AF, the application of a maximal concentration of 5-HT (10 μM) was followed by the co-application of a maximal concentration of ISO (1 μM), in order to assess the ability of β-adrenoceptors to increase peak I_{CaL} after saturation of 5-HT_4 receptors. Figures 4A and 4B show examples of this protocol, from a cell from a patient in SR or with AF, respectively. 5-HT produced an increase in peak I_{CaL} that was significantly greater in cells from patients in SR than that in cells from patients with AF (232±33 vs 115±21% above control; $p<0.05$; Figure 4C). Subsequent co-application of ISO further

increased peak I_{CaL} in both groups, but by a relatively greater increase in cells from patients with AF (256±94% greater than with 5-HT alone) than in cells from patients in SR (22±6% greater than with 5-HT; $p<0.05$). Although the absolute I_{CaL} density in the presence of 5-HT with or without ISO was significantly less in cells from patients with AF than that in cells from patients in SR (Figure 4C), the relative increase with ISO (to 273±38% above control, $p<0.05$, with SR; and to 324±56% above control, $p<0.05$, with AF) was not significantly different between the groups ($p>0.05$). Following washout of 5-HT and ISO, the subsequent application of ISO alone increased $I_{CaL,}$ in a similar manner to the co-application of ISO with 5-HT, in both groups (Figure 4C).

In the same set of cells, we analysed the kinetics properties of the time-dependent inactivation of basal and stimulated peak I_{CaL} (Table 2). The fast (τ_1) and the slow (τ_2) inactivation time constants were increased in cells from patients with AF compared to cells from patients in SR. 5-HT had no significant effect on τ_1 and τ_2 in cells from patients with and without AF. However, their respective fractions A_1 and A_2 were altered by shifting the I_{CaL} inactivation from the fast to the slow phase. Co-application of 5-HT with ISO decreased τ_1, but not τ_2, in both group of cells, and this was associated with a reduced A_1 and an increased A_2.

Effects of 5-HT on APD characteristics and influence of chronic AF

The basal APD_{50} was longer in cells from patients with AF (40±3 ms; n=37 cells, 14 patients) compared to cells from patients in SR (17±2 ms, $p<0.05$; n=58 cells, 24 patients). By contrast, the APD_{90} was significantly shortened in cells from patients with AF (203±8 ms) compared to cells from patients in SR (239±11 ms; $p<0.05$). Within the AF group of patients, no significant differences were found in basal APD characteristics between patients who had (n=5) and who had not (n=9) undergone MVR surgery. Figure 5A shows original traces of action potential

recordings illustrating the effects of 5-HT in a cell from a patient in SR (left panel) or with AF (right panel), both treated with a β-adrenoceptor antagonist. In these examples, 5-HT (10 μM) caused a prolongation of the APD_{50}, without affecting the late repolarisation and the cERP. Mean data demonstrated that the 5-HT-induced prolongation of the APD_{50} in the AF group was significantly smaller than that in the SR group (Figure 5B; $p<0.05$). This effect was fully reversible after 3 min washout. There was no significant effect of 5-HT on other action potential measurements including APD_{75}, APD_{90}, cERP, overshoot, amplitude and maximum upstroke velocity in either the cells from patients in SR or with AF.

Effects of 5-HT on APD characteristics and influence of chronic β-blockade

In cells from patients in SR, 5-HT produced a significantly greater prolongation of APD_{50} in cells from β-blocked (36±6 ms; n=37 cells, 15 patients) than non β-blocked patients (10±3 ms, $p<0.05$; n=21 cells, 9 patients). By contrast, in cells from patients with AF, there was no significant difference in the 5-HT-induced prolongation of the APD_{50} between cells from the β-blocked (18±5 ms; n=25 cells, 8 patients) and non β-blocked patients (9±3 ms, $p>0.05$; n=12 cells, 7 patients). These findings were confirmed by using patient means for both β-blocked patients and non β-blocked patients in SR (33±6 vs 9±3 ms; $p<0.05$) or AF (16±4 vs 9±4 ms; $p>0.05$), respectively.

Effects of 5-HT, with and without ISO, on APD characteristics, and influence of chronic AF

In a subset of cells, we investigated effects on action potentials of the co-application of ISO (1 μM) and 5-HT (10 μM), following 5-HT alone, i.e. using the same protocol described earlier for

I_{CaL}. Figure 6A shows examples of such effects in a cell from a patient in SR (left panel) or with AF (right panel). Mean data from 10 cells (3 patients) indicated that ISO may further prolong APD_{50} in cells from patients with AF (from 60±7 to 74±9 ms; $p<0.05$; n= 10 cells, 3 patients), but not in cells from patients in SR (from 63±9 to 69±9 ms; $p>0.05$; n=16 cells, 5 patients), with no significant effects on APD_{75}, APD_{90} and the cERP. GR-113808 (1 μM) prevented the increase induced by 5-HT (10 μM) of APD_{50} in cells from patients in SR (n=8 cells, 3 patients) or with AF (n=3 cells, 1 patient), but not that of ISO (1 μM) in cells from patients in SR (7 cells, 3 patients) or with AF (2 cells, 1 patients).

Effects of 5-HT, with and without ISO, on cADs, and influence of chronic β-blockade

Cellular arrhythmic depolarisations were not observed in any of the 37 cells from 14 patients with AF to which 10 μM 5-HT was applied. In contrast, when 10 μM 5-HT was applied in 58 cells from 24 patients in SR, cADs, consistent with early and delayed afterdepolarisations, occurred in 13 of the cells studied (22%) from 9 patients (38%) ($p<0.05$; Fisher's exact test). These cADs in response to 5-HT were observed only in cells obtained from β-blocked patients (13 of 37 cells), while none were observed in cells from the non β-blocked patients (0 of 21 cells; $p<0.05$). This difference remained significant when the comparison was made by patient means ($p<0.05$). In a subset of cells from patients with and without AF (Figure 6B), when ISO (1 μM) was co-applied with 5-HT (10 μM), we observed the appearance of cADs in 15 out of 16 cells (94%) studied from 5 patients in SR and in 3 out of 11 cells (27%) from 3 patients with AF. Both nifedipine and GR-113808 prevented the 5-HT-induced cADs in cells from patients in SR (4 cells from 2 patients and 8 cells from 3 patients, respectively). However, while GR-113808 did not prevent the ISO-induced cADs in cells from patients in SR (7 cells, 3 patients)

or with AF (2 cells, 1 patients), nifedipine did in cells from patients in SR (2 cells, 1 patient) or with AF (3 cells, 2 patients).

Discussion

This study is the first, to our knowledge, to report that AF is associated with a reduction of the effects of 5-HT on I_{CaL} and APD_{50} in human atrial cells. This was associated with a loss of arrhythmic activity in cells from patients previously treated with a β-adrenoceptor antagonist. The pro-arrhythmic actions of 5-HT in humans have been known for a long time [14] and support for its pro-arrhythmic effects has been reported in several studies from patients in SR [2,9,10], but there are no reports from patients with AF. Our study supports the hypothesis that 5-HT may play a role in initiating AF by promoting ectopic activity in patients in SR, that is enhanced by chronic treatment of patients with a β-adrenoceptor antagonist. However, once the arrhythmia is maintained and the remodelling process has occurred, 5-HT has a reduced efficacy and potency in activating the L-type Ca^{2+} channels. Therefore, despite the possible release of 5-HT in the fibrillating atria by platelets [2], its arrhythmic influence may be attenuated. This suggests that the remodelling processes induced by chronic AF may involve an adaptive response including protection from these effects of 5-HT. In addition, since late repolarisation and cERP were unaffected by application of 5-HT in cells from patients with and without AF, it is unlikely that 5-HT plays a role in maintaining the arrhythmia by affecting the minimum path length required for re-entry [15]. The absence of arrhythmic activity by 5-HT in cells from patients with AF, and the low incidence of arrhythmic activity found in cells from patients in SR, are in line with a lack of clinical evidence to support 5-HT as a major factor involved in initiating and maintaining AF in humans [3]. Indeed, even in 5-HT-secreting tumour diseases like the carcinoid syndrome, in which high plasma levels of 5-HT have been detected, the incidence of AF is low [2,3].

The mechanisms involved in the reduction of the electrophysiological actions of 5-HT in cells from patients with AF are not clear. The involvement of the $5\text{-}HT_4$ receptors and the L-type Ca^{2+} channels in the actions of 5-HT in these cells has been established by the prevention of the

effects by the 5-HT_4 receptor antagonist, GR-113808, or the Ca^{2+} channel blocker, nifedipine.

In our study, ISO, with or without 5-HT, induced a relatively greater I_{CaL} increase in cells from patients with AF compared to cells from patients in SR, suggesting that the L-type Ca^{2+} channel was not the limiting factor for the 5-HT response. The enhanced I_{CaL} response to ISO in cells from patients with AF has been reported by others [16-18], but the effects of 5-HT have not been studied previously. A reduction of mRNA expression of 5-HT_4 receptors in chronic AF has been reported [12], but other mechanisms may also contribute to the reduced effects of 5-HT in AF. For example, increased activity of protein phosphatases in chronic AF has been shown to be associated with reduced basal and ISO-activated I_{CaL} currents [18], and changes in protein phosphatases and/or kinases with AF could contribute to the reduced efficacy of 5-HT on I_{CaL} current density. The ability of 5-HT to increase cyclic AMP and cyclic AMP-dependent protein kinase activity has been shown to be significantly less than that of ISO [6,19,20], although similar increases in I_{CaL} were induced by 5-HT and ISO in atrial cells from patients in SR [8,19,21]. In addition, changes in the coupling of the stimulatory or inhibitory G-proteins with 5-HT or ISO receptors may differently affect adenylate cyclase activity leading to the phosphorylation/dephosphorylation of the L-type Ca^{2+} channel [22,23]. These and other factors, such as the expression of different 5-HT_4 receptor subtypes in human atrial cells that couple to different Gs/i proteins [24-26], may possibly reflect a physiological adaptation to limit the Ca^{2+} overload associated with AF [27]. The reduced potency of 5-HT on the I_{CaL} response with AF may support altered receptor/channel coupling, in line with the observation of a reduced potency of ISO on contractile responses in atrial tissue from patients with AF [28].

The proarrhythmic activity induced by β-adrenoceptor agonists (e.g. isoproterenol) has been known for a long time [29] and their ability to increase I_{CaL} via activation of the cyclic AMP cascade has been highlighted as the main trigger to induce afterdepolarisations in human atrial cells [29,30]. Similarly, 5-HT, which activates the same biochemical pathway, has been

demonstrated to induce pro-arrhythmic activity in atrial cells from patients in SR, particularly in those previously treated with β-adrenoceptor antagonists [9,10]. The reduced efficacy and potency of 5-HT to increase I_{CaL} in cells from patients with AF in the present study was associated with a loss of pro-arrhythmic activity by 5-HT, which may be directly linked to the reduced Ca^{2+} entry [31]. ISO caused a greater increase in I_{CaL} when compared to 5-HT in both the SR and AF groups, and this was associated with a greater incidence in the occurrence of arrhythmic activity. Similarly, it has been shown previously that the partial 5-HT_4 agonist prucalopride, in cells from patients in SR, caused reduced Ca^{2+} entry when compared to 5-HT, and did not induce arrhythmic activity, while 5-HT, in the same cells, did [32]. In addition, the L-type Ca^{2+} channel blocker nifedipine prevented the appearance of 5-HT/ISO-induced cADs, suggesting that increased I_{CaL} and cellular Ca^{2+} overload may play a major role in inducing cADs in human atrial cells [9,31,32]. Further studies, for example using a combination of electrophysiology and Ca^{2+} imaging techniques, are needed to elucidate the mechanisms of the observed arrhythmic activity.

The slowing of the time-dependent I_{CaL} inactivation in AF is consistent with other studies in which τ_1 and/or τ_2, were increased in AF [17,33], possibly reflecting an adaptation to an altered activity of the SR Ca^{2+} release channels and/or a reduced Ca^{2+} influx through L-type Ca^{2+} channels [34,35]. By contrast, 5-HT and ISO decreased the fast time constant (τ_1) of I_{CaL} inactivation in both the SR and AF groups, and shifted the fast phase to the slow phase, suggesting an enhanced Ca^{2+}-dependent inactivation probably due the increased release of Ca^{2+} by ryanodine receptors during stimulation [34].

One important factor that may affect 5-HT response in human atrium is prior treatment with β-adrenoceptor antagonists [2,9,10,36]. The present study indicated that chronic β-blockade increased the effects of 5-HT on I_{CaL} in cells from patients with and without AF. However, while chronic β-blockade potentiated the effects of 5-HT on the APD_{50} and arrhythmic activity

in cells from patients in SR, it did not in cells from patients with AF. In our study we report a prolonged basal APD_{50}, together with a reduced basal APD_{90}, in cells from patients with AF, consistent with other studies [37,38], but in contrast to other reports of no change in the APD_{50} [39] or reduced APD_{50} with AF [33,40]. Since a maximal concentration of ISO was capable of further prolonging the APD_{50} during perfusion with 5-HT, the prolonged basal APD_{50} found with AF in our study was not preventing a further increase with 5-HT. While it has been reported that the levels of mRNA of human atrial 5-HT_4-, β_1- and β_2-adreno-receptors were unaffected by chronic β-blockade in cells from patients with and without AF [12], the interaction of complex "electrophysiological" [41] and "pharmacological" [42] remodelling processes may alter the balance of outward and inward currents which determine the APD_{50} and attenuate the effect of chronic β-blockade on the I_{CaL} responses to 5-HT.

In conclusion, AF is associated with a reduced efficacy and potency of 5-HT to increase I_{CaL} in human atrial cells. The reduced Ca^{2+} current is associated with a reduced duration of the early plateau phase of the action potential and an absence of arrhythmic activity with 5-HT. The ability of ISO to cause a relatively greater increase in I_{CaL} than 5-HT in cells from patients with AF, compared with those in SR, suggests that the reduced efficacy of 5-HT observed with AF was not due to reduction in L-type Ca^{2+} channels but may involve other factors, such as reduced density of the 5-HT_4 receptors or alterations of coupling mechanisms. Thus, the potentially arrhythmogenic influence of 5-HT may be suppressed in human atrium that has been remodelled by AF.

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Legends for Figures and Tables

Table 1.

Patients' pre-operative clinical characteristics

Values are numbers of patients (*n* and % of total, respectively) with selected clinical characteristics, who were in SR or with AF on the day of surgery. Age is expressed as mean±SEM. CABG=coronary artery bypass graft surgery, AVR=aortic valve replacement, MVR=mitral valve replacement, RAA=replacement ascending aorta, ACE=angiotensin converting enzyme, MI=myocardial infarction, LVD=left ventricular dysfunction.

Table 2.

Kinetic properties of the time-dependent inactivation of I_{CaL}

Time-dependent inactivation of I_{CaL} was fitted by a bioexponential function 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±SEM in cells from patients in SR or with AF, in the absence or in the presence of 5-HT (10 μM), or co-applied with ISO (1 μM). The asterisks indicates $p<0.05$ vs SR control (*) or vs AF control (**), using paired or unpaired Student's t-tests, as appropriate.

Figure 1.

Effects of 5-HT on I_{CaL} current-voltage relationship

An example of original I_{CaL} traces obtained from a cell from a patient in SR (left panel) or with AF (right panel), during depolarising voltage clamp pulses (250 ms) from -40 mV to +40 mV, in 10 mV incremental steps, from a holding potential of -40 mV, under control conditions (open symbols) and in the presence of 5-HT at 10 μM (closed symbols) is shown. As an inset, current-voltage relationships of I_{CaL} expressed in terms of current density, pA/pF, in cells from patients in SR (n=28 cells, 16 patients) or with AF (n=21 cells, 11 patients) are shown. Values are expressed as means± SEM.

Figure 2.

Concentration-dependent effects of 5-HT on peak I_{CaL}

Comparison of the concentration-response relationship for 5-HT-induced increase in peak I_{CaL} density (pA/pF) in cells from patients in SR (0.001-10 μM; solid squares; n=23-52 cells, 11-31 patients) or with AF (0.001-10 μM; solid circles; n=26-31 cells, 12-14 patients) is shown in Figure 2A. Values are means ±SEM. The increase in I_{CaL} is expressed as absolute value of change compared to before the addition of 5-HT. Comparison of the calculated best-fit values for the E_{max} (Figure 2B) and EC_{50} (Figure 2C) are shown between cells from patients in SR or with AF. The asterisk indicates $p<0.05$ (unpaired Student's t-test).

Figure 3.

Influence of chronic β-blockade on concentration-dependent effects of 5-HT on peak I_{CaL}

Figure 3A shows a comparison of concentration-response curves for 5-HT-induced increase of peak I_{CaL} density (pA/pF) between cells from patients in SR treated (BByes; n=15-37 cells, 7-22 patients) and not treated (BBno; n=8-17 cells, 4-9 patients) with a β-adrenoceptor antagonist (open symbols), and those from patients with AF treated (BByes; n=11-18 cells, 5-7 patients) and not treated (BBno; n=13-16 cells, 5-6 patients) with a β-adrenoceptor antagonist (closed symbols). Comparison of the calculated best-fit values for the E_{max} (Figure 3B) and EC_{50} (Figure 3C) are shown between groups of patients. Asterisk denotes $p<0.05$ (paired or unpaired Student's t-test), with NS equal to $p>0.05$ (not statistically significant).

Figure 4.

Effects of 5-HT, with and without ISO, on peak I_{CaL}

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 5-HT, followed by the co-application of ISO at 1 μM, and the subsequent washout of drugs in a cell from a patient in SR or with AF are shown in Figure 4A and 4B, respectively. Inset traces (a-d) show original currents recorded at the time points labelled. Figure 4C shows mean (±SEM) I_{CaL} data, in the absence (open bars), in the presence of 10 μM 5-HT (closed bars) or co-applied with 1 μM ISO (crossed bars), measured in cells from patients in SR (n=27 cells, 12 patients; open circles) or with AF (n=10 cells, 6 patients; closed circles). Subsequent re-application of ISO alone following the washout of 5-HT+ISO is also shown for both SR (n=7 cells from 5 patients) and AF (4 cells from 2 patients) groups. Asterisk denotes $p<0.05$ (paired or unpaired Student's t-test).

Figure 5.

Effects of 5-HT on action potentials characteristics

Representative examples of original action potential recordings before (open circles), in the presence of 10 μM 5-HT (closed circles), and then following 3 min wash (open squares), from cells from patients in SR (left panel) or with AF (right panel), both treated with a β-adrenoceptors antagonist, are shown in Figure 5A. Cells were paced at 75 bpm. Dotted lines in bold show the level of 50% of the action potential amplitude. The cellular ERP is indicated by solid bars and was defined as described in the Methods section. Figure 5B shows the increase in APD_{50} (ms) by 5-HT (10 μM), measured in cells from patients in SR (n= 58 cells, 24 patients) or with AF (n=37 cells, 14 patients). Asterisk denotes $p<0.05$ (unpaired Student's t-test).

Figure 6.

Effects of 5-HT, with and without ISO, on arrhythmic activity

Figure 6A shows an example of original recordings of action potentials obtained from a cell from a patient in SR (left panel) or with AF (right panel), before (open circle), in the presence of 10 μM 5-HT (solid circle), 10 μM 5-HT+1 μM ISO (striped circle) and then following 3 min washout (open square) or attenuation of ISO-induced cADs by 10 μM nifedipine (solid square). Figure 6B shows the incidence of cADs (%) in cells from patients with and without AF, which occurred during the application of 5-HT at 10 μM (closed bars) or co-applied with ISO at 1μM (crossed bars). Asterisks denotes $p<0.05$ (Fisher's exact test).

Table 1. Patient's preoperative clinical characteristics

	SR		**AF**	
	n	**(%)**	***n***	**(%)**
Patients	66		15	
Male/female	46/20	70/30	9/6	60/40
Age	65±1		69±2	
Surgery				
CABG	55	(83)	1	(7)
AVR	2	(3)	3	(20)
MVR	1	(2)	7	(47)
AVR+MVR	0	(0)	1	(7)
CABG+AVR	7	(11)	1	(7)
CABG+MVR	1	(2)	1	(7)
MVR+RAA	0	(0)	1	(7)
Drugs				
Ca^{2+} channel blocker	31	(47)	3	(20)
Beta blocker	47	(71)	8	(53)
ACE inhibitor	38	(58)	7	(47)
Nitrate	42	(64)	3	(20)
Diuretic	22	(33)	9	(60)
Lipid lowering	56	(85)	7	(47)
Digoxin	0	(0)	9	(60)
Warfarin	1	(2)	12	(80)
Symptoms				
Angina	61	(92)	8	(53)
Palpitations	12	(18)	6	(40)
Hypertension	40	(61)	8	(53)
Hyperlipidaemia	52	(79)	5	(33)
Previous history				
MI	29	(44)	1	(7)
Diabetes	12	(18)	2	(13)
LV function				
-normal	48	(73)	9	(60)
-mild-moderate LVD	17	(26)	5	(33)
-severe LVD	1	(2)	1	(7)

Table 2. Kinetic properties of the time-dependent inactivation of I_{CaL}

	τ_1 (ms)	τ_2 (ms)	A_1 (%)	A_2 (%)
SR Control	6.2±0.5	41±4	72±2	28±2
SR 5-HT	5.7±0.5	40±2	66±2*	34±2*
SR 5-HT+ISO	5.5±0.4*	41±2	60±3*	40±3*
SR Wash	7.8±0.9	52±3	71±2	29±2
AF Control	14.7±1.7*	69±11*	80±4	20±4
AF 5-HT	12.4±2.6	71±14	74±3**	26±3**
AF 5-HT+ISO	9.9±1.8**	65±12	62±7**	38±7**
AF Wash	16.9±3.3	85±12	77±3	23±3

Figure 1.

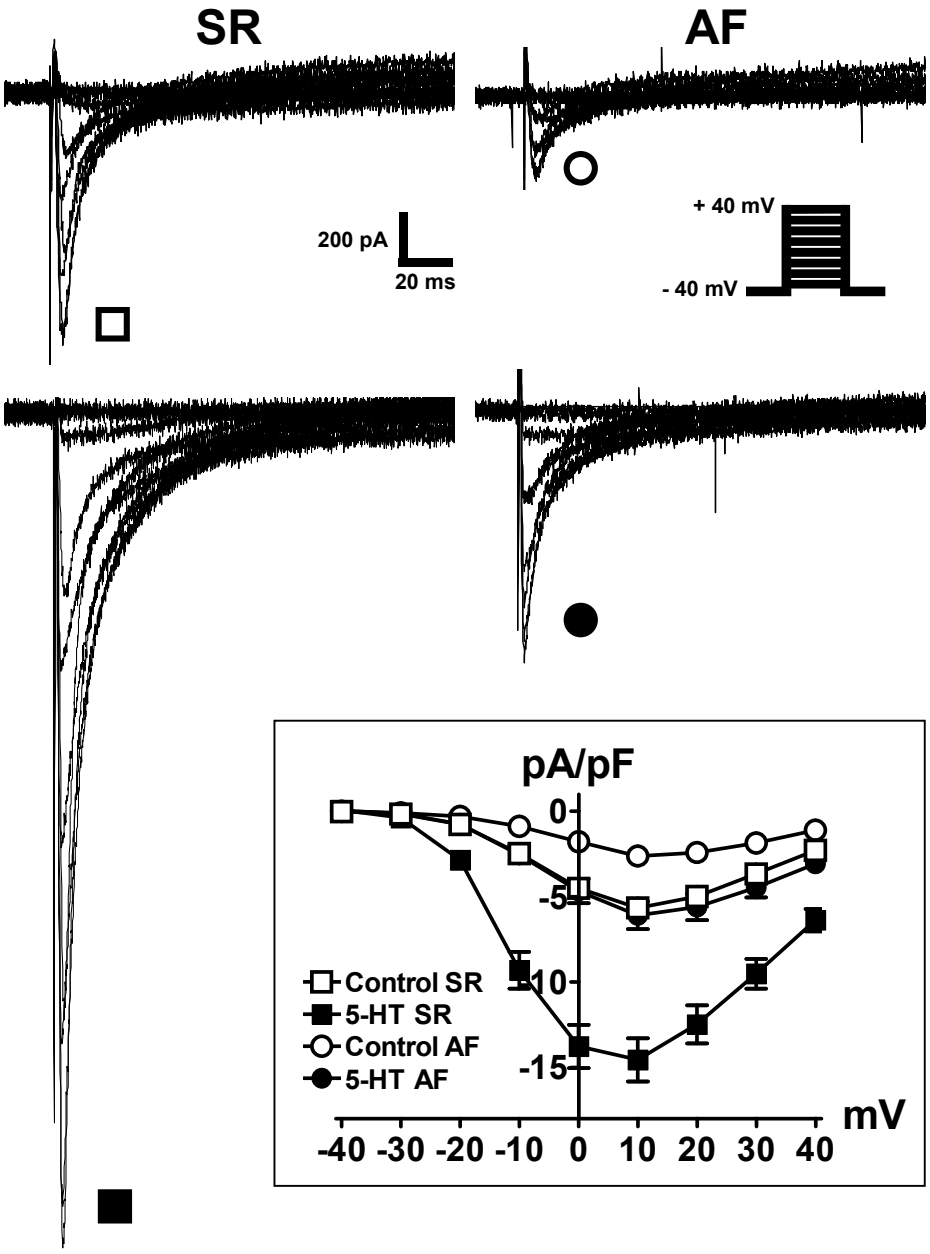
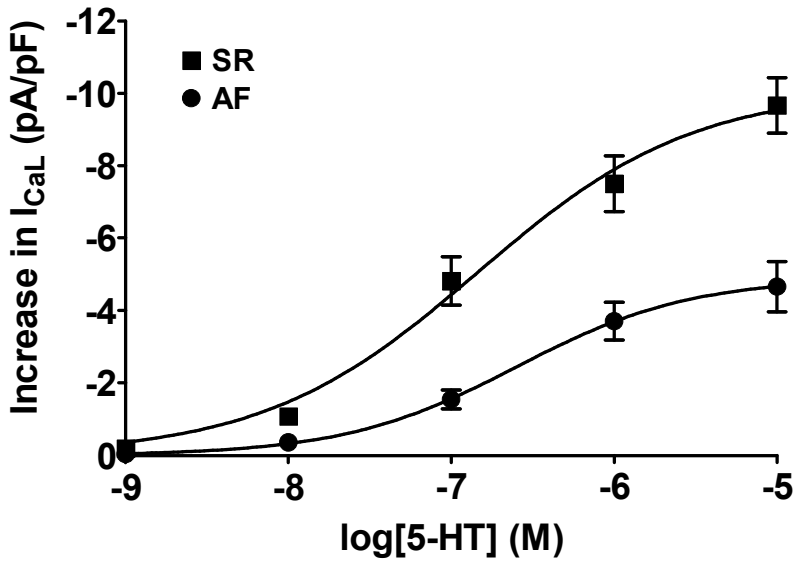
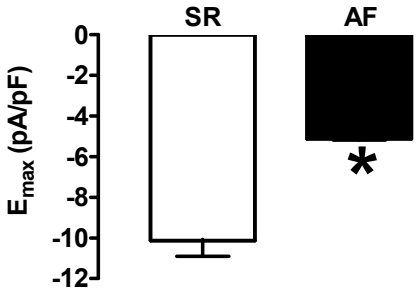

SR
AF
200 pA
20 ms
+ 40 mV
- 40 mV
pA/pF
0
-5
-10
-15
Control SR
5-HT SR
Control AF
5-HT AF
mV
-40 -30 -20 -10 0 10 20 30 40

Figure 2.

A.


SR
AF
Increase in I_{CaL} (pA/pF)
-12
-10
-8
-6
-4
-2
0
-9
-8
-7
-6
-5
log[5-HT] (M)

B.


SR
AF
E_{max} (pA/pF)
0
-2
-4
-6
-8
-10
-12
*

C.

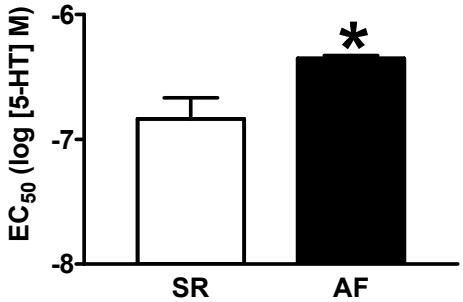
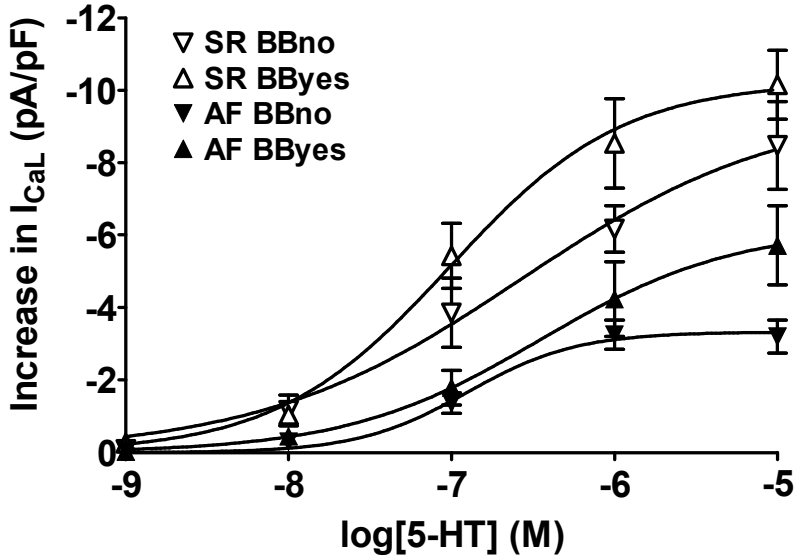
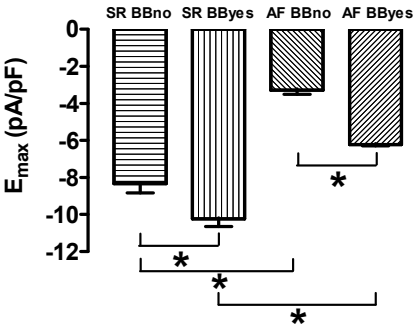

EC_{50} (log [5-HT] M)
-6
-7
-8
*
SR
AF

Figure 3.

A.


Increase in I_{CaL} (pA/pF)
-12
-10
-8
-6
-4
-2
0
-9
-8
-7
-6
-5
log[5-HT] (M)
SR BBno
SR BByes
AF BBno
AF BByes

B.


SR BBno
SR BByes
AF BBno
AF BByes
E_{max} (pA/pF)
0
-2
-4
-6
-8
-10
-12
*
*
*
*

C.

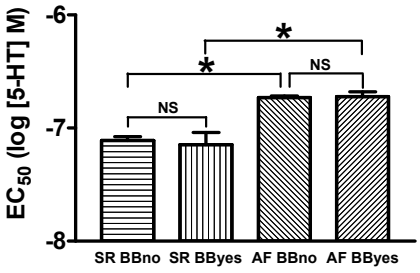
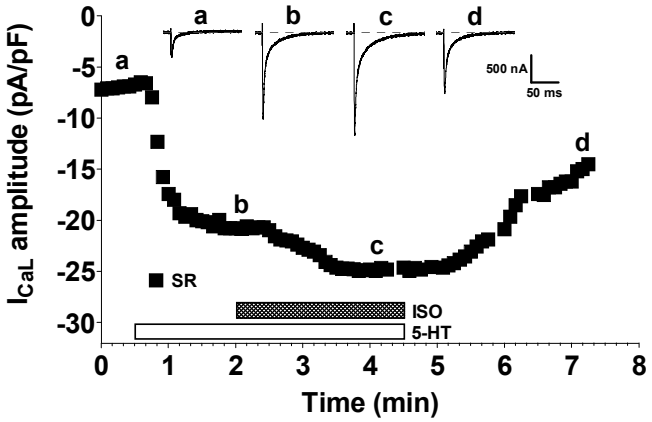
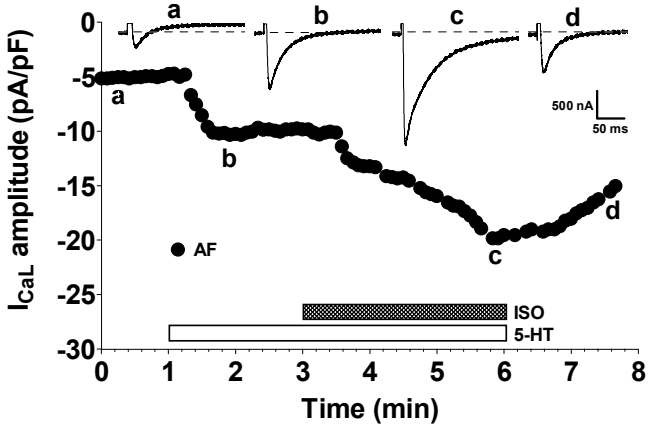

EC_{50} (log [5-HT] M)
-6
-7
-8
*
*
NS
NS
SR BBno
SR BByes
AF BBno
AF BByes

Figure 4.

A.


a
b
c
d
500 nA
50 ms
SR
ISO
5-HT
I_{CaL} amplitude (pA/pF)
Time (min)

B.


a
b
c
d
500 nA
50 ms
AF
ISO
5-HT
I_{CaL} amplitude (pA/pF)
Time (min)

C.

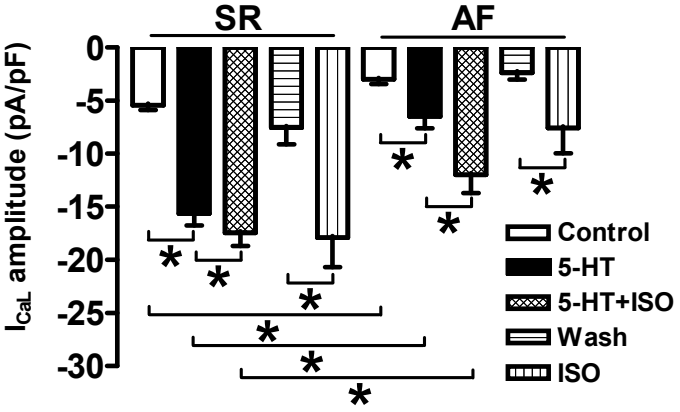
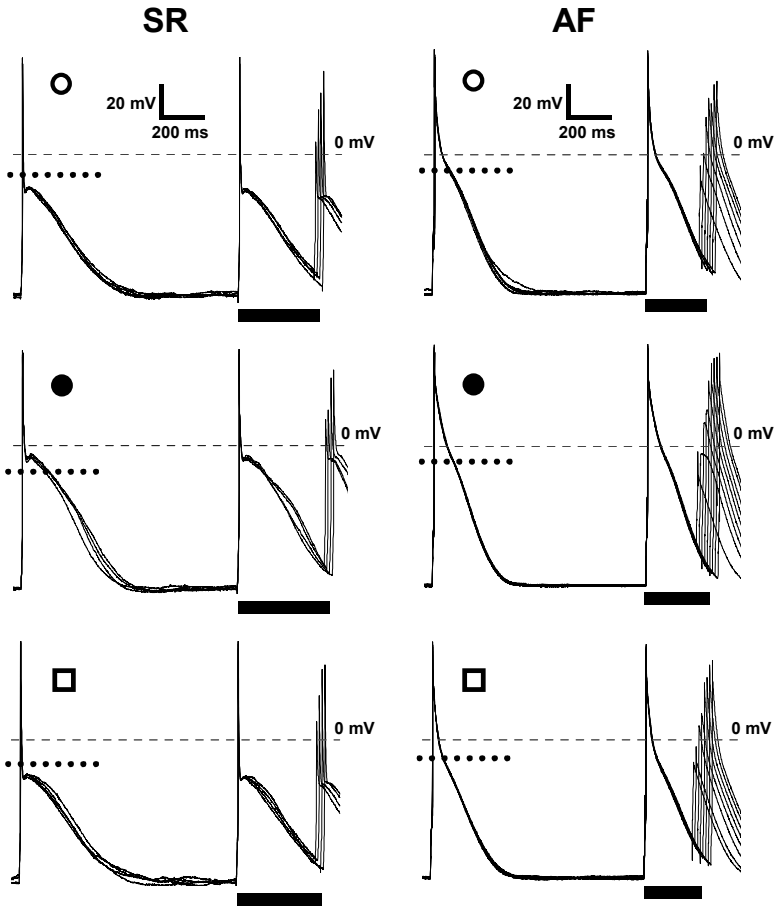

SR
AF
Control
5-HT
5-HT+ISO
Wash
ISO
I_{CaL} amplitude (pA/pF)

Figure 5.

A.


SR
AF
20 mV
200 ms
0 mV

B.

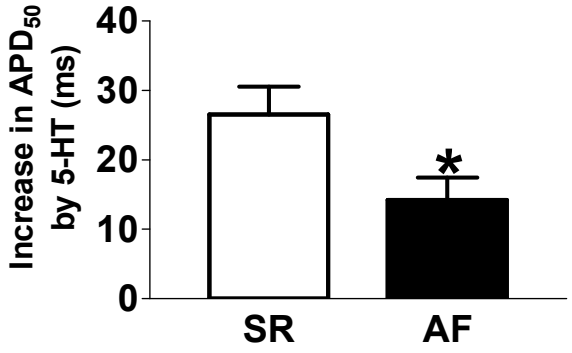
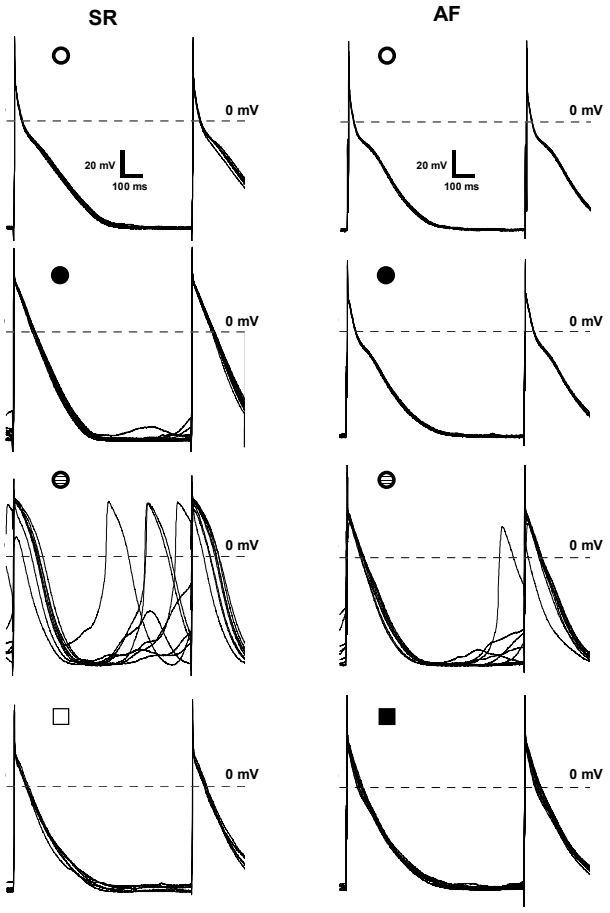
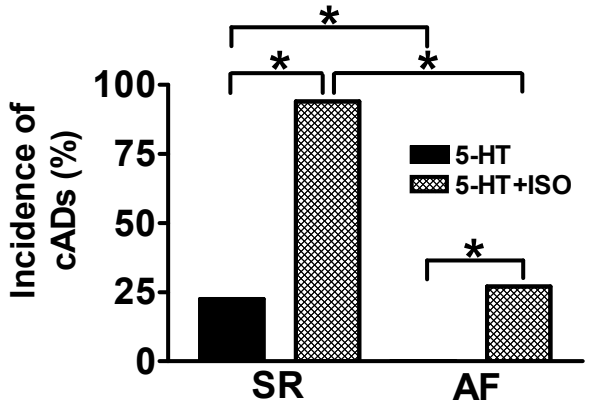

Increase in APD50 by 5-HT (ms)
40
30
20
10
0
*
SR
AF

Figure 6.

A.


SR
AF
0 mV
20 mV
100 ms

B.


Incidence of cADs (%)
100
75
50
25
0
5-HT
5-HT+ISO
SR
AF
*