

α₁-adrenergic stimulation increases ventricular action potential duration in the intact mouse heart

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Abstract

The role of α_1 -adrenergic receptors (α -ARs) in the regulation of myocardial function is less wellunderstood than that of β -ARs. Previous reports in the mouse heart have described that α_1 -adrenergic stimulation shortens action potential duration in isolated cells or tissues, in contrast to prolongation of the action potential reported in most other mammalian hearts. It has since become appreciated, however, that the mouse heart exhibits marked variation in inotropic response to α_1 -adrenergic stimulation between ventricles and even individual cardiomyocytes. We investigated the effects of α_1 -adrenergic stimulation on action potential duration at 80% of repolarization in the right and left ventricles of Langendorff-perfused mouse hearts using optical mapping. In hearts under β -adrenergic blockade (propranolol), phenylephrine or noradrenaline perfusion both increased action potential duration in both ventricles. The increased action potential duration was partially reversed by subsequent perfusion with the α -adrenergic antagonist phentolamine (1 µmol L⁻¹). These data show that α_1 -receptor stimulation may lead to a prolonging of action potential in the mouse heart and thereby refine our understanding of how action potential duration adjusts during sympathetic stimulation.

Key words: fight-or-flight, APD, sympathetic activation, adrenergic receptors, myocardium

Introduction

Adrenergic stimulation modulates myocardial function by acting upon α - and β -adrenergic receptors (ARs). The latter, especially β 1-ARs, are found in the greatest abundance (Baker 2014; Myagmar et al. 2017), and their functions, including mediating a powerful positive inotropic effect in the heart, are well-established (Lymperopoulos et al. 2013; Baker 2014). Conversely, α -ARs, namely the α_1 -ARs expressed in cardiomyocytes, remain much more enigmatic and controversial (Mohl et al. 2011; Endoh 2016), as the literature reveals considerable variation in cardiac effects between different species, tissues, and experimental preparations (Wagner and Brodde 1978; Tohse et al. 1992; Zhang et al. 1998; Ross et al. 2003; Endoh 2016; Joyce and Wang 2020). Nevertheless, it is clear that as β -adrenergic function declines during heart failure, due to the desensitization of β 1-AR, the relative importance of cardiac α_1 -ARs increases (Skomedal et al. 1997; Sjaastad et al. 2003; Jensen et al. 2011) due to the desensitization of β 1-ARs (Bristow et al. 1982). Accordingly, the α_1 -adrenergic

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signalling pathway is emerging as a potential therapeutic target (Jensen et al. 2011; Perez and Doze 2011; Baker 2014; O'Connell et al. 2014; Endoh 2016).

The mouse has become a cornerstone to study α -adrenergic regulation in the heart, particularly given the genetic amenability that enabled the generation of various α_1 -AR sub-type specific transgenic and knockout lines (Koch et al. 2000; Ross et al. 2003; Turnbull et al. 2003; Mohl et al. 2011; Jensen et al. 2014). However, whilst in most mammals (Brückner et al. 1978; Wagner and Brodde 1978; Brückner and Scholz 1984; Endoh et al. 1991; Endoh 2016), including humans (Landzberg et al. 1991; Janssen et al. 2018), α_1 -adrenergic stimulation of ventricular myocardium results in a positive inotropic effect, the effects in mice are particularly complex, and both positive and negative inotropic have been reported in cardiomyocytes, isolated muscle, and whole heart preparations (Ross et al. 2003; Petrashevskaya et al. 2004; Wang et al. 2006; Mohl et al. 2011). In accordance with the positive inotropic effect, most mammals exhibit a lengthening of action potential duration (APD) with α_1 adrenergic stimulation (Brückner and Scholz 1984; Tohse et al. 1987, 1990; Apkon and Nerbonne 1988; Endoh et al. 1991; Fedida and Bouchard 1992). By contrast, previous studies in mice utilizing direct measurements of transmembrane potentials in isolated right ventricular papillary muscle or isolated cardiomyocytes have reported shortened APD in response to α_1 -adrenergic stimulation (Nishimaru et al. 2001; Petrashevskaya et al. 2004).

To get insight into the regulation of APD during sympathetic stimulation, we investigated the effects of α_1 -adrenergic stimulation on APD using optical mapping in whole Langendorff-perfused mouse hearts. In our first series of experiments, Protocol 1, we studied the effect of selective α -adrenergic stimulation with the pharmacological agonist phenylephrine. Because the heart primarily relies on stimulation via the neurotransmitter noradrenaline in vivo, in Protocol 2 we investigated if this non-selective agonist of adrenergic receptors is also able to induce electrophysiological effects mediated by α_1 -ARs when β -ARs are inhibited by propranolol, which mimics the β -AR desensitization during heart failure.

Methods

Experimental animals and preparation

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the European Commission Directive 2010/63/EU and was approved by the institutional review board (Amsterdam University Medical Centers).

Six-week-old male FVB/N wild-type mice were anesthetized in CO_2 and killed by cervical dislocation. The heart was rapidly excised and the aorta was cannulated and Langendorff- perfused with Tyrode's solution (mmol L⁻¹: NaCl (128), KCl (4.7), CaCl₂ (1.45), MgCl₂ (0.6), NaHCO₃ (27), NaH₂PO₄ (0.4), glucose (11), and maintained at pH 7.4 by bubbling with carbogen (95% O₂, 5% CO₂)) within 10 min. The preparation was placed in a water-jacketed organ bath, containing Tyrode's solution, with the ventral surface facing upwards. Both the perfusate reservoir and organ bath were maintained at 37 °C. For the duration of the experiment, the heart was perfused at a rate of 3–4 mL min⁻¹.

Immediately after starting perfusion, blebbistatin (\pm) (Bio-Techne Ltd, United Kingdom) was added to Tyrode's reservoir to achieve a final concentration of 16 µmol L⁻¹, which was maintained throughout the experiment. Five min later, 100–125 µL of 0.1 mmol L⁻¹ di-4-ANEPPS (Molecular Probes, Eugene, Oregon, USA) (dissolved in ethanol) was diluted into 300 µL of Tyrode's solution and delivered as bolus into the perfusate line. Optical action potentials were measured with an excitation light provided by a 5-W power LED (filtered 510 ± 20 nm). Fluorescence (filtered >610 nm) was





Fig. 1. The experimental apparatus used in this study. (A) Langendorff perfused mouse heart with pseudoelectrocardiogram (ECG) electrode placement and stimulator electrodes (Stim.). Pseudo-ECG electrodes are labelled consistent with in vivo equivalents as defined in humans (Boukens et al. 2014); right arm (R), left arm (L) and left leg (F). (B) Experimental protocol, consisting of sequential addition of propranolol (Prop.; 1 µmol L^{-1}), phenylephrine (PE; 1 µmol L^{-1} ; protocol 1) or noradrenaline (NA; 500 µmol L^{-1} ; protocol 2), and phentolamine (1 µmol L^{-1}) to the perfusate. Arrows mark periods of pacing and recordings of optical action potentials. (C) Example of data output from ECG electrodes and optical camera.

transmitted through a lens system on a CMOS sensor (100×100 elements; MICAM Ultima), and the data were acquired with MICAM Ultima software on a personal computer. Pseudo-electrograms were recorded by placing 3 electrodes at ± 0.5 cm distance from the heart in the Einthoven configuration as published previously (Boukens et al. 2013). Electrode R and L were placed alongside the right and left atrium, respectively, whereas electrode F was placed alongside the apex. Electrode R was used as negative input for both lead I and lead II. Recordings were made using Labchart amplifier (ADInstruments, Model 15T, sample frequency 1 kHz) and analyzed in LabChart Pro v8.1.13. A silver electrode was used for stimulation at the apex via a custom build current controlled stimulator (Antronics).

Experimental protocol

Ten to fifteen minutes after the di-4-ANEPPS treatment, the experimental protocol started. For each trial, optical action potentials were measured during apical simulation at 200 ms intervals before the duration was decreased in 20 ms steps to 120 ms, or until the heart failed to capture. At each frequency, stimulation was maintained for approximately 40–60 s to allow sufficient stabilisation before action potentials were measured.

Two protocols were employed (Fig. 1); each included serial additions of pharmacological agents to the Tyrode's solution perfusate. Each initially used propranolol to block stimulation of β -adrenergic receptors (Brückner and Scholz 1984; Nishimaru et al. 2001; Montgomery et al. 2002; Ross et al. 2003) and ended with phentolamine, an α -adrenergic antagonist. In the first protocol (N = 4), the trial was conducted under control conditions with propranolol (1 µmol L⁻¹), phenylephrine (1 µmol L⁻¹), and phentolamine (1 µmol L⁻¹). In the second protocol (N = 5), phenylephrine was substituted with noradrenaline (500 nmol L⁻¹). Each agent was perfused for 10 min prior to the pacing trial to achieve



steady state (Ross et al. 2003; Wang et al. 2006; Baker 2014). We additionally conducted a series of trials (N = 3) without the addition of phenylephrine/noradrenaline and phentolamine to account for time-dependent changes in APD after the addition of propranolol. Noradrenaline bitartrate, phenylephrine hydrochloride, propranolol hydrochloride, and phentolamine hydrochloride were purchased from Sigma-Aldrich (St Louis, MO, USA).

Data analysis

Optical action potentials were analysed with custom software in MATLAB (version 2017a, MathWorks, Inc. Nattick, Massachusetts, USA). In brief, action potentials were normalized based on the peak of the upstroke after spatial binning of 7×7 . Ten action potentials were averaged after which APD80 was determined based on 80% of repolarization. We took the average APD80 of a region of 1 mm² at the base of the left and right ventricles at each frequency and with each drug treatment. Total ventricular activation time and repolarization time were measured during pacing at 180 ms intervals using computer generated activation maps.

Statistical analysis was performed in GraphPad Prism version 8.4.0 (GraphPad Software, San Diego, CA, USA). Mixed-effects models (with repeated measures for stimulation frequency and drug treatment) followed by Dunnett's post-hoc tests were used to determine significant (p < 0.05) effects of propranolol, noradrenaline/phenylephrine, and phentolamine on APD80 at different stimulation frequencies. A repeated measurement one-way analysis of variance was performed to analyse the effect of propranolol, noradrenaline, and phentolamine on end QRS-complex time, end T-wave time, repolarisation time, and activation time. Data are presented as means \pm standard error of the mean (SEM). In **Supplementary Material 1**, we present figures depicting data points of individual hearts.

Results

To investigate the role of α_1 -ARs on ventricular repolarization, we administered phenylephrine (Protocol 1) and noradrenaline (Protocol 2) to Langendorff-perfused mouse hearts (Fig. 1). We first administered propranolol to exclude possible binding of phenylephrine and noradrenaline to β -adrenergic receptors. We also tested the effect of propranolol alone on action potential duration in a separate set of hearts (N = 3) to account for time-dependent changes during the course of the experiment. Figure 2 shows optical action potentials recorded 0, 15, and 45 min after administration of propranolol, during which APD did not change.

In Protocols 1 and 2 propranolol administration increased intrinsic RR interval (sinus rhythm) by ~40 ms and ~20 ms, respectively. By the end of both protocols, intrinsic RR interval had increased from 201 ± 21 ms to 300 ± 26 ms in protocol 1 and from 184 ± 22 ms to 247 ± 25 ms in protocol 2 (Table 1). To exclude the influence of heart rate on QT interval, we therefore measured the effect of phenylephrine and noradrenaline on repolarization during stimulation at the ventricular apex at 180 ms interval. Figures S1 and S2 show that neither QRS duration nor QT time changed during administration of either drug.

During ventricular pacing we also determined local moments of activation and repolarization (RT80). Panel A and B of Fig. 3 show two typical examples of the activation (upper) and repolarization (lower) sequence at the ventral side of the heart during stimulation on the apex. In both protocols, last moment of activation delayed significantly after administration of propranolol but remained unchanged after adding the other drugs. On the other hand, last moment of repolarization occurred later after administration of phenylephrine and noradrenaline. Additional administration of phentolamine, during perfusion with phenylephrine or noradrenaline, did not affect repolarization (see Fig. S3 for data points of the individual hearts).





Fig. 2. Propranolol does not affect action potential duration in the electrically paced heart. Panel (A) shows optical action potentials of the left (upper) and right (lower) ventricle at 0, 15 and 45 min after administration of propranolol (120 ms cycling interval). Panel (B) shows maps of the activation potential duration at similar time intervals. The graph in panel (C) shows that action potential duration did not change throughout the duration of the protocol (N = 3). Propra, propranolol; ms, miliseconds.

	RR		PR		QRS		QT	
	Average	SEM	Average	SEM	Average	SEM	Average	SEM
Protocol 1								
Before	200.7	21.4	42.2	7.7	13.3	2.6	63.0	3.7
Propranol	243.4	12.0	61.3	10.7	13.9	2.4	65.1	6.8
Phenylephrine	271.2	20.6	71.5	5.0	16.4	3.9	78.3	8.9
Phentolamine	299.5	25.5	91.0	13.1	22.8	6.2	83.1	4.4
Protocol 2								
Before	184.2	21.7	45.3	2.6	18.3	1.8	89.1	17.6
Propranol	202.0	26.2	47.9	4.4	19.6	4.3	74.1	6.9
Noradrenaline	234.7	20.5	63.9	10.3	19.1	3.3	88.0	3.6
Phentolamine	247.3	24.8	62.8	8.7	20.7	2.0	90.3	7.5

Table 1. Changes in intrinsic RR interval and pseudo-ECG parameters during drug perfusions.

Figure 4 shows APD maps during Protocol 1 (**Fig. 4A**) and Protocol 2 (**Fig. 4B**). The initial treatment with the general β -adrenergic receptor blocker propranolol did not affect APD in either ventricle (**Fig. 4**). Administration of either phenylephrine or noradrenaline prolonged APD, which then





Fig. 3. α_1 -adrenergic stimulation prolongs local repolarization (RT80). The maps in panel (A) and (B) show activation (upper) and repolarization patterns during protocol 1 and 2, respectively. The graphs in (C) and (D) show the change in last local activation (grey, Last Act) and final local repolarization (black, Last repol) during protocol 1 (N = 4) and 2 (N = 5), respectively. Asterisks indicate significant (p < 0.05) differences between data points. Data are means ± SEM.

became shorter after administration of phentolamine (Figs. 5A and 5B). Phenylephrine significantly (p < 0.05) increased APD80 in the right ventricle at 200 ms stimulus intervals and in the left ventricle at 200 and 160 ms intervals (p = 0.052 at 180 ms). Following phentolamine, APD80 was reduced at 200 and 160 ms in the right ventricle and 180 ms in the left ventricle (p < 0.05) (Figs. 5C and 5D). Noradrenaline significantly increased APD80 (p < 0.05) at all frequencies in the right ventricle, with the exception at 120 ms, which was borderline significant (p = 0.057). Likewise, noradrenaline increased APD80 at all frequencies (p < 0.05) in the left ventricle with the marginal exception at 180 ms stimulus interval (p = 0.059). APD80 was significantly reduced towards control levels by α -AR block with phentolamine at 200 and 180 ms stimulus intervals in the left ventricle (Figs. 5E, 5F, and S4).

Discussion

Our data show that stimulation of α_1 -ARs prolongs APD in both the left and right ventricle of mice at various stimulation frequencies. Moreover, we show that noradrenaline is able to prolong APD in mice when β -ARs are blocked.

The important role of α_1 -ARs in the regulation of myocardial function is less well appreciated than that of β -ARs (Brückner et al. 1985; Baker 2014; Endoh 2016). It has previously been shown that α_1 -AR stimulation shortens the APD in mice (Nishimaru et al. 2001; Petrashevskaya et al. 2004).







Nishimaru et al. (2001) demonstrated that APD reduces rapidly at the onset of phenylephrine treatment and remained shortened for at least 15 min, whereas our measurements within the same timeframe (10 min after the initiation of α_1 -AR stimulation) indicated an increased APD. The disparate aspects of the results between previous work and our own remain to be clarified, but may be due to the complex cellular heterogeneity in the heart (Chu et al. 2013; Myagmar et al. 2017). Indeed, having established that the positive inotropic effect in response to α_1 -AR stimulation in a subset of cells was not due to altered sarcoplasmic reticulum Ca²⁺ load, Chu et al. (2013) speculated that increased APD may be implicated in at least some cardiomyocytes. As we solely measured epicardial action potentials, it is possible that the difference stems from transmural differences. APD is shorter in epicardial than endocardial cardiomyocytes in mice (Bondarenko and Rasmusson 2010), potentially providing more scope for it to increase during α_1 -AR stimulation. It is also possible that studies on isolated cells or tissues interrupt the integrity of the preparation and lead to differences in α_1 -AR signalling (Ross et al. 2003). Indeed, it was previously reported that α_1 -AR subtype expression changed considerably during isolation of hepatocytes (González-Espinosa et al. 1999).

The previous studies operated with a very low stimulation frequency (1 Hz; 60 beats min⁻¹), whereas our study was performed close to the physiological range (>300 beats min⁻¹; (Kramer et al. 1993)), which is an additional possible source of variation. However, whilst frequency dependency of α_1 -AR stimulation has been demonstrated, greater positive inotropic effects are typically observed at low frequency, making this explanation unlikely. Considerable inter-strain variability has also been reported with regards to cardiac electrophysiology in mice (Waldeyer et al. 2009), which may also contribute to the distinction.

Wang et al. (2006) described prominent interventricular differences in response to α_1 -AR stimulation in the mouse heart. Whilst trabeculae of the left ventricle exhibit a positive inotropic response, trabeculae of the right ventricle have an overall negative inotropic response to α_1 -adrenergic stimulation (Wang et al. 2006). We were therefore interested to see if the APD response differed between ventricles. However, in both ventricles we observed an increase in APD of a similar magnitude





Fig. 5. α_1 -adrenergic stimulation prolongs ventricular action potential duration in left and right ventricles. (A) and (B) show individual optical action potentials from the base of the left and right ventricle at a cycle interval of 180 ms. (C) and (D) show the results of protocol 1 (with phenylephrine). N = 4 preparations and for all measurements for control and propranolol conditions. After phenylephrine, N = 2 for 140 and 120 ms cycle intervals. After phentolamine, N = 3 for frequencies ≤ 180 ms and N = 1 at 120 ms. (E) and (F) show the results of protocol 2 (with noradrenaline). N = 5 for all measurements except for after phentolamine (N = 4). Asterisks significant effect (p < 0.05) of noradrenaline (i.e., propranolol vs. noradrenaline). Double daggers significant difference between noradrenaline and phentolamine (p < 0.05). Data are means \pm SEM.



response to α_1 -adrenergic stimulation and the effects were similar regardless of whether the adrenergic stimulus was phenylephrine or noradrenaline in the presence of propranolol (Fig. 5).

The ionic basis for the prolonging of APD that we report remains to be dissected. Although Nishimaru et al. (2001) observed a decrease in APD with α_1 -adrenergic stimulation, they reported an increased L-type Ca²⁺ current, which may be an effective means to increase APD in the mouse heart (Wang et al. 2019). The α_1 -adrenergic regulation of the L-type Ca²⁺ current in mammalian myocardium has proven to be particularly controversial (van der Heyden et al. 2005). Increased L-type Ca²⁺ current was initially suggested to be involved in the prolonged APD in several species (Miura et al. 1978; Brückner and Scholz 1984), but was subsequently largely discredited in favour of reduced outward currents (Apkon and Nerbonne 1988; Hartmann et al. 1988; Hescheler et al. 1988; Tohse et al. 1990; Endoh et al. 1991; Fedida and Bouchard 1992). Later work, however, indicated that the regulation of the L-type Ca²⁺ current is particularly sensitive to disturbances to intracellular milieu with conventional whole-cell patch-clamp techniques. With perforated patch-clamp, α -adrenergic stimulation has been convincingly shown to directly increase L-type Ca²⁺ current in rat ventricular myocytes (Liu and Kennedy 1998; Zhang et al. 1998, 2005; O-Uchi et al. 2005).

Recently, it was demonstrated that a transient prolonging of APD during sympathetic stimulation is optimised to increase calcium transient amplitude in the mouse heart (Wang et al. 2019). This effect was abolished by propranolol, suggesting that it is primarily mediated by β -ARs. However, this study only considered the effects of 1 min of sympathetic stimulation. The complex effects of α_1 -AR stimulation, however, develop more slowly than β -ARs (Baker 2014), perhaps because the receptors reside in the nucleus and hence require prior internalisation of the signal (O'Connell et al. 2014). Indeed, with α_1 -adrenergic stimulation of the mouse ventricle, during the first few minutes a negative inotropic effect dominates α -adrenergic stimulation, even when a positive inotropic effect ultimately transpires (Ross et al. 2003; Wang et al. 2006). Our data thus complement the study of Wang et al. (2019), and suggest that during tonic adrenergic stimulation, as may occur during more extended bouts of exercise, α_1 -ARs may reinforce and fine-tune the prolonging of APD.

Limitations

Optical mapping during Langendorff-perfusion is the only method that allows the measurement of APD and repolarization differences on the epicardial surface of the mouse heart. To reduce motion artefacts in the optical signals the heart was immobilized by administering blebbistatin. It has been proposed that blebbistatin affects intracellular Ca²⁺ homeostasis as it inhibits myosin ATPase-II, and the actin-myosin interaction relies on Ca²⁺. However, L-type Ca²⁺ current appears unaffected in the mouse ventricle in the presence of blebbistatin (Dou et al. 2007). In addition, in our experiments the heart was retrograde perfused at a constant rate. In the in vivo situation, α -adrenergic stimulation elicits profound effects on the vasculature, including arterial vasoconstriction and venoconstriction (Joyce and Wang 2020), which would also influence cardiac performance. However, we believe our strategy was well suited to studying the direct pharmacological effects of α_1 -adrenergic stimulation of the ventricle.

Future directions

Due to the nature of our protocol involving sequential drug administrations, we were restricted to making a single measurement of APD 10 min after the initiation of each treatment. This was intended to allow us to focus on the effects of the drug treatments once they reached steady state (Ross et al. 2003; Wang et al. 2006). However, in doing so we neglected the early stages of α_1 -AR signalling, which may be relevant during brief periods of adrenergic stimulation. Multiphasic contractile responses are known to occur in the mouse heart during α -adrenergic stimulation in the initial phases



(Ross et al. 2003; Wang et al. 2006), so it may prove fruitful for future work to study the time course in greater temporal resolution to reveal how APD may change in the mouse heart during immediate and sustained stimulation. In addition, unravelling the mechanistic basis for the increased APD in both ventricles of the mouse heart reported here may provide clarification on the complex role of L-type Ca^{2+} current in the myocardium of mammals (van der Heyden et al. 2005). α_1 -AR signalling is known to activate protein kinase C (Talosi and Kranias 1992) and Ca^{2+} /calmodulin-dependent PK II (CaMKII) (O-Uchi et al. 2005), which in turn will catalyse the phosphorylation of a host of proteins that may directly or indirectly affect APD, and could be identified by future work. The APD prolongation may also depend on stimulation of phosphoinositide hydrolysis (Endoh et al. 1991).

Conclusions

Our data show that epicardial action potentials prolong in response to α -AR stimulation in the intact mouse heart. This suggests the electrophysiological response to α -AR stimulation in the mouse heart could share more similarities with other mammals than previously acknowledged, consolidating it as a model to advance our understanding of general α -adrenergic control.

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Author contributions

WJ and BJB conceived and designed the study. WJ, KTS, and BJB performed the experiments/collected the data. WJ and BJB analyzed and interpreted the data. KTS, BJ, TW, and BJB contributed resources. WJ, KTS, BJ, TW, and BJB drafted or revised the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Data availability statement

All relevant data are within the paper and in the Supplementary Material.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2020-0081.

Supplementary Material 1

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