Adrenoceptor sub-type involvement in Ca^{2+} current stimulation by noradrenaline in human and rabbit atrial myocytes

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Abstract
Atrial fibrillation (AF) from elevated adrenergic activity may involve increased atrial L-type Ca^{2+} current (ICaL) by noradrenaline (NA). However, the contribution of the adrenoceptor (AR) sub-types to such ICaL-increase is poorly understood, particularly in human. We therefore investigated effects of various broad-action and sub-type-specific α- and β-AR antagonists on NA-stimulated atrial ICaL. ICaL was recorded by whole-cell-patch clamp at 37 °C in myocytes isolated enzymatically from atrial tissues from consenting patients undergoing elective cardiac surgery and from rabbits. NA markedly increased human atrial ICaL, maximally by ~2.5-fold, with EC_{75} 310 nM. Propranolol (β_{1} + β_{2}-AR antagonist, 0.2 microM) substantially decreased NA (310 nM)-stimulated ICaL, in human and rabbit. Phentolamine (α_{1} + α_{2}-AR antagonist, 1 microM) also decreased NA-stimulated ICaL. CGP20712A (β_{1}-AR antagonist, 0.3 microM) and prazosin (α_{1}-AR antagonist, 0.5 microM) each decreased NA-stimulated ICaL in both species. ICI118551 (β_{2}-AR antagonist, 0.1 microM), in the presence of NA + CGP20712A, had no significant effect on ICaL in human atrial myocytes, but increased it in rabbit. Yohimbine (α_{2}-AR antagonist, 10 microM), with NA + prazosin, had no significant effect on human or rabbit ICaL. Stimulation of atrial ICaL by NA is mediated, based on AR sub-type antagonist responses, mainly by activating β_{1}- and α_{1}-ARs in both human and rabbit, with a β_{2}-inhibitory contribution evident in rabbit, and negligible α_{2} involvement in either species. This improved understanding of AR sub-type contributions to noradrenergic activation of atrial ICaL could help inform future potential optimisation of pharmacological AR-antagonism strategies for inhibiting adrenergic AF.

Keywords Cardiac electrophysiology · Adrenoceptors · Noradrenaline · Calcium current · Atrial myocyte · Atrial fibrillation

Introduction
The catecholamine noradrenaline (NA) is released by sympathetic (adrenergic) post-ganglionic nerves terminating on cardiac myocytes. It is substantially involved in regulating cardiac excitation–contraction and the fight-or-flight response, and sometimes in the generation of cardiac arrhythmias including the most common, atrial fibrillation (AF) [39, 46]. The generation of AF by NA probably involves its marked effect to increase atrial L-type Ca^{2+} current, ICaL, as shown in human [4, 6] and rat [3] atrial myocytes, in turn contributing to triggered activity from atrial spontaneous depolarisations or afterdepolarisations [9, 19, 44, 46]. This ICaL increase results, in large part, from activation of cell surface beta-adrenoceptors (β-AR), supported by numerous studies showing marked effects of the synthetic broad action (β_{1}-, β_{2}- and β_{3}-AR) agonist isoprenaline (ISO) on human atrial ICaL (e.g. [4, 21, 31]). Furthermore, ISO infusion in patients produced AF [29]. β-AR antagonists are used in the pharmacological treatment of patients with AF, primarily for controlling the associated rapid ventricular rates (rate control), but they may also be effective in suppressing AF (rhythm control) when adrenergic tone is elevated, e.g. β_{1}-AR sub-type antagonists in patients with postoperative AF (bisoprolol, metoprolol) [8] or with heart failure (metoprolol) [37]. However, NA activates α- as well as β-ARs, and each main AR sub-type has been identified in human atrial myocardium [46]. Moreover, since the mixed α_{1}-, β_{1}-, β_{2}-AR antagonist, carvedilol, was more effective at preventing postoperative AF than the β_{1}-antagonists...
metoprolol or atenolol [15, 23], this suggests the possibility of identifying specific mixed AR sub-type antagonism profiles for optimising rhythm control drug efficacy during adrenergic AF.

To do so, however, requires an improved understanding of the contributions of activation of the individual AR sub-types to the effect of NA on human atrial I_Ca_L. So far, this has been addressed using AR sub-type selective agonists, with β2-agonism (salbutamol) increasing human atrial I_Ca_L [50], β3-agonism (BRL37344) having no effect [5] and selective β1-agonism not yet studied for human atrial I_Ca_L. Reports of α-AR agonism in human atrium are so far restricted to contraction, e.g. positive inotropic effect of the α1-agonist phenylephrine [14], although I_Ca_L has been studied in other species, with a marked increase in the current by phenylephrine in cat atrial myocytes [41], and no effect of phenylephrine or methoxamine in rabbit or rat atrial myocytes [12, 18]. Mixed effects of α-AR agonists have also been reported for ventricular I_Ca_L [36]. It is important, however, to investigate the AR sub-type contribution to the I_Ca_L response when using the naturally occurring catecholamine, NA, because this will stimulate all the ARs, simultaneously as would occur in vivo if desired, with consequent physiologically relevant relative activation levels amongst the different AR sub-types, as well as physiologically relevant interactions amongst their associated signalling pathways.

However, there are no reports, to our knowledge, of studies investigating the independent contributions to the I_Ca_L response of the different AR sub-types in this way, i.e. using NA in the presence of AR sub-type selective antagonists, in human atrial myocytes. Potential species-differences in I_Ca_L responses to NA and AR antagonists should also be considered, in order that data from animal species used in models of AF from adrenergic stimulation and/or altered pathology can be adequately compared with those from human. Rabbits have been studied previously to investigate atrial cellular electrophysiological mechanisms of AF promotion by β-AR stimulation with ISO [20], but I_Ca_L responses to NA with AR antagonists have yet to be studied in this species.

The aim, therefore, is to investigate effects, on NA-stimulated I_Ca_L, of various broad-action and sub-type-specific α- and β-AR antagonists, alone or in combination, in human and rabbit atrial myocytes.

Methods

Patients and animals

Right atrial tissues were obtained from 15 adult patients who were undergoing cardiac surgery, predominantly for coronary artery bypass grafting. All patients were in sinus rhythm on the day of surgery, and none had a history of AF. See Table 1 for patients’ clinical characteristics and drug treatments. Rabbits (n = 15; strain: New Zealand White; supplier: Envigo UK; sex: male; age (mean ± SE [range]): 20 ± 1 [15–29] weeks; weight: 3.2 ± 0.1 [2.9–3.7] kg; feeding: ad libitum) were humanely killed by intravenous injection of anaesthetic (100 mg/kg Na+-pentobarbital, via the left marginal ear vein) and removal of the heart, which was retrogradely perfused via the aorta before isolating cardiomyocytes.

Atrial cardiomyocytes and voltage-clamp technique

Human and rabbit atrial cardiomyocytes were isolated by enzymatic dissociation (Collagenase Type 1, Lorne Laboratories, Lower Earley, UK) and mechanical disaggregation [22, 47] and stored (≤ 9 h, ~ 20 °C) in cardioplegic solution (mM): KOH (70), KCl (40), L-glutamic acid (50), taurine (20), KH₂PO₄ (20), MgCl₂ (3), glucose (10), HEPES (10), EGTA (0.5), pH 7.2. The whole-cell-patch voltage-clamp technique was used to record membrane current, in ruptured-patch mode, with an AxoClamp 2B

| Table 1 Patients’ clinical characteristics | Average, n (total n = 15) |
|-------------------------------------------|---------------------------|
| Age                                       | 65 ± 3 (range 45–83) years, 15 |
| Sex                                       | 93% male, 15               |
| Cardiac rhythm                            | 100% sinus rhythm, 15      |
| Operation                                 |                           |
| Coronary artery bypass graft surgery      | 87%, 15                    |
| Aortic valve replacement                  | 33%, 15                    |
| Mitral valve replacement                  | 7%, 15                     |
| Atrial septal defect repair               | 0%, 15                     |
| Ventricular septal defect repair          | 7%, 15                     |
| Cardiac drugs                             |                           |
| β1-blocker (bisoprolol)                   | 77%, 13                    |
| Angiotensin-converting enzyme inhibitor   | 43%, 14                    |
| Angiotensin receptor blocker              | 23%, 13                    |
| Calcium channel blocker                   | 29%, 14                    |
| Digoxin                                   | 0%, 13                     |
| Nicorandil                                | 15%, 13                    |
| Eplerenone                                | 0%, 13                     |
| Nitrate                                   | 60%, 15                    |
| Statin                                    | 100%, 14                   |
| Disease                                   |                           |
| Angina                                    | 67%, 15                    |
| History of myocardial infarction          | 36%, 14                    |
| History of hypertension                   | 87%, 15                    |
| Diabetes                                  | 7%, 14                     |
| Left ventricular function                 |                           |
| Left ventricular ejection fraction        | 57 ± 3 (range 38–73) %, 12 |
amplifier (Axon Instruments) and WinWCP software (J Dempster). Cardiomyocytes were superfused at 35–37 °C with a physiological salt solution containing (mM) NaCl (140), KCl (4), CaCl₂ (1.8), MgCl₂ (1), glucose (11) and HEPES (10); pH 7.4. Microelectrodes (1.5–3.0 MΩ resistance) contained (mM) K-aspartate (130), KCl (15), NaCl (10), MgCl₂ (1), HEPES (10) and EGTA (0.1); pH 7.25. The resulting liquid–liquid junction potential (+ 9 mV; bath relative to pipette) was compensated for a priori [26]. The low [EGTA]ᵢ allows physiological oscillations in cytosolic [Ca²⁺] during IC₅₉ₓ recordings [20]. IC₅₉ₓ was stimulated with 300 ms duration voltage steps to 0 mV from a holding potential (HP) of − 50 mV (to avoid Na⁺ current), delivered at 0.33 Hz. Signals were low-pass filtered at 10 kHz. 4-aminopyridine (4-AP; 5 mM) and niflumic acid (0.1 mM) were added to the superfusion solution to suppress contaminating K⁺ currents (mainly I_TO and I_Kur) and I_Cl(Ca), respectively.

Drugs and reagents
Noradrenaline (Merck Life Science, Glasgow, UK) was used at 0.01–10 µM [4]; propranolol (Merck) at 0.2 µM [13]; phenolamine (Abcam, Cambridge, UK) at 1 µM [17]; CGP20712A (Merck) at 0.3 µM [5]; ICI118551 (Tocris Bioscience, Bristol, UK) at 0.1 µM [1]; prazosin (Merck) at 0.5 µM [2] and yohimbine (Merck) at 10 µM [16]. Propranolol was racemic, which may affect additionally I_Na and I_K; although this was largely mitigated by the HP and [4-AP] used. The AR sub-type antagonists were chosen for their high selectivity (e.g. CGP: ~ 500-fold selectivity for β₁ > β₂; ICI > 500-fold β₂ > β₁ [1]), to effectively dissect individual sub-type responses rather than to mimic clinically used drugs. Indeed, metoprolol, atenolol and bisoprolol may have rather poor selectivity for β₁ > β₂ [1]. All reagents for storage-, pipette- and superfusion-solutions were supplied by Merck, except for niflumic acid (Tocris).

Data and statistical analysis
Data are expressed as mean ± SEM. Comparisons amongst three or more groups were made using (for matched, parametric data) repeated measures one-way ANOVA or (for un-matched, parametric data) ordinary one-way ANOVA, each followed by Tukey’s multiple comparisons test or (for matched, non-parametric) Friedman test and Uncorrected Dunn’s test. Comparisons between two groups of un-matched data were made using either an un-paired t-test (for parametric data) or Mann–Whitney (non-parametric).

P<0.05 was regarded as statistically significant. All statistical and curve fitting analyses were done using Graphpad Prism 7.00.

Results
Noradrenaline increases human atrial L-type Ca²⁺ current in a concentration-dependent manner
In human atrial myocytes, NA produced a marked, concentration-dependent increase in IC₅₉ₓ, shown by the original current traces and concentration–response curve in Fig. 1A and B, respectively. At maximally effective concentration, NA increased IC₅₉ₓ ~ 2.5-fold (Fig. 1B). A near-maximally effective, but not saturating, NA concentration (EC₇₅) was chosen for use in all subsequent AR antagonist experiments, calculated from Fig. 1B, i.e. 310 nM.
Effects of broad action β- and α-adrenoceptor antagonists on NA-stimulated ICaL in human and rabbit atrial myocytes

Broad-action β-, and α-, AR antagonism of ICaL-responses to this NA concentration was studied using propranolol, and phentolamine, applied in a step-wise cumulative fashion, in atrial cells from patients, and also from rabbits for direct comparison (Fig. 2). Following a control period to allow for stabilisation of the normal rate of ICaL run-down (time-dependent decrease following cell rupture), NA superfusion caused a rapid and substantial increase in peak ICaL in all cells studied, with the response stabilising within ~1–1.5 min (e.g. Figure 2A and C). In two representative human atrial cells (Fig. 2Ai and ii), propranolol, still in the presence of NA, caused a rapid and substantial decrease in ICaL to below the NA-stimulated response, and subsequently applied phentolamine caused a rapid, and relatively smaller, decrease in ICaL. In one of these cells (Fig. 2Aii), propranolol and phentolamine were simultaneously washed off; the resulting increase in ICaL (itself reversible) shows that the NA-stimulatory effect had been preserved throughout the preceding superfusion of the antagonists. In each of nine human atrial cells studied in this way, propranolol decreased, then phentolamine further decreased, the NA-stimulated ICaL. The mean data (Fig. 2B) show that both propranolol and phentolamine significantly decreased ICaL, and that the degree of reduction from phentolamine was significantly smaller than that from propranolol. In rabbit atrial cells, NA also produced a rapid and significant increase in ICaL, and propranolol then caused a rapid, substantial and significant decrease in NA-stimulated ICaL, in each of 7 cells studied (Fig. 2C and D). However, by contrast with the human atrial cells, phentolamine (following propranolol) produced a mixed response, either decreasing (e.g. Figure 2Ci) or increasing (e.g. Figure 2Cii) ICaL, in both cases reversible upon phentolamine-washout. The spread of

![Fig. 2](image-url)
these phentolamine responses can be seen in Fig. 2D: with a decrease in 4/7 cells (by 21, 29, 44 and 48%; reversible in 3/4), and an increase in 3/7 cells (by 16, 159 and 331%; reversible in each). Moreover, there was no significant effect of phentolamine on average, i.e. in contrast to its significant antagonistic effect in human atrial cells under the same conditions.

The bi-exponential time course of $I_{CaL}$ inactivation was also examined. NA (310 nM) had no significant effect on the fast ($\tau_1$) or slow ($\tau_2$) time constants in either species: in human, control $\tau_1$ and $\tau_2$ were $9.77 \pm 1.26$ and $112.1 \pm 23.2$ ms, respectively, vs $7.30 \pm 0.64$ and $236.9 \pm 66.4$ ms with NA ($P=0.087$ and 0.126, respectively; $n=9$ cells); in rabbit: control $\tau_1$ and $\tau_2$ were $12.58 \pm 4.40$ and $91.9 \pm 20.1$ ms, vs $13.54 \pm 2.96$ and $88.7 \pm 12.4$ ms with NA ($P=0.781$ and 0.797; $n=7$ cells).

Comparison of independent anti-adrenergic effects of propranolol and phentolamine

In rabbit atrial cells, effects of broad-action $\alpha$- and $\beta$-antagonism were also studied independently of one other, by using phentolamine in the absence of propranolol (for $\alpha$-antagonism without concurrent $\beta$-antagonism) and, in a different group of cells, vice versa. Propranolol alone again caused a consistent, marked and significant decrease in NA-stimulated $I_{CaL}$ (Fig. 3Ai and Bi). However, phentolamine alone (Fig. 3Aii and Bii), by contrast with phentolamine in the continued presence of propranolol (Fig. 2C and D), also caused a consistent (i.e. in each of 9 cells studied), marked and significant decrease in NA-stimulated $I_{CaL}$. Furthermore, the degree of the inhibitory effect of phentolamine was not significantly different from that of propranolol.

Comparison of $\beta$-AR sub-type contributions to $I_{CaL}$-stimulation by NA, between human and rabbit atrial myocytes

Having established a substantial $\beta$-AR contribution to the stimulatory effect of NA on atrial $I_{CaL}$, the relative contributions to this of the main $\beta$-AR subtypes ($\beta_1$ and $\beta_2$) were investigated using CGP20712A (CGP) and ICI1118551 (ICI), respectively, again applied in a step-wise cumulative fashion and compared between species. In each of 6 human atrial cells studied (e.g. Figure 4Ai and ii), CGP caused a rapid and marked decrease in NA-stimulated $I_{CaL}$, with a significant average inhibitory effect (Fig. 4B) similar to that from propranolol ($\beta_1$ + $\beta_2$-antagonist) earlier (Fig. 2B). In rabbit atrial cells, CGP had similar effects, both in terms of the comparison with human (Fig. 4C and D vs A and B) and with propranolol (Figs. 4C and D vs 2 C and D). However, the effects of ICI on NA-stimulated $I_{CaL}$ differed substantially, both when compared with CGP, and between species. Thus, amongst 5 human atrial cells studied with ICI, there was a mixed response: a reversible (upon ICI-washout) increase in 3 cells (e.g. Figure 4Ai), by 12, 35 and 37% (Fig. 4B), and a marked and reversible decrease in 2 cells (e.g. Figure 4Aii), by 72 and 78% (Fig. 4B). There was no significant effect of ICI on average, contrasting with the consistent and significant inhibitory effect of CGP in the same cells (Fig. 4B). In the rabbit atrial cells, by contrast with the human atrial cells under the same conditions, ICI consistently and reversibly (in each of 5 cells studied) increased $I_{CaL}$ (e.g. Figure 4Ci and ii), an effect which was significant on average (Fig. 4D). The degree of $I_{CaL}$ increase by ICI was not significantly different ($P=0.391$) to the degree of $I_{CaL}$ decrease by CGP in these cells.
α-AR sub-type contributions to NA-stimulation of ICaL in human and rabbit atrial myocytes

The relative contributions of the main α-AR subtypes (α₁ and α₂), to the broad α-AR contribution to the stimulatory effect of NA on ICaL, were investigated using prazosin and yohimbine (still in the presence of NA + prazosin) produced with a significant effect on average (Fig. 5B). By contrast, a mixed ICaL response: a moderate decrease in 4 cells (e.g. (Fig. 5D). The degree of the ICaL decrease by prazosin was lier (compare Fig. 5D with Fig. 4D). Yohimbine, by contrast not significantly different (∗P=0.073) from that by CGP earlier (compare Fig. 5D with Fig. 4D). Yohimbine, by contrast, produced a mixed ICaL response: a decrease in 6 of these 7

cells (e.g. Figure 5Ci), an increase in the other (Fig. 5Cii) and no significant effect on average (Fig. 5D).

Discussion

Investigation of independent AR sub-type contributions to NA’s effect on human atrial ICaL first required establishing the NA-ICaL concentration–response relationship, to select a suitable NA concentration for testing with the AR sub-type selective antagonists. We found NA to have a marked, concentration-dependent stimulatory effect on ICaL, with an EC₅₀ of 156 nM, comparable with that in another human atrial study (200 nM) [4], although a markedly higher value has also been reported [6]. Whilst NA circulates in the sub-low-nanomolar range in humans [35], it is expected to be substantially more concentrated at the adrenergic nerve endings and in cardiac tissues [51]. We selected our EC₅₀ for use in all subsequent experiments (in human and rabbit for their direct comparison) because whilst near maximally effective, this would not saturate the stimulatory response, therefore permitting the antagonists to readily exert their effects. Whilst NA consistently increased ICaL, its subsequent “run-down” (line graphs, Figs. 2, 3, 4 and 5), an accepted limitation of the ruptured-patch technique (due to “a decrease in channel activity with time during recording in dialyzed cells” [43]), required the antagonist responses to be normalised with respect to the previous intervention (bar graphs,
Fig. 5  α-AR sub-type contributions to NA-stimulation of I_{CaL} in human and rabbit atrial myocytes. A Typical I_{CaL} changes in two human atrial cells (i and ii) in response to an α₁-antagonist (prazosin, 0.5 µM: “Pra”), then an α₂-antagonist (yohimbine, 10 µM: “Yoh”), both with NA at 310 nM. B Mean responses to interventions in A. n = 6 cells, 3 patients; * = P < 0.05, NS = not significant (ANOVA). C Corresponding I_{CaL} changes in two rabbit atrial cells (i and ii). D Mean responses (n = 7 cells, 3 rabbits) to same interventions as in C.

Figs. 2, 3, 4 and 5) to compensate for this rundown and thus adequately assess average antagonist effects. Broad action β-AR antagonism (with propranolol) revealed a substantial and consistent contribution to NA’s stimulatory effect on human atrial I_{CaL} from either β₁- or β₂-ARs or both (since β₁-ARs are not expected to be involved in this response [5, 24]). This is congruent with numerous studies in which the broad action AR agonist ISO substantially increased human atrial I_{CaL} [4, 21, 31], although no previous atrial I_{CaL} study could be found in which propranolol was applied following either ISO or NA. In the continued presence of NA plus propranolol, i.e. with the β₁- and β₂-ARs still antagonised and the α-ARs thus adrenergically activated and solely (independently) amenable to antagonism, broad action α-AR antagonism with phentolamine revealed a substantial and consistent contribution to NA’s stimulatory effect on human atrial I_{CaL} from either α₁- or α₂-ARs or both. Furthermore, we found that the α-AR contribution to the stimulatory effect of NA on I_{CaL} was significantly smaller (at 37%) than that of the β-AR contribution (at 60%), in human atrial cells. Use of the same protocol in the rabbit atrial cells, i.e. stepwise cumulative addition of NA, propranolol and phentolamine, revealed important species similarities, but also a curious difference regarding the contribution of α-ARs. Thus, whilst propranolol consistently, markedly and significantly antagonised NA’s stimulatory effect on rabbit as well as human atrial I_{CaL}, in rabbit, by contrast with human, phentolamine had a mixed response following propranolol, producing increases in I_{CaL} in some cells, as well as the decreases as seen in human. These I_{CaL} increases by phentolamine were clear, marked and reversible and occurred in approximately half of the rabbit atrial cells studied. By contrast, no I_{CaL} increase was produced by phentolamine in any of the nine human atrial cells studied in this way. Since only the α-ARs were noradrenergically activated at this point in these experiments (β-AR activation prevented by propranolol in both species), such I_{CaL} increases by the α-AR antagonist indicate an inhibitory contribution of independent α-AR activation to the effect of NA on I_{CaL} in those rabbit atrial cells, i.e. attenuating, but not overcoming, the overall effect of NA to increase I_{CaL}. The reason for this mixed effect of phentolamine in the rabbit atrial cells is unknown, but the resulting net (average) absence of effect, as presumably would occur in the syncytium (multicellular), suggests a potentially important species difference that whilst noradrenergic activation of human atrial I_{CaL} involves a significant contribution from α-ARs, this may not be the case in rabbit, at least when the α-ARs are activated independently of the β-ARs. To assess the α-AR contribution to NA’s effect on rabbit atrial I_{CaL}, this time in the presence of simultaneously activated β-ARs, phentolamine was applied in the absence of propranolol and, in a different group of cells, propranolol in the absence of phentolamine for comparison. In this case, we found either α- or β-AR antagonism to consistently (in every cell), markedly and significantly decrease (and by a similar degree between α- and β-) NA-stimulated
suggesting that the attenuating influence of independent α-AR activation on the stimulatory influence of NA on I_{CaL} as seen above is prevented when α- and β- ARs are simultaneously activated. This finding likely relates to the highly complex interactions which can occur between α- and β-ARs and their signalling pathways [48]. It also highlights another complex, potentially limiting, yet intriguing aspect of this type of study, the relevance of the order of application of AR-antagonist(s) following NA.

Having established a substantial broad β-AR contribution to NA’s stimulatory effect on atrial I_{CaL} in both species, we then dissected the β_{1-} versus β_{2-}AR involvement, using CGP and ICI, respectively, and showed β_{1-}AR activation to mediate a consistent, substantial and significant contribution to noradrenergic activation of human and rabbit atrial I_{CaL}. The similarity in the magnitude of effect of CGP with that of propranolol, in both species, indicated the prominence of the β_{1-}AR involvement. By contrast, we found β_{2-}AR activation, amongst human atrial cells, to have a mixed, and on average negligible, involvement in the overall β-adrenergic activation of I_{CaL}. This mixed response could relate to stimulatory and inhibitory responses known to result from β_{2-}activation, via G_{s} and G_{i} signalling pathways, respectively [45]. In the only similar human atrial I_{CaL} study, in which a synthetic agonist rather than NA was used to activate β_{2-}ARs [50], salbutamol increased the current, which would suggest a stimulatory contribution of β_{2-}activation to its adrenergic activation under their conditions. We found an important species difference regarding β_{2-}AR activation, amongst human atrial cells, independent β_{2-}AR antagonism with ICI (since β_{1-}AR activation prevented by CGP in both species) produced a consistent, reversible, substantial and on average significant increase in I_{CaL}. This indicated a significant inhibitory contribution of β_{2-}AR activation to the effect of NA on rabbit (but not human) I_{CaL}, attenuating the overall effect of NA to increase I_{CaL}, presumably relating to a relatively enhanced G_{i} signalling response to β_{2-}AR activation [45]. Consistent with this, in rat atrial tissues, β_{2-}antagonism (butoxamine) potentiated the effect of ISO to produce spontaneous contractions [2]. Furthermore, and also in line with the present data, recent studies comparing effects of β_{1-} and β_{2-}AR agonism on rat ventricular I_{CaL}, intracellular Ca^{2+}-cycling and action potentials found that initial β_{2-}AR stimulation suppressed most of the well-characterised changes of cardiac excitation–contraction coupling commonly seen when adding a β_{1-}AR agonist [27, 49].

Dissection of the respective α_{1-} versus α_{2-}AR involvement in NA’s effect on human atrial I_{CaL} (with prazosin and yohimbine) revealed α_{1-}AR activation to mediate a consistent, substantial and significant contribution to noradrenergic activation of the current, but an overall negligible contribution from α_{2-}AR activation. The stimulatory contribution from this α_{1-}AR activation was, nevertheless, significantly smaller (at 37%) than that observed from the β_{1-}AR activation (at 71%). Although no studies of effects of synthetic α-AR agonists on human atrial I_{CaL} could be found, the α_{1-}AR agonist phenylephrine had positive inotropic effects on human atrial muscle strips [14]. These could potentially be explained, at least in part, by the presently observed stimulatory contribution of α_{1-}AR activation on I_{CaL}. However, it should be noted that such inotropic effects could also be due, at least in part, to inhibition of repolarising K^{+} current, as shown with phenylephrine for human atrial I_{K1}, I_{TO} and I_{CaL} [33], or to increased IP_{3}-dependent sarcoplasmic reticular Ca^{2+} release [41]. No human atrial I_{CaL} studies using prazosin or yohimbine could be found, although there are reports of attenuation by prazosin of NA-induced positive inotropy [32], again congruent with the observed effects of prazosin on NA-stimulated I_{CaL}. In the rabbit atrial cells, we also found a consistent, substantial and significant stimulatory contribution of α_{1-}AR activation to the NA-stimulation of I_{CaL} and a negligible contribution from α_{2-}AR activation. Previous atrial I_{CaL} studies, using synthetic α_{1-}agonists rather than NA, showed either no effect (in rabbit [12] and rat [18]), or a stimulatory effect, in cat [41]. In mice, NA-induced AF was inhibited by prior injection of the α_{1-}antagonist prazosin [34]. Both NA and α_{1-}agonism inhibit rabbit atria I_{TO} [12], carried prominently by Kv1.4 [42]. We blocked I_{TO} using 4-AP, to avoid contaminating I_{CaL} recordings. However, in vivo, I_{TO} decrease from α_{1-}stimulation could exert an action potential prolonging influence additional to that from the present I_{CaL} increase, and other effects of α-stimulation, including pre-synaptic, should also be considered.

Taking our results together, we find that stimulation of atrial I_{CaL} by NA is mediated, based on responses to AR subtype-antagonists (applied in a set order: sub-type1, followed by sub-type2), mainly by activating β_{1-} and α_{1-}ARs, in both human and rabbit. Whilst α_{1-}AR involvement was negligible in both species and β_{2-}AR involvement negligible in human, in rabbit, β_{2-}activation can attenuate the stimulatory effect of NA on I_{CaL}. Finally, in human (but not rabbit), the contribution of β_{1-}activation to the I_{CaL} stimulatory response to NA was larger than that of α_{1-}activation. An overview of these AR sub-type contributions, with a qualitative estimation of their relative weights, and differences between human and rabbit, is given in Table 2. These findings have relevance to the electrophysiological mechanisms and potential inhibition of NA-induced AF. Delayed afterdepolarisations (DADs) were produced by catecholamines in dog atria [19], identified as such by their rate-dependent increase in amplitude and decrease in coupling interval [19, 44]. Furthermore, afterdepolarisations of various types were produced or facilitated by ISO in human atrial tissues or cells [28, 31, 40]. DADs are caused by increased inward Na^{+}/Ca^{2+} exchange current...
The present data suggest that potential therapeutic targeting of AR sub-types as a means of inhibiting NA-evoked atrial arrhythmias should be most effective with β1-AR antagonists, and potentially more effective with concurrent α1-AR antagonism. This would be consistent both with the clinical use of β1-AR antagonists for preventing post-operative AF [8], and the observation that carvedilol (α1-, β1-, β2-AR-antagonist) was more effective at preventing this arrhythmia than β1-AR antagonists [15, 23], although extra-AR actions of carvedilol [11] might also contribute.

However, since α1-AR activation might exert various cardioprotective effects, α1-AR antagonism should nevertheless be considered with caution [52]. Furthermore, potentially therapeutic targeting of selected AR sub-types must be considered in the context of highly complex, dynamic and pathology-dependent interactions between each of the various AR sub-types and their associated signalling pathways [48].

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Author contribution Conception or design of the work: PS, RM, GS, AW. Acquisition, analysis or interpretation of data: PS, RM, GS, AW. Drafting the work or revising it critically for important intellectual content: PS, RM, GS, AW.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Ethical approval number: REC 17/WS/0134. Written, informed consent was obtained from all patients. Procedures and experiments involving human atrial myocytes were approved by West of Scotland Research Ethics Service (REC: 17/WS/0134). Written, informed consent was obtained from all patients. The investigation conformed to the principles outlined in the Declaration of Helsinki. Procedures and experiments involving rabbit left atrial myocytes (UK Project Licence: 70/8835) were approved by Glasgow University Ethics Review Committee and conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Consent for publication All authors agree with the content and all gave explicit consent to submit and obtained consent from the responsible authorities at the institute where the work was carried out.

Competing interests The authors declare no competing interests.

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