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**Anti-adrenergic effects of endothelin on human atrial  
action potentials are potentially anti-arrhythmic.**

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Running head: Anti-adrenergic effects of ET on atrial action potential.

**Abstract.**

Endothelin-1 (ET-1) is elevated in patients with atrial fibrillation (AF) and heart failure. We investigated effects of ET-1 on human atrial cellular electrophysiological measurements expected to influence the genesis and maintenance of AF. Action potential characteristics and L-type  $\text{Ca}^{2+}$  current ( $I_{\text{CaL}}$ ) were recorded by whole cell patch clamp, in atrial isolated myocytes obtained from patients in sinus rhythm. Isoproterenol (ISO) at  $0.05 \mu\text{M}$  prolonged the action potential duration at 50% repolarisation ( $\text{APD}_{50}$ :  $54 \pm 10$  vs  $28 \pm 5$  ms;  $P < 0.05$ ,  $n = 15$  cells, 10 patients), but neither late repolarisation nor cellular effective refractory period (ERP) were affected. ET-1 ( $10 \text{ nM}$ ) reversed the effect of ISO on  $\text{APD}_{50}$ , and had no basal effect (in the absence of ISO) on repolarisation or ERP. During repetitive stimulation, ISO ( $0.05 \mu\text{M}$ ) produced arrhythmic depolarisations ( $P < 0.05$ ). Each was abolished by ET-1 at  $10 \text{ nM}$  ( $P < 0.05$ ). ISO ( $0.05 \mu\text{M}$ ) increased peak  $I_{\text{CaL}}$  from  $-5.5 \pm 0.4$  to  $-14.6 \pm 0.9 \text{ pA/pF}$  ( $P < 0.05$ ;  $n = 79$  cells, 34 patients). ET-1 ( $10 \text{ nM}$ ) reversed this effect by  $98 \pm 10\%$  ( $P < 0.05$ ), with no effect on basal  $I_{\text{CaL}}$ . Chronic treatment of patients with a  $\beta$ -blocker did not significantly alter basal  $\text{APD}_{50}$  or  $I_{\text{CaL}}$ , the increase in  $\text{APD}_{50}$  or  $I_{\text{CaL}}$  by  $0.05 \mu\text{M}$  ISO, nor the subsequent reversal of this effect on  $\text{APD}_{50}$  by  $10 \text{ nM}$  ET-1. The marked anti-adrenergic effects of ET-1 on human atrial cellular action potential plateau, arrhythmic depolarisations and  $I_{\text{CaL}}$ , without affecting ERP and independently of  $\beta$ -blocker treatment, may be expected to contribute a potentially anti-arrhythmic influence in the atria of patients with AF and heart failure.

**Keywords.** Endothelin, atrium, myocyte, action potential, repolarisation, refractory period, abnormal automaticity, afterdepolarisation, calcium current, arrhythmia mechanisms, atrial fibrillation.

## Introduction.

Endothelin-1 exerts a wide variety of cardiovascular effects [1-3]. The consequences of these on the cardiac rhythm are currently under investigation. They may be influenced by the concentration of ET-1, the species and cardiac chamber studied, the presence of adrenergic stimulation and the degree of any coronary vasoconstriction by ET-1 [1,3]. Endothelin-1 provoked arrhythmias in the ventricles, by prolonging action potentials and promoting afterdepolarisations, particularly in dogs *in-vivo* [4-6]. The effects of ET-1 on atrial arrhythmias, such as atrial fibrillation (AF), however, are less clear. Recent reports have highlighted potentially pro-arrhythmic effects on intracellular  $\text{Ca}^{2+}$  ( $(\text{Ca}^{2+})_i$ ) handling, via activation of inositol-1,4,5 trisphosphate ( $\text{IP}_3$ ) receptors [7-10]. Endothelin-1 also produced arrhythmic contractions in human atria [11]. However, to our knowledge, the effects of ET-1 on human atrial electrophysiological activity likely to influence the genesis and/or maintenance of AF have not been studied.

Endothelin-1 has consistently been reported to exert a marked anti-adrenergic reduction in L-type  $\text{Ca}^{2+}$  current ( $I_{\text{CaL}}$ ) in both the atria and ventricles [12-15]. This effect is invariably and substantially larger than any change by ET-1 in basal (non-adrenergically-stimulated)  $I_{\text{CaL}}$  [12-15], including in human atria [13,14]. The  $I_{\text{CaL}}$  is a major determinant of action potential morphology, including in human atrial cells [16]. This current also may play a role in the development of abnormal automaticity and afterdepolarisations, both early (EAD) and delayed (DAD) [17]. Plasma ET-1 elevation is independently associated with AF in patients with congestive heart failure (CHF) [18,19]. Since catecholamines also are elevated in CHF [20], it is conceivable that such anti-adrenergic reduction in atrial  $I_{\text{CaL}}$  by ET-1 might attenuate AF genesis, by inhibiting abnormal automaticity, EADs or DADs. However, it might also promote re-entrant AF, by shortening the action potential and effective refractory period (ERP). The effects of ET-1 on either action potentials or these arrhythmogenic electrophysiological mechanisms have not been studied in human atrial cells.

Beta-adrenoceptor antagonists ( $\beta$ -blockers) are increasingly used to treat patients with AF and CHF. These drugs may oppose pro-arrhythmic influences of elevated sympathetic drive. They may also potentiate atrial arrhythmic contractions induced by ET-1 and catecholamines [11,21]. We have demonstrated that long term treatment of patients with a  $\beta$ -blocker was associated with prolongation of late repolarisation and the ERP, in atrial isolated myocytes [22]. However, the influences of such treatment on atrial cellular electrophysiological responses to ET-1 are unknown.

The aim of this study, therefore, was two-fold. Firstly, to investigate effects of ET-1 and the sympathomimetic isoproterenol (ISO), alone and in combination, on human atrial cellular action potential

morphology, ERP, arrhythmogenic electrophysiological activity and  $I_{CaL}$ . Secondly, to compare these effects between groups of patients treated and not treated chronically with a  $\beta$ -blocker.

### **Materials and Methods.**

Right atrial appendage tissue (weight:  $0.39 \pm 0.03$  g) was obtained from 66 consenting patients in sinus rhythm undergoing cardiac surgery. Procedures were approved by the institutional research ethics committee. Atrial cells were isolated by enzymatic dissociation and mechanical disaggregation, using protease (Type XXIV, 4 U/ml, Sigma) and collagenase (Type 1, 330 U/ml, Worthington), as described previously [16,22].

Action potentials and  $I_{CaL}$  were recorded using the whole-cell patch clamp technique, in perforated-patch configuration to limit intracellular dialysis. Glass microelectrodes were pulled and heat-polished to 1.5-5 M $\Omega$ . Cells were superfused at 35-37°C, at 1.5-2 ml/min with a physiological salt solution, containing (mM): NaCl (130), KCl (4), CaCl<sub>2</sub> (2), MgCl<sub>2</sub> (1), glucose (10) and HEPES (10); pH 7.4. An Axopatch-1D amplifier (Axon Instruments) and “WinWCP” software (donated by J Dempster, Strathclyde University) were used to stimulate and record electrical activity. Signals were low-pass filtered at 5 kHz prior to digitisation (Digidata 1200, Axon). Following seal formation, the time course of series resistance ( $R_s$ ) reduction due to perforation was bi-exponential, with mean fast and slow phase half lives of 93 and 297 s, respectively ( $n=165$  cells), with stabilisation (typically after  $\sim 10$  min) at  $7.2 \pm 0.2$  M $\Omega$ . Cells with  $R_s > 20$  M $\Omega$  were excluded. Mean cell capacity was  $83 \pm 1$  pF. Capacitive transients were subtracted electronically from the recordings. The voltage drop across  $R_s$  was routinely compensated electronically, by  $68 \pm 0\%$ , limiting maximum voltage errors to  $< 3$  mV. Action potentials were recorded using a K<sup>+</sup> based intracellular solution, containing (mM): nystatin (0.18), K methanesulfonic acid (100), KCl (30), NaCl (5), MgCl<sub>2</sub> (1), HEPES (5); pH 7.3. A liquid junction potential (+5 mV, bath relative to pipette) was compensated prior to sealing. Action potentials were stimulated with 5 ms current pulses of 1.2 x threshold, with an 8-pulse ( $S_1$ ) conditioning train at 75 beats/min. All cells were current-clamped (with hyperpolarising current of  $< 150$  pA) to a diastolic potential of -75 to -80 mV, with holding current kept constant in each cell thereafter. Action potential traces were scanned for the presence of “cellular arrhythmic depolarisations” (CADs), defined as any phase 3 transient depolarisation or phase 4 depolarisation of  $> 3$  mV. Action potential restitution was investigated with progressively premature test pulses ( $S_2$ ) following the  $S_1$  trains, with  $S_1$  and  $S_2$  of equal magnitude. The

cells' ERP was calculated as the longest S<sub>1</sub>-S<sub>2</sub> interval failing to elicit an S<sub>2</sub> response of amplitude >80% of that of the preceding S<sub>1</sub> [16,22]. I<sub>CaL</sub> was recorded using a Cs<sup>+</sup> based pipette solution to eliminate outward K<sup>+</sup> currents, containing (mM): nystatin (0.18), Cs methanesulphonic acid (100), CsCl (30), NaCl (5), MgCl<sub>2</sub> (1), HEPES (5); pH 7.3. I<sub>CaL</sub> was measured from a holding potential of -40 mV, with 250 ms voltage pulses (0.33 Hz), increasing from -30 to +60 mV in 10 mV steps. The time course of change in peak I<sub>CaL</sub> was examined using repetitive (0.2 Hz) 250 ms pulses from -40 to +10 mV. All drugs were prepared as stock solutions in H<sub>2</sub>O, stored frozen and protected from light. ET-1, ISO, and the ET<sub>B</sub> agonist sarafotoxin were obtained from Sigma. The ET<sub>A</sub> antagonist FR139317 was obtained from SNPE England.

Details of patients' clinical characteristics and drug treatments were obtained from the case notes, and are shown in Table 1. Values are means±SE. ISO concentration-I<sub>CaL</sub> response data were fitted iteratively with a variable slope sigmoidal curve, using the Hill equation and Graphpad Prism software. Differences between means were assessed using paired or unpaired Student's *t* tests, as appropriate. The incidences of occurrence of CADs were compared using a  $\chi^2$  test, with Yates' Correction. *P*<0.05 was taken as statistically significant.

## Results.

### *Effects of isoproterenol, endothelin-1 and their combination, on human atrial L-type $Ca^{2+}$ current.*

Isoproterenol (0.05  $\mu$ M) produced a marked and significant increase in peak  $I_{CaL}$ , from  $-5.5\pm 0.4$  to  $-14.6\pm 0.9$  pA/pF ( $P<0.05$ ,  $n=79$  cells, 34 patients). Figure 1A, upper panel shows original  $I_{CaL}$  recordings from a single atrial cell, representative of this response. The effect of ISO peaked within 2 min (Figure 1A, middle panel). Only cells displaying a stable response to ISO were used for subsequent investigation. The  $I_{CaL}$  response to ISO was concentration-dependent ( $10^{-10}$ - $10^{-6}$  M), with  $E_{max}$  of  $210\pm 15\%$  above control, and  $EC_{50}$  of  $2.3\times 10^{-8}$  M ( $n=4-79$  cells, 3-34 patients). The co-application of ET-1 and ISO reversed the ISO-induced  $I_{CaL}$  increase (Figure 1A, upper panel), returning  $I_{CaL}$  to approximately basal amplitude (middle panel). This was confirmed by mean data from 11 patients (lower panel). The degree of this anti-adrenergic effect, expressed as the absolute  $I_{CaL}$  reduction by ET-1 from the ISO value as a function of the absolute  $I_{CaL}$  increase by ISO, was  $98\pm 10\%$  with 10 nM ET-1 ( $n=14$  cells, 11 patients). ET-1 at lower concentrations also produced marked and significant anti-adrenergic effects on  $I_{CaL}$ : by  $96\pm 17\%$  at 1 nM ( $n=4$  cells, 2 patients),  $91\pm 22\%$  at 0.1 nM ( $n=4$  cells, 2 patients), and by  $93\pm 26\%$  at 0.01 nM ( $n=4$  cells, 4 patients). By contrast, ET-1 (10 nM) had no significant effect on basal  $I_{CaL}$ , i.e. in the absence of adrenergic stimulation. This is demonstrated in Figure 1B by original  $I_{CaL}$  traces (upper panel), by the lack of change in  $I_{CaL}$  during ET-1 application and washout (middle panel), and by the lack of a significant effect of ET-1 on mean basal  $I_{CaL}$  (lower panel).

### *Endothelin-receptor involvement in L-type $Ca^{2+}$ current response to ET-1.*

The  $ET_A$  antagonist FR139317 (10 nM) partially reversed the anti-adrenergic effect of 10 nM ET-1 on  $I_{CaL}$ . Figure 2A shows that FR139317 produced a gradual increase in  $I_{CaL}$  after the characteristic ET-1 response, in the continued presence of ISO and ET-1. FR139317 exerted a similar effect in each of 6 cells (4 patients), with a significant reversal of the anti-adrenergic effect of ET-1 by  $22\pm 7\%$  ( $P<0.05$ ). Figure 2B shows that FR139317 had no effect on ISO-stimulated  $I_{CaL}$ , and when co-applied prior to ET-1, prevented the anti-adrenergic effect of ET-1. The  $ET_B$  agonist sarafotoxin (S6c) at 10 nM had no effect on basal (non-ISO-stimulated)  $I_{CaL}$  (Figure 2C).  $I_{CaL}$  in the absence and presence of S6c was  $-3.9\pm 1.1$  and  $-3.3\pm 0.8$  pA/pF, respectively ( $P>0.05$ ,  $n=5$  cells, 3 patients). Following S6c washout, ISO stimulated an increase in  $I_{CaL}$  which was unaffected by the re-addition of S6c, but which was reversed by ET-1 in the continued presence of S6c (Figure 2C).

### ***Effects of ISO and ET-1 on action potentials and effective refractory period.***

Isoproterenol at 0.05  $\mu\text{M}$  elevated the action potential plateau, as shown by the original recordings in Figure 3A (upper panel). This was associated with a marked prolongation of early and mid repolarisation, but later repolarisation was unaffected (Figure 3A, upper panel). The action potential duration at 50% repolarisation ( $\text{APD}_{50}$ ) was  $54 \pm 10$  ms in the presence of ISO, significantly longer (by 93%) than in its absence, at  $28 \pm 5$  ms ( $P < 0.05$ ), but neither the  $\text{APD}_{75}$  ( $163 \pm 21$  vs  $154 \pm 13$  ms,  $P > 0.05$ ) nor  $\text{APD}_{90}$  ( $204 \pm 16$  vs  $220 \pm 14$  ms;  $P > 0.05$ ;  $n = 22$  cells, 12 patients) were significantly affected. Endothelin-1 (10 nM) fully reversed the effect of 0.05  $\mu\text{M}$  ISO on the action potential, returning its morphology approximately to the pre-ISO state. This is shown in Figure 3A, upper panel, with confirmatory mean data from 10 patients given in the lower panel. Figure 3B (upper panel) shows that 0.05  $\mu\text{M}$  ISO had no effect on either action potential restitution or the cellular ERP, in line with its lack of effect on  $\text{APD}_{90}$ . These measurements also were unaffected by the combination of 0.05  $\mu\text{M}$  ISO and 10 nM ET-1. The absence of changes in ERP were confirmed by mean data in Figure 3B (lower panel). Figure 4A shows that ET-1 (10 nM) had no significant effect on action potential shape or mean  $\text{APD}_{50}$  in the absence of ISO. Endothelin-1 (10 nM) also had no significant effect, in the absence of ISO, on  $\text{APD}_{75}$  ( $132 \pm 13$  ms in control vs  $142 \pm 13$  ms with ET-1),  $\text{APD}_{90}$  ( $210 \pm 17$  vs  $226 \pm 20$  ms;  $n = 21$  cells, 5 patients), nor action potential maximum upstroke velocity,  $V_{\text{max}}$  ( $198 \pm 23$  vs  $183 \pm 20$  V/s;  $n = 9$  cells, 5 patients;  $P > 0.05$  for each). Furthermore, ET-1 also had no significant effect on basal action potential restitution or the cellular ERP (Figure 4B).

### ***Cellular arrhythmic depolarisations induced by ISO, and anti-adrenergic effect of ET-1.***

Action potentials recorded using a repetitive stimulation protocol in the presence of ISO (0.05  $\mu\text{M}$ ) were frequently interrupted by low amplitude, usually sub-threshold, transient depolarisations. We have termed these “cellular arrhythmic depolarisations” (CADs). Figure 5Ai shows original recordings of phase 4 CADs produced by ISO. The control  $\text{APD}_{50}$  was 24 ms, and this was increased by ISO to 51 ms. Endothelin-1 (10 nM) abolished these ISO-induced CADs, associated with reversal of the plateau elevation ( $\text{APD}_{50}$ : 32 ms). Figure 5Aii shows action potentials with a control  $\text{APD}_{50}$  of above average value (67 ms at steady-state). In this cell, ISO produced relatively large amplitude CADs, associated with a relatively large increase in  $\text{APD}_{50}$  compared with that of Figure 5Ai. Many, but not all of these CADs occurred during phase 3: a single CAD reached threshold just prior to the 6th driven beat (Figure 5Aii). Each of these CADs was abolished by ET-1 (Figure 5Aii), again with an associated anti-adrenergic effect on early repolarisation. Figure 5Aiii shows the



typical response to ET-1 in the absence of ISO, i.e. lack of production of CADs. Figure 5B shows that ISO produced CADs in a significant number of cells (panel i) and that, in cells in which ISO and ET-1 were co-applied, ET-1 abolished ISO-induced CADs (panel ii). By contrast, ET-1 alone had no significant effect on the incidence of CADs (panel iii).

***Effects of  $\beta$ -blocker therapy on basal electrophysiology and responses to ISO and ET-1.***

The atrial cell basal ERP, i.e. in the absence of ISO, was significantly longer in a group of patients treated with a  $\beta$ -blocker for  $\geq 7$  days pre-operatively ( $221 \pm 9$  ms; 50 cells, 13 patients), than in a group of non- $\beta$ -blocked patients ( $184 \pm 15$  ms; 22 cells, 7 patients;  $P < 0.05$ ). However, there were no significant differences between these patient groups in the basal electrophysiological measurements which were shown subsequently to be affected by either ISO or ET-1. The basal peak  $I_{CaL}$  density and the basal  $I_{CaL}$  voltage-dependency were similar in the two patient groups (Figure 6, panels A and B). The basal  $APD_{50}$  also was not significantly affected (panel C). Furthermore, the magnitude of neither of the observed significant effects of  $0.05 \mu\text{M}$  ISO, i.e. to increase peak  $I_{CaL}$  and  $APD_{50}$ , was significantly different between these patient groups (panels D and E, respectively). Finally, there was no significant difference between  $\beta$ -blocked and non- $\beta$ -blocked patient groups in the subsequent ET-1 ( $10 \text{ nM}$ )-induced reduction in  $APD_{50}$  (panel F).

## Discussion.

The effects of ET-1 on action potentials were studied for the first time to our knowledge in human atrium. Endothelin-1 exerted a marked anti-adrenergic action, abolishing the prolongation of APD produced by ISO. By contrast, ET-1 had no significant basal action, i.e. in the absence of adrenergic stimulation. The effects of ET-1 on action potentials have been studied only rarely in the atrium of any species [23-25]. Anti-adrenergic effects have not been investigated and the basal responses are equivocal [23-25]. Endothelin-1 raised the plateau of “slow response action potentials” in guinea-pig atrium [23]. It also shortened the APD in guinea-pig [24] and canine [25] atria. In the ventricle, by contrast, numerous studies, particularly canine have demonstrated an APD prolonging action of ET-1 [4-6,26].

In the present study, ET-1 abolished atrial cellular arrhythmic depolarisations (CADs) provoked by ISO during action potential trains. We studied single cells, so this can be considered to be a direct electrophysiological effect of ET-1, not secondary to, for example, coronary vasoconstriction. Endothelin-1 did not produce significant numbers of CADs in the absence of adrenergic stimulation, even at a  $10^3$ -fold higher concentration than was anti-adrenergic on  $I_{CaL}$ . By contrast, ET-1 induced arrhythmic contractions in human atrial tissues [9,11]. Atrial contraction was not measured in the present study, and ET-1 exerts various contractile effects not requiring electrophysiological changes [1,2]. These may include enhanced  $Ca^{2+}$ -induced  $Ca^{2+}$  release (CICR) by  $IP_3$  activation, phosphorylation of myosin light chains, and enhancement of myofilament  $Ca^{2+}$  responsiveness. Some may account, at least in part, for the apparent discrepancy between the effect of ET-1 to induce arrhythmic contractions but not to produce CADs. Methodological differences between studies may contribute also, e.g. with blockade of  $\beta_1$  and  $\beta_2$  adrenoceptors in both contraction studies [9,11]. Furthermore, arrhythmic contractions induced by ET-1 are not mediated by  $ET_A$  receptors [11]. Since effects of ET-1 on  $I_{CaL}$  were mediated by  $ET_A$ , in the present and other studies [13,14], the arrhythmic contractions may be largely independent of  $I_{CaL}$ . In support, effects of ET-1 on atrial contractility were shown to not require an involvement of  $I_{CaL}$ , even though its involvement could also be demonstrated [27]. Endothelin-1 elevated  $(Ca^{2+})_i$  concentration,  $Ca^{2+}$  transient amplitude and spark frequency in rat, cat and mouse atria, via  $IP_3$  activation [7,8,10]. It also induced diastolic premature  $Ca^{2+}$  transients [7] and facilitated arrhythmogenic  $Ca^{2+}$  waves [8]. In one of these studies [7], premature  $Ca^{2+}$  transients were accompanied by action potentials. These effects of ET-1 on  $(Ca^{2+})_i$  could exert an arrhythmogenic influence in the atria of patients. Such extrapolation should be made with caution, however. The atrial studies [7,8,10] were not on human tissues, and the lowest concentration of ET-1 used in each (100 nM) was  $\sim 10^4$ -fold

greater than the highest levels reported in patients (6-12 pM) [20,28]. In the studies showing pro-arrhythmic effects of ET-1 on contractility [9,11] and  $(Ca^{2+})_i$  handling [7,8,10], the atrial tissues were not under adrenergic stimulation. However, ET-1 produced marked negative inotropism in human atrium in the presence of catecholamines [29]. Therefore, ET-1 exerts marked anti-adrenergic effects on atrial contractility, as well as on CADs.

The CADs produced by catecholamine in this study occurred in cells with a relatively marked prolongation of the APD. This is consistent with the reported association between APD lengthening and the development of afterdepolarisations [17]. Many of these CADs displayed characteristics of abnormal automaticity, and possibly EADs or DADs. Their suppression by ET-1 could, therefore, contribute an inhibitory influence on non re-entrant mechanisms of AF. Consistent with that, intracoronary ET-1 prevented AF produced by intravenous ISO in dogs [5]. Furthermore, ET-1 lacked effect on either the cellular ERP or the action potential  $V_{max}$ . This suggests that ET-1 may not alter the minimum path length for re-entry, and therefore not promote (or inhibit) re-entrant AF. The anti-adrenergic effects of ET-1 were observed at extremely low, clinically relevant concentrations (down to 10 pM), despite near-maximal adrenergic stimulation. They might be expected to occur also, therefore, in patients with CHF whose catecholamine levels are elevated [20]. Nevertheless, extrapolation should be made with caution, since it is presently unknown whether such effects of ET-1 would be preserved in patients with AF or CHF. Of note, the modulation of  $I_{CaL}$  by PKC, which is involved in ET-1 signalling [30,31], was impaired in chronic AF [32]. It is also unclear whether AF causes ET-1 elevation in the absence of CHF or other myocardial diseases [33]. Furthermore, the chronic effects of ET-1, rather than the relatively acute effects studied here and elsewhere, are presently unknown. These could include, for example, a long term adaptive response akin to “pharmacological remodelling” [22].

Afterdepolarisations are affected by changes in  $I_{CaL}$  [17]. In the present study, this current was reduced markedly by ET-1 when adrenergically stimulated, whilst non-adrenergically stimulated  $I_{CaL}$  was unaffected. We limited cytosolic dialysis, and thus efflux of intracellular messengers required for ET-1 signalling, by using the perforated patch configuration. In a study using ruptured patch, ET-1 reduced basal  $I_{CaL}$  in human atrium [13]. However, in line with the present data, it had no effect on basal  $I_{CaL}$  when intracellular GTP was present [13]. In another study, ET-1 either increased or decreased human atrial basal  $I_{CaL}$ , depending on the pre-ET-1  $I_{CaL}$  density, again using ruptured patch [14]. In the atria of other species, either an absence of effect of ET-1 on basal  $I_{CaL}$  [12,34] or a decrease [24] have been demonstrated. However, no reports of  $I_{CaL}$

increase by ET-1 were found in atrium. The effects of ET-1 on basal  $I_{CaL}$  in the ventricles have been studied more widely. These include a decrease [26,35], no change [15,36] or an increase [37,38]. In each of these atrial and ventricular studies in which basal and anti-adrenergic effects of ET-1 were compared [12-15,34,36,37], the most consistent and predominant effect was an anti-adrenergic reduction in  $I_{CaL}$ . This has been shown to involve cAMP and  $G_i$  protein in human atrial cells [14]. The present conformity of effects of ISO, ET-1 and their combination on  $I_{CaL}$ , APD and CADs suggests that CAD abolition by ET-1 involved the anti-adrenergic reduction in  $I_{CaL}$ . Consistent with this, an  $I_{CaL}$  agonist produced APD lengthening and EADs in canine myocardial bundle branch, and each effect was abolished by an  $I_{CaL}$  blocker [4]. The reduction in  $I_{CaL}$  by ET-1 also may involve  $(Ca^{2+})_i$ -induced inactivation of this current, via enhanced CICR [9,10]. Furthermore, a  $Cl^-$  current activated by ISO and cAMP was inhibited by ET-1 in human atrial cells [39]. However, this current was present in ~20% of cells and required 30 nM ET-1 to affect. Endothelin-1 increased the human atrial  $Na^+$  current ( $I_{Na}$ ) [13]. This was measured using low extracellular  $Na^+$  concentration ( $[Na^+]_o$ ) at 22°C. However, we and others [6,26] found no significant effect of ET-1 on action potential  $V_{max}$ , a reliable indicator of  $I_{Na}$ , at physiological  $[Na^+]_o$  and temperature. Nevertheless, effects of ET-1, either basal or anti-adrenergic, on currents additional to  $I_{CaL}$  cannot be excluded.

Chronic treatment of patients with a  $\beta$ -blocker was associated with a prolonged atrial cellular ERP, in the absence of adrenergic stimulation. This is considered to be a component of “pharmacological remodelling”, since the  $\beta$ -blocker is presumed to be removed by the repeated washing of cells during their isolation [22]. The basal  $APD_{50}$  and  $I_{CaL}$  were unchanged by chronic  $\beta$ -blockade, also consistent with the earlier study [22]. In the present study, chronic  $\beta$ -blockade did not influence the effects of ISO or the anti-adrenergic effects of ET-1 on these measurements. Such patient treatment has been associated with potentiation of atrial arrhythmic contractions by  $\beta_2$ -adrenoceptor stimulation [21] and by ET-1 [11], though anti-adrenergic actions of ET-1 were not investigated [11]. The present data are in line with a reported lack of influence of chronic  $\beta$ -blockade on human atrial  $I_{CaL}$  responses to ET-1 [14]. The concentrations of ISO and ET-1 we used indicate that the  $E_{max}$  of the electrophysiological responses was unaffected by chronic  $\beta$ -blockade. However, the possibility that the potency of these responses may be affected, cannot be excluded.

The present data suggest that ET-1 may contribute a potentially anti-arrhythmic electrophysiological influence in human atrium in the presence of adrenergic stimulation. This influence may not be affected by the treatment of patients with  $\beta$ -blockers. It should be considered alongside the potentially pro-arrhythmic

influences of ET-1 on  $(Ca^{2+})_i$  handling and myocardial contractility in the absence of adrenergic stimulation. Elevation of ET-1 is an adverse prognostic marker in CHF [40]. However, ET-receptor antagonists do not reduce mortality, despite their short term haemodynamic benefit, in patients with CHF [41]. It is conceivable that potentially beneficial electrophysiological influences of ET-1 might be blunted by the blockade of cardiac ET-receptors.

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## References.

- [1] Russell FD, Molenaar P. The human heart endothelin system: ET-1 synthesis, storage, release and effect. *Trends Pharmacol Sci* 2000; 21: 353-359.
- [2] Sugden PH. An overview of endothelin signaling in the cardiac myocyte. *J Mol Cell Cardiol* 2003; 35: 871-886.
- [3] Wainwright CL, McCabe C, Kane KA. Endothelin and the ischaemic heart. *Curr Vasc Pharmacol* 2005; 3: 333-341.
- [4] Yorikane R, Shiga H, Miyake S, Koike H. Evidence for direct arrhythmogenic action of endothelin. *Biochem Biophys Res Comm* 1990; 173: 457-462.
- [5] Kiss O, Zima E, Soos P, Kekesi V, Juhasz-Nagy A, Merkely B. Intracoronary endothelin-1 infusion combined with systemic isoproterenol treatment: antagonistic arrhythmogenic effects. *Life Sci* 2004; 75: 537-548.
- [6] Geller L, Szabo T, Kiss O, Solti F, Juhasz-Nagy A, Merkely B. Fundamental electrophysiological differences between low-dose intracoronary endothelin-1 infusion and myocardial ischemia revealed by multiple monophasic action potential recording. *J Cardiovasc Pharmacol* 2000; 36 (Suppl 1): S167-S171.
- [7] Mackenzie L, Bootman MD, Laine M, Berridge MJ, Thuring J, Holmes A, Li W-H, Lipp P. The role of inositol 1,4,5-trisphosphate receptors in  $Ca^{2+}$  signalling and the generation of arrhythmias in rat atrial myocytes. *J Physiol* 2002; 541: 395-409.
- [8] Zima AV, Blatter LA. Inositol-1,4,5-trisphosphate-dependent  $Ca^{2+}$  signalling in cat atrial excitation-contraction coupling and arrhythmias. *J Physiol* 2004; 555: 607-615.
- [9] Kockskamper J, Sigirci E, Rubertus S-U, Pieske B. Endothelin-1 elicits arrhythmias in human atrium via modulation of intracellular calcium release. *Circulation* 2004; 110: III-163 (Abstract).
- [10] Li X, Zima AV, Sheikh F, Blatter LA, Chen J. Endothelin-1-induced arrhythmogenic  $Ca^{2+}$  signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate ( $IP_3$ )-receptor type 2-deficient mice. *Circ Res* 2005; 96: 1274-1281.

- [11] Burrell KM, Molenaar P, Dawson PJ, Kaumann AJ. Contractile and arrhythmic effects of endothelin receptor agonists in human heart in vitro: blockade with SB 209670. *J Pharmacol Exp Ther* 2000; 292: 449-459.
- [12] Delpéch N, Soustre H, Potreau D. Antagonism of  $\beta$ -adrenergic stimulation of L-type  $\text{Ca}^{2+}$  current by endothelin in guinea-pig atrial cells. *Eur J Pharmacol* 1995; 285: 217-220.
- [13] Cheng TH, Chang CY, Wei J, Lin CI. Effects of endothelin 1 on calcium and sodium currents in isolated human cardiac myocytes. *Can J Physiol Pharmacol* 1995; 73: 1774-1783.
- [14] Boixel C, Dinanian S, Lang-Lazdunski L, Mercadier JJ, Hatem SN. Characterization of effects of endothelin-1 on the L-type  $\text{Ca}^{2+}$  current in human atrial myocytes. *Am J Physiol* 2001; 281: H764-H773.
- [15] Watanabe T, Endoh M. Antiadrenergic effects of endothelin-1 on the L-type  $\text{Ca}^{2+}$  current in dog ventricular myocytes. *J Cardiovasc Pharmacol* 2000; 36: 344-350.
- [16] Workman AJ, Kane KA, Rankin AC. The contribution of ionic currents to changes in refractoriness of human atrial myocytes associated with chronic atrial fibrillation. *Cardiovasc Res* 2001; 52: 226-235.
- [17] Fozzard HA. Afterdepolarizations and triggered activity. *Basic Res Cardiol* 1992; 87 (Suppl 2): 105-113.
- [18] Tuinenburg AE, Van Veldhuisen DJ, Boomsma F, Van Den Berg MP, De Kam PJ, Crijns HJGM. Comparison of plasma neurohormones in congestive heart failure patients with atrial fibrillation versus patients with sinus rhythm. *Am J Cardiol* 1998; 81: 1207-1210.
- [19] Masson S, Gorini M, Salio M, Lucci D, Latini R, Maggioni AP. Clinical correlates of elevated plasma natriuretic peptides and Big endothelin-1 in a population of ambulatory patients with heart failure. A substudy of the Italian Network on Congestive Heart Failure (IN-CHF) registry. *Ital Heart J* 2000; 1: 282-288.
- [20] Bolger AP, Sharma R, Li W, Leenarts M, Kalra PR, Kemp M, Coats AJS, Anker SD, Gatzoulis MA. Neurohormonal activation and the chronic heart failure syndrome in adults with congenital heart disease. *Circulation* 2002; 106: 92-99.

- [21] Hall JA, Kaumann AJ, Brown MJ. Selective  $\beta_1$ -adrenoceptor blockade enhances positive inotropic responses to endogenous catecholamines mediated through  $\beta_2$ -adrenoceptors in human atrial myocardium. *Circ Res* 1990; 66: 1610-1623.
- [22] Workman AJ, Kane KA, Russell JA, Norrie J, Rankin AC. Chronic beta-adrenoceptor blockade and human atrial cell electrophysiology: evidence of pharmacological remodelling. *Cardiovasc Res* 2003; 58: 518-525.
- [23] Ishikawa T, Yanagisawa M, Kimura S, Goto K, Masaki T. Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria. *Am J Physiol* 1988; 255: H970-H973.
- [24] Ono K, Tsujimoto G, Sakamoto A, Eto K, Masaki T, Ozaki Y, Satake M. Endothelin-A receptor mediates cardiac inhibition by regulating calcium and potassium currents. *Nature* 1994; 370: 301-304.
- [25] Yorikane R, Koike H, Miyake S. Electrophysiological effects of endothelin-1 on canine myocardial cells. *J Cardiovasc Pharmacol* 1991; 17 (Suppl 7): S159-S162.
- [26] Magyar J, Iost N, Kortvely A, Banyasz T, Virag L, Szigligeti P, Varro A, Opincariu M, Szecsi J, Papp JG, Nanasi PP. Effects of endothelin-1 on calcium and potassium currents in undiseased human ventricular myocytes. *Pflugers Arch* 2000; 441: 144-149.
- [27] Vigne P, Breittmayer JP, Marsault R, Frelin C. Endothelin mobilizes  $Ca^{2+}$  from a caffeine- and ryanodine-insensitive intracellular pool in rat atrial cells. *J Biol Chem* 1990; 265: 6782-6787.
- [28] McMurray JJ, Ray SG, Abdullah I, Dargie HJ, Morton JJ. Plasma endothelin in chronic heart failure. *Circulation* 1992; 85: 1374-1379.
- [29] Saetrum Opgaard O, Moller S, de Vries R, Edvinsson L, Saxena PR. Positive inotropic responses mediated by endothelin  $ET_A$  and  $ET_B$  receptors in human myocardial trabeculae. *Clin Sci* 2000; 99: 161-168.
- [30] Woo SH, Lee CO. Effects of endothelin-1 on  $Ca^{2+}$  signaling in guinea-pig ventricular myocytes: role of protein kinase C. *J Mol Cell Cardiol* 1999; 31: 631-643.
- [31] Kilts JD, Grocott HP, Kwatra MM.  $G\alpha_q$ -coupled receptors in human atrium function through protein kinase C  $\epsilon$  and  $\delta$ . *J Mol Cell Cardiol* 2005; 38: 267-276.



- [32] Christ T, Boknik P, Wohrl S, Wettwer E, Graf EM, Bosch RF, Knaut M, Schmitz W, Ravens U, Dobrev D. L-type  $\text{Ca}^{2+}$  current downregulation in chronic human atrial fibrillation is associated with increased activity of protein phosphatases. *Circulation* 2004; 110: 2651-2657.
- [33] Chen M-C, Wu C-J, Yip H-K, Chang H-W, Chen C-J, Yu T-H, Hung WC. Increased circulating endothelin-1 in rheumatic mitral stenosis. Irrelevance to left atrial and pulmonary artery pressures. *Chest* 2004; 125: 390-396.
- [34] Delpech N, Soustre H, Potreau D. Endothelin-1 inhibits L-type  $\text{Ca}^{2+}$  current enhanced by isoprenaline in rat atrial myocytes. *J Cardiovasc Pharmacol* 1997; 29: 136-143.
- [35] Tohse N, Hattori Y, Nakaya H, Endou M, Kanno M. Inability of endothelin to increase  $\text{Ca}^{2+}$  current in guinea-pig heart cells. *Br J Pharmacol* 1990; 99: 437-438.
- [36] Thomas GP, Sims SM, Karmazyn M. Differential effects of endothelin-1 on basal and isoprenaline-enhanced  $\text{Ca}^{2+}$  current in guinea-pig ventricular myocytes. *J Physiol* 1997; 503: 55-65.
- [37] Watanabe T, Endoh M. Characterization of the endothelin-1-induced regulation of L-type  $\text{Ca}^{2+}$  current in rabbit ventricular myocytes. *Naunyn-Schmiedeberg's Arch Pharmacol* 1999; 360: 654-664.
- [38] Kelso E, Spiers P, McDermott B, Scholfield N, Silke B. Dual effects of endothelin-1 on the L-type  $\text{Ca}^{2+}$  current in ventricular cardiomyocytes. *Eur J Pharmacol* 1996; 308: 351-355.
- [39] Tsai CS, Cheng TH, Lin CI, Chen JJ, Lee FY, Li CY, Hong HJ, Loh SH. Inhibitory effect of endothelin-1 on the isoproterenol-induced chloride current in human cardiac myocytes. *Eur J Pharmacol* 2001; 424: 97-105.
- [40] Pousset F, Isnard R, Lechat P, Kalotka H, Carayon A, Maistre G, Escolano S, Thomas D, Komajda M. Prognostic value of plasma endothelin-1 in patients with chronic heart failure. *Eur Heart J* 1997; 18: 254-258.
- [41] Kirchengast M, Luz M. Endothelin receptor antagonists: clinical realities and future directions. *J Cardiovasc Pharmacol* 2005; 45: 182-191.

## Table and Figure legends.

### Table 1. Patients' characteristics.

Values are numbers of patients (*n*, % of total) with selected clinical characteristics, except for age (mean±SE). CCB=calcium channel blocker, CABG=coronary artery bypass graft surgery, AVR=aortic valve replacement, LVD=left ventricular dysfunction, MI=myocardial infarction.

### Figure 1.

#### Anti-adrenergic effect of endothelin-1 on human atrial L-type Ca<sup>2+</sup> current.

Comparison of effect of 10 nM ET-1 (striped symbols) on peak I<sub>CaL</sub> (at +10 mV) in the presence, A, and absence, B, of 0.05 μM ISO (filled symbols), relative to basal (non-ISO-stimulated) I<sub>CaL</sub> (open symbols). Upper panels: original, superimposed I<sub>CaL</sub> recordings, with chronological order labelled, from single atrial cells. Dotted lines=zero pA. Calibration applies to both panels. Middle panels: time course of change in I<sub>CaL</sub> by drugs, represented by horizontal bars (*n*=1 cell/panel). Lower panels: histograms of mean±SE I<sub>CaL</sub>. Data are paired: 14 cells, 11 patients in A; 5 cells, 3 patients in B. Asterisks=*P*<0.05; NS=not significant.

### Figure 2.

#### Effects of ET-receptor antagonist and agonist on I<sub>CaL</sub> response to ET-1.

Time course of change in peak I<sub>CaL</sub> by application (horizontal bars) of an ET<sub>A</sub> antagonist, FR139317, 10 nM (FR, ▨), an ET<sub>B</sub> agonist, sarafotoxin, 10 nM (S6c, ▩), 10 nM ET-1 (▧) and/or 0.05 μM ISO (■). *n*=1 cell/panel. A, Partial reversal by FR139317 of anti-adrenergic effect of ET-1 on I<sub>CaL</sub>. B, Prevention by FR139317 of the ET-1 anti-adrenergic effect, and lack of effect of FR139317 on ISO-induced I<sub>CaL</sub> increase. C, Lack of effect of S6c on basal or ISO-stimulated I<sub>CaL</sub>, or on anti-adrenergic action of ET-1.

### Figure 3.

#### Effects of ISO and ET-1 on action potentials and effective refractory period.

A, Action potential waveform and duration at 50% repolarisation (APD<sub>50</sub>) and B, Restitution and effective refractory period (ERP) in atrial cells, in the absence of a drug (○), following 0.05 μM ISO (●) and subsequent co-application of 10 nM ET-1 (⊗). Upper panels: original, superimposed action potentials, from the same cell, produced by (A) the 7th of a train of conditioning current pulses (75 beats/min) and (B) the 7th and 8th conditioning pulses followed by progressively premature test pulses. Horizontal bars=cell ERP. Lower panels: histogram data; paired means (*n*=15 cells, 10 patients).

#### Figure 4.

##### Lack of effect of ET-1 on basal action potential repolarisation and ERP.

A, Action potential waveform and mean APD<sub>50</sub>, and B, Restitution and mean ERP, in absence of a drug (○) and following 10 nM ET-1 (⊗). Upper panels: superimposed responses to (A) 7th conditioning pulse and (B) in a different cell, to the 7th and 8th conditioning pulses followed by premature test pulses. Horizontal bars=ERP. Lower panels: paired means of APD<sub>50</sub> (21 cells, 5 patients) and ERP (16 cells, 5 patients).

#### Figure 5.

##### Cellular arrhythmic depolarisations induced by ISO, and abolished by ET-1.

A, Original action potentials (APs) evoked by trains of current pulses (75 beats/min) in the absence of a drug (○), with 0.05 μM ISO (●) and subsequent 10 nM ET-1 (⊗) in (i) a cell with a normal AP plateau, and (ii) a cell with relatively high plateau. Arrows indicate “cellular arrhythmic depolarisations” (CADs): any AP phase 3 transient depolarisation or phase 4 depolarisation >3 mV. Dots indicate driven beats. iii, typical absence of response to ET-1 without ISO. All initial APs are post-rest. B, Histograms showing (i) significant production of CADs by ISO (■), (ii) abolition of CADs by ET-1, (iii) lack of significant (NS) CAD production by ET-1 (▨). Numbers within columns=*n* cells displaying CADs/total studied.

#### Figure 6.

##### Lack of effect of chronic β-blocker therapy on atrial electrophysiological responses to ISO and ET-1.

Comparison of L-type Ca<sup>2+</sup> current (I<sub>CaL</sub>), action potential duration at 50% repolarisation (APD<sub>50</sub>) and their responses to isoproterenol at 0.05 μM (ISO) and endothelin-1 at 10 nM (ET-1), between patients treated ≥7 days with a β-blocker (▨&▲; “+BB”) and those not treated with a β-blocker (□&○; “-BB”). A, Basal (i.e. in absence of a drug) peak I<sub>CaL</sub>. B, Basal I<sub>CaL</sub> voltage-dependency, with I<sub>CaL</sub> expressed at each voltage as a function of peak current. C, Basal APD<sub>50</sub>. D, Absolute increase in I<sub>CaL</sub> produced by ISO. E, Absolute increase in APD<sub>50</sub> produced by ISO. F, Absolute reduction in APD<sub>50</sub> produced by ET-1 in the presence of ISO.

Values are means±SE. NS=not significant. Group sizes (c=cells, p=patients): A, +BB: 114c, 34p, -BB: 52c, 13p; B, +BB: 62c, 21p, -BB: 22c, 7p; C, +BB: 55c, 13p, -BB: 23c, 7p; D, +BB: 61c, 25p, -BB: 18c, 9p; E, +BB, -BB: 11c, 6p each; F, +BB: 7c, 5p, -BB: 8c, 5p.

**Table 1.**

		<i>n</i>	<i>%</i>
Patient details	Male/female	52/14	79/21
	Age (years)	64±1	-
Drug treatments	Lipid lowering	57	86
	β-blocker	48	73
	Nitrate	40	61
	ACE inhibitor	32	48
	CCB	21	32
	Diuretic	20	30
Operation type	CABG	59	89
	AVR	6	9
	CABG+AVR	1	2
LV function	Normal	33	50
	Mild/moderate LVD	28	42
	Severe LVD	4	6
Disease	Angina	61	92
	Hyperlipidaemia	53	80
	Hypertension	38	58
	Previous MI	33	50
	Diabetes	7	11

Figure 1.

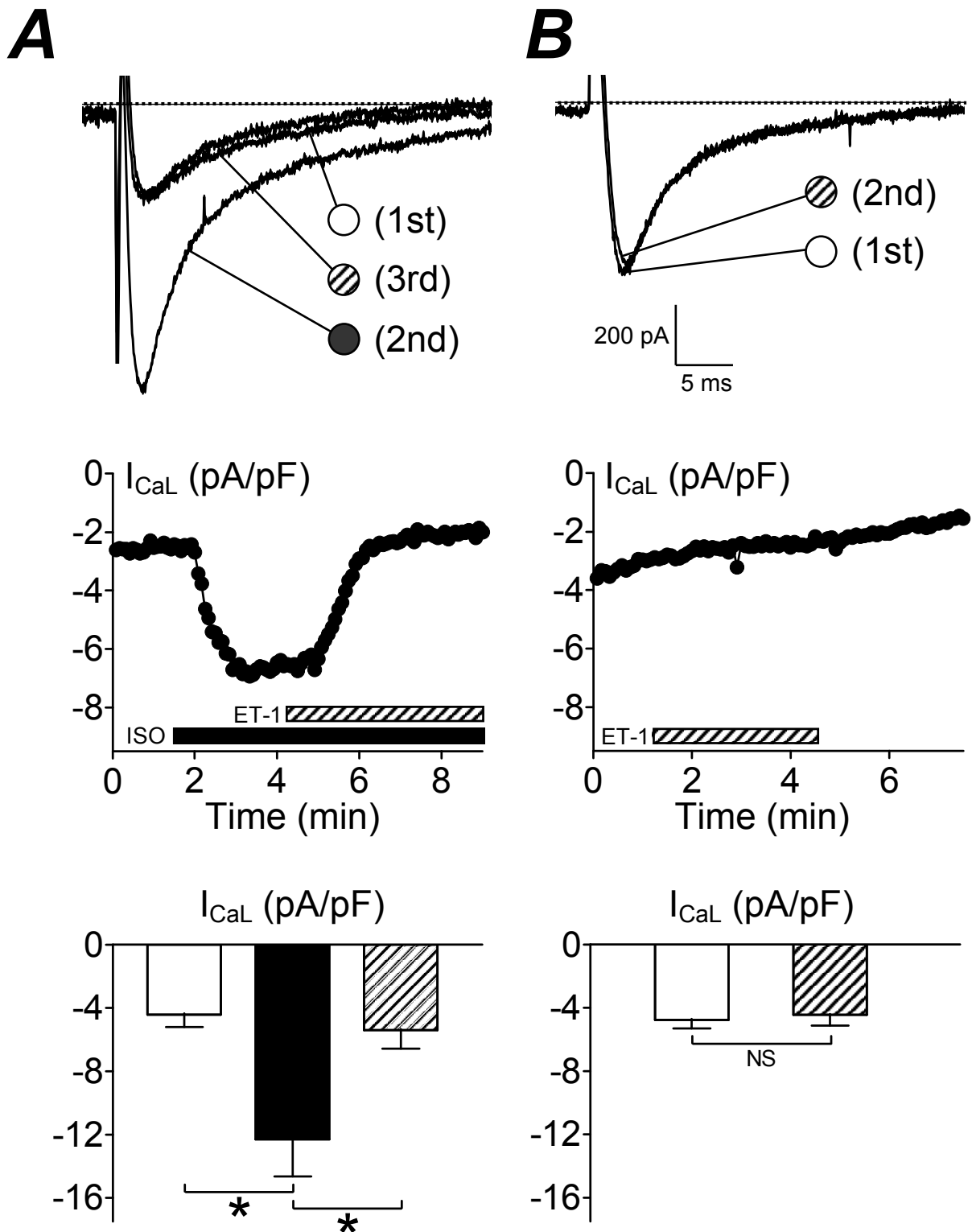


Figure 2.

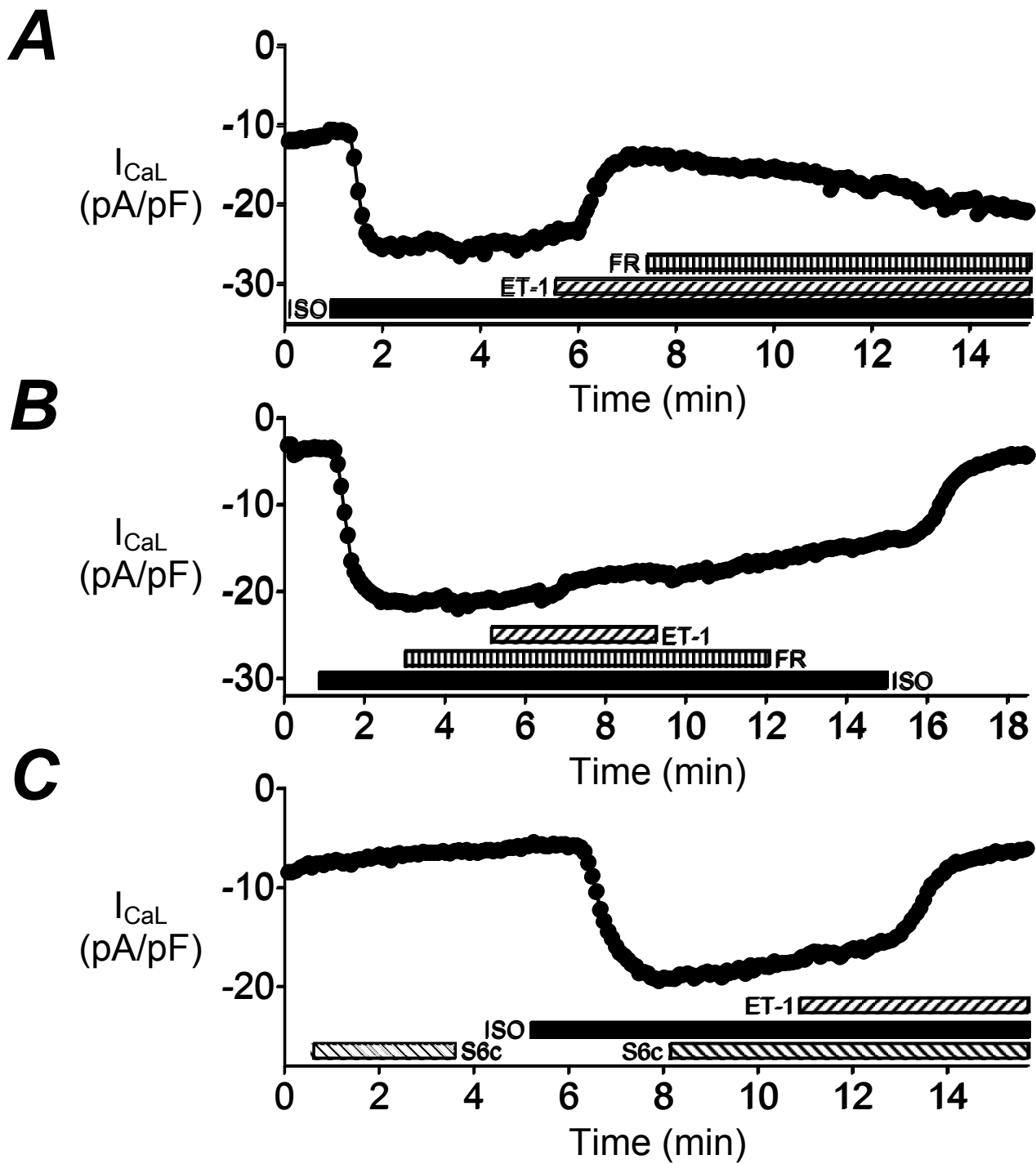


Figure 3.

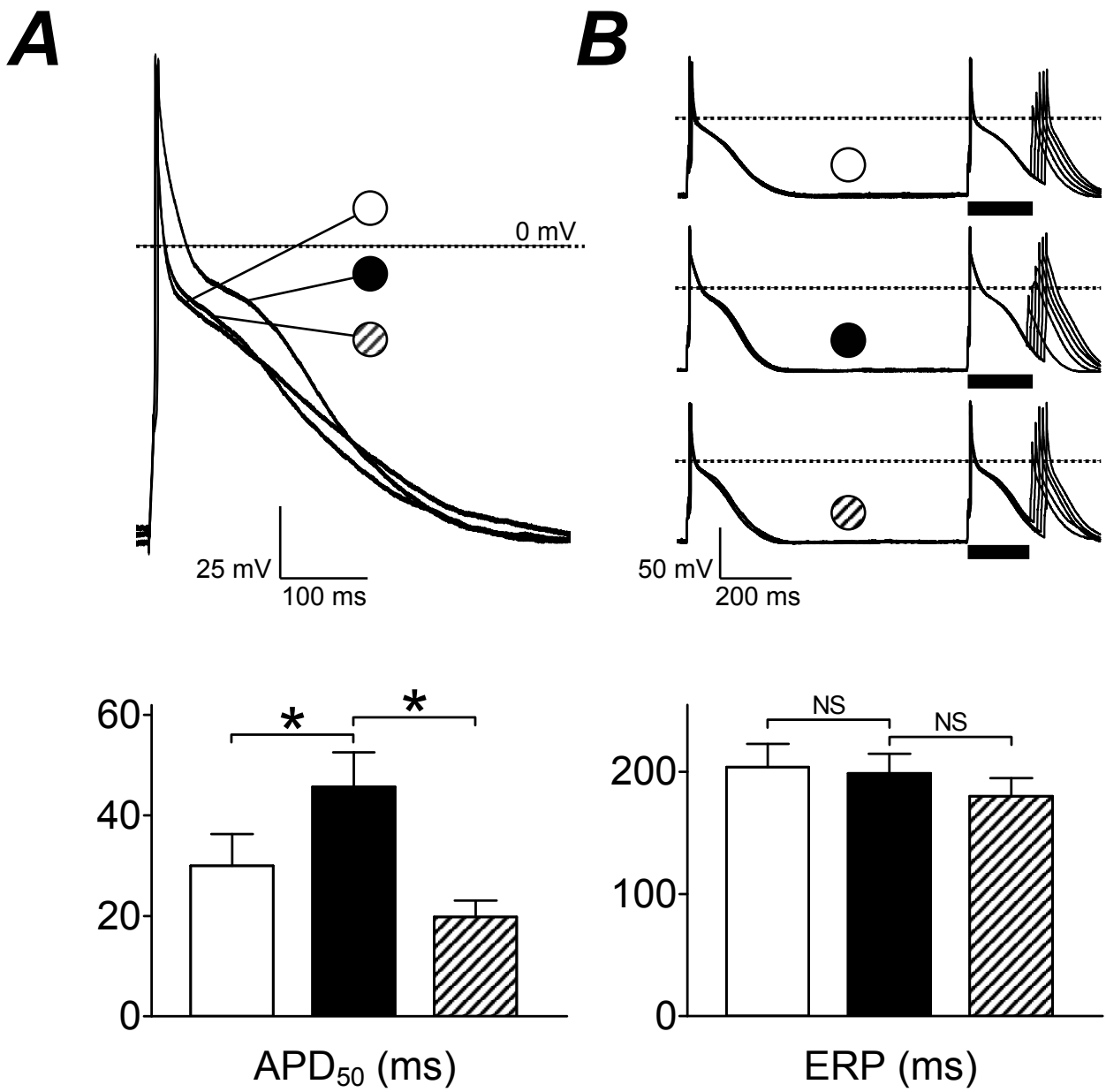


Figure 4.

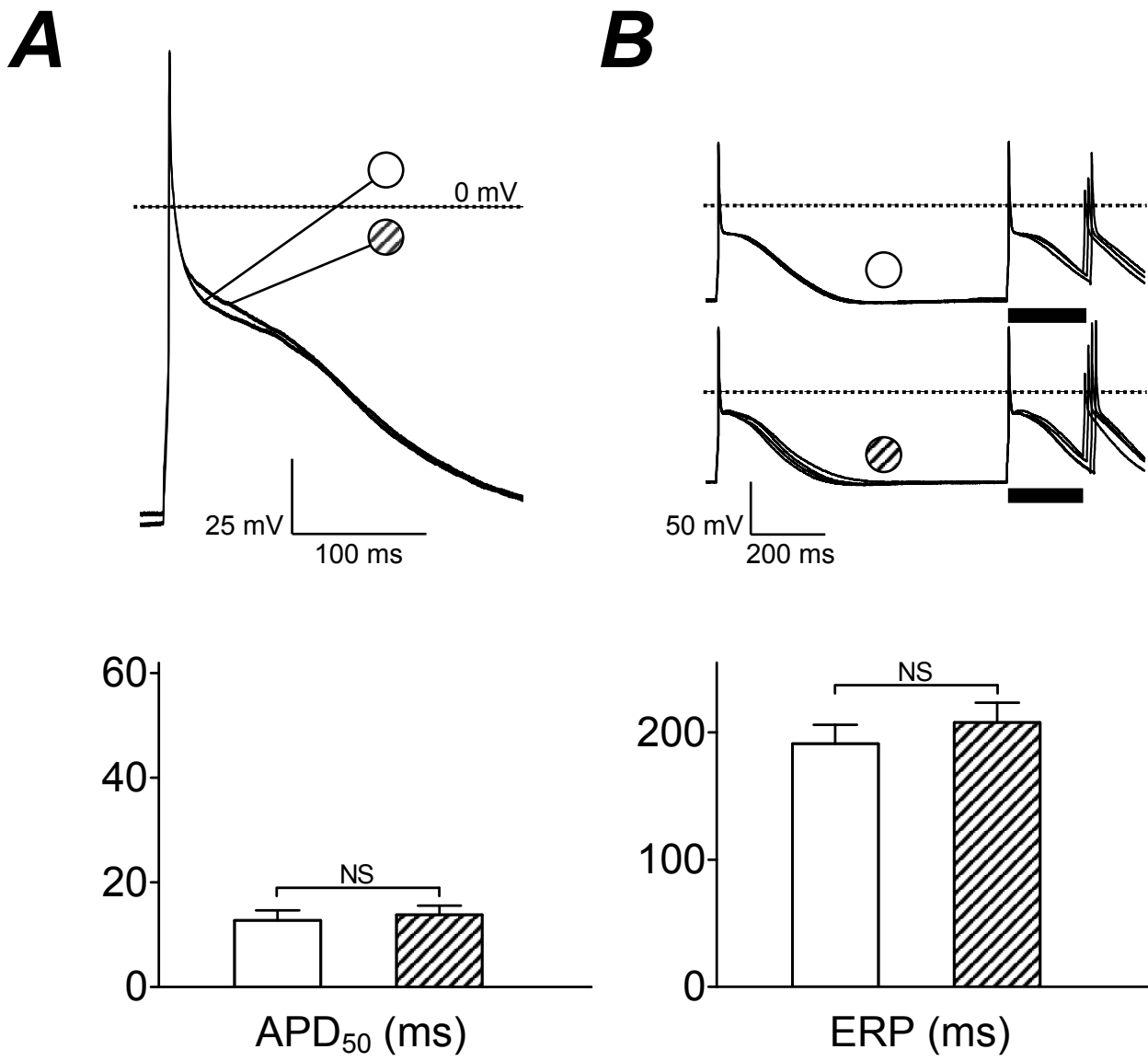
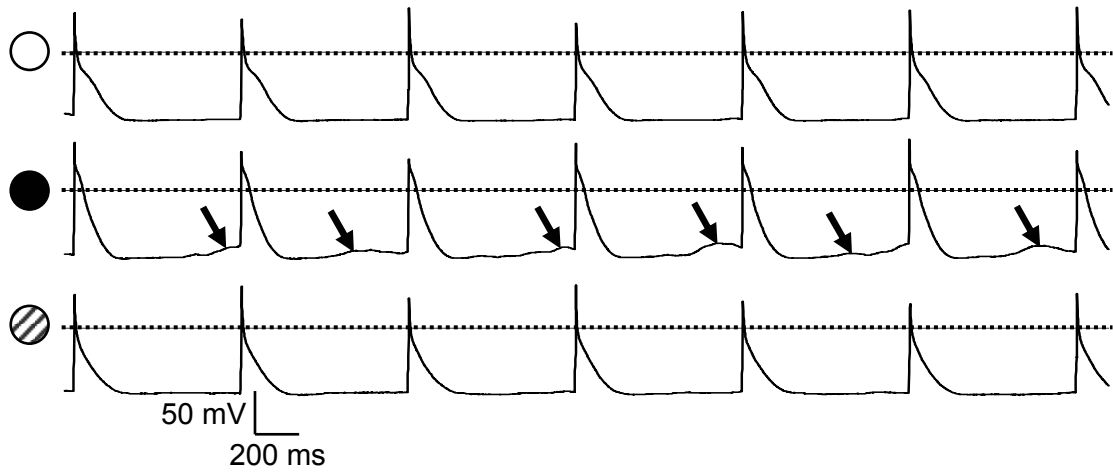


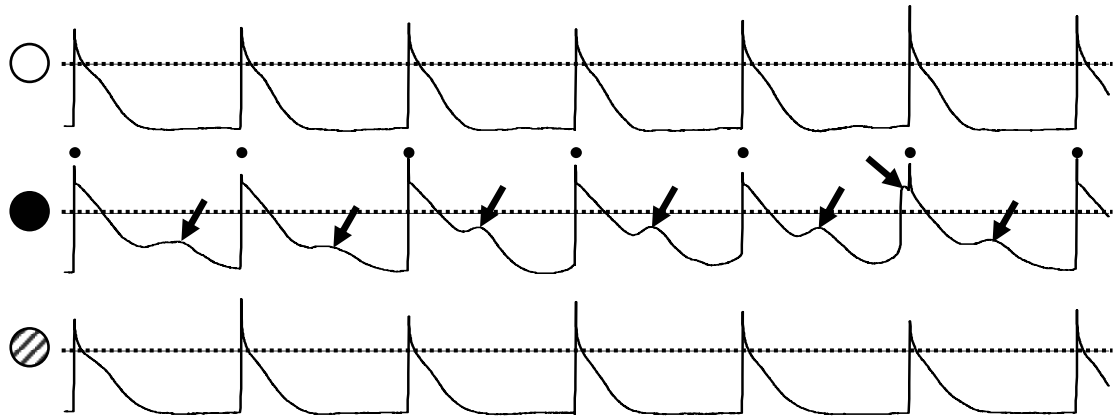


Figure 5.

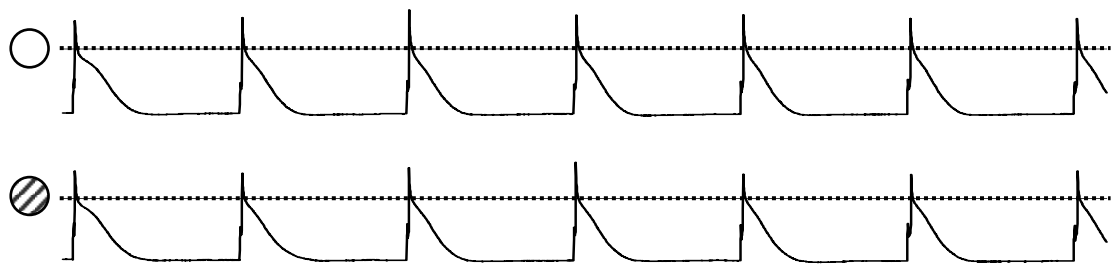
**Ai**



**ii**



**iii**



**Bi**

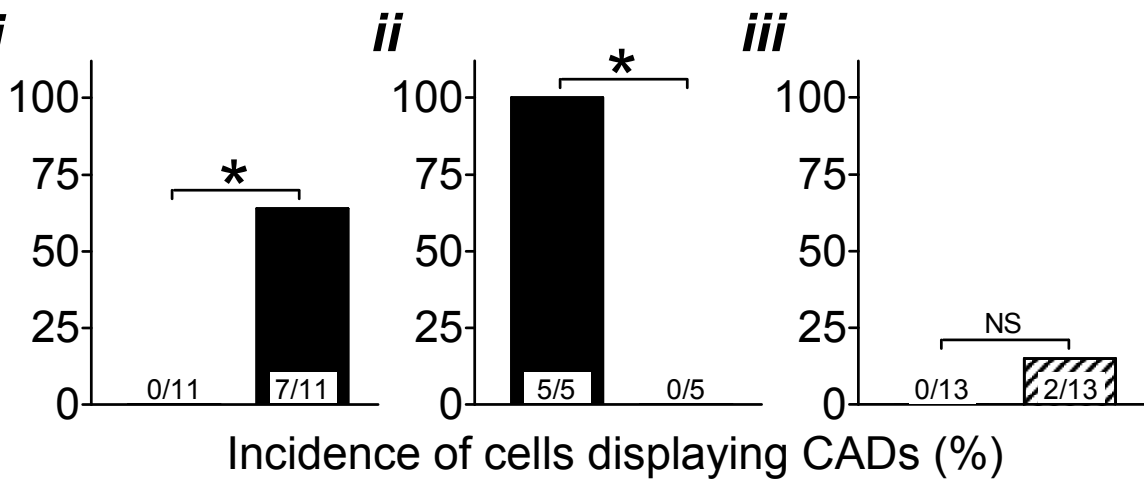


Figure 6.

