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ß-Adrenergic receptor signaling increases NAADP and cADPR levels in the heart

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ABSTRACT

Evidence suggests that ß-Adrenergic receptor signaling increases heart rate and force through not just cyclic AMP but also the Ca^{2+}-releasing second messengers NAADP (nicotinic acid adenine dinucleotide phosphate) and cADPR (cyclic ADP-ribose). Nevertheless, proof of the physiological relevance of these messengers requires direct measurements of their levels in response to receptor stimulation. Here we report that in intact Langendorff-perfused hearts ß-adrenergic stimulation increased both messengers, with NAADP being transient and cADPR being sustained. Both NAADP and cADPR have physiological and therefore pathological relevance by providing alternative drug targets in the ß-adrenergic receptor signaling pathway.

1. Introduction

Although the principle process driving contraction of cardiac myocytes is Ca^{2+}-induced Ca^{2+} release, the rate and force of contraction are regulated by the second messenger cyclic AMP [1]. In addition to cyclic AMP, evidence is accumulating that the Ca^{2+}-releasing second messengers cyclic ADP-ribose (cADPR) [2–4] and nicotinic acid adenine dinucleotide phosphate (NAADP) [5] also affect rate and force of contraction. Both cADPR [2–4,6,7] and NAADP [5] enhance Ca^{2+} transient amplitude and duration, as well as the force of contraction during excitation–contraction coupling.

Cyclic AMP levels are under the control of the autonomic nervous system, and decrease upon stimulation of muscarinic receptors and increase upon stimulation of ß adrenergic receptors. However, ß adrenergic receptor stimulation might increase rate and force of contraction through multiple redundant pathways. Moderate stimulation of the ß adrenergic receptor results in potentiation of contraction and intense stimulation induces arrhythmias, both of which can be reversed with cADPR antagonists [8]. Additionally, ß adrenergic receptors couple to the enzyme ADP-ribosyl cyclase [9]. Unusually, this enzyme produces both cADPR and NAADP [10,11]. Consistent with this, ß adrenergic receptor stimulation is coupled to NAADP synthesis [5]. Intriguingly, several inhibitors of ADP-ribosyl cyclase, identified with a high-throughput screen, were found to be anti-arrhythmic in a porcine cardiac model [12]. Surprisingly, there are no reports of ß adrenergic receptor stimulation on cADPR levels and a single report on NAADP levels [5]. We now report that in Langendorff-perfused hearts, ß-adrenergic stimulation results in a transient increase in NAADP and a sustained increase in cADPR.

2. Materials and methods

2.1. Langendorff-perfused heart preparations

Guinea pig hearts were prepared on a langendorff apparatus as described previously [5]. The hearts were either perfused with a solution of 100 nM isoprenaline for a finite period of time or perfused with the physiological salt solution as controls. For some experiments we recorded heart rate and force of contraction to confirm the action of ß-adrenergic stimulation (Chart 5, AD Instruments).

2.2. Measurement of NAADP and cADPR levels

Total protein in each sample was determined using the standard bicinchoninic acid analysis microwell technique (Pierce) using bovine serum albumen as the standard as reported previously [13]. Sea urchin egg homogenate was prepared as described previously [14]. [^{32}P]cADPR was synthesized as described

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previously [10,15] and separated in the same manner as [^{32}P]NAADP [13]. cADPR levels were measured as previously described [16,17]. [^{32}P]NAADP was synthesized in a two-step reaction as described previously [18,19]. NAADP levels were measured as reported in detail previously [13].

2.3. Statistics

Data are presented as mean ± standard error of the mean. For comparison between two groups of equal variance, a *t*-test was used as either one-tailed or two-tailed and either paired or unpaired as appropriate. If variance was not equal between the groups being compared (Bartlett's Test), then a *t*-test with Welch's correction was used. For multiple comparison between groups, data were compared with a one-way analysis of variance and if significant at $p \leqslant 0.05$, differences between means were evaluated by Dunnett's Multiple Comparison Test against the control. All statistical tests were performed with GraphPad Prism 4.0 for the Mac (GraphPad Software, La Jolla, CA, USA).

3. Results and discussion

3.1. ß-Adrenergic receptor stimulation increases cADPR

To investigate the effect of stimulating ß-adrenergic receptors on cADPR levels over time in cardiac tissue, we used Langendorff-perfused whole hearts [5]. Addition of isoprenaline (100 nM) to intact hearts resulted in an increase in cADPR levels that persisted for at least 5 min (Fig. 1A). This sustained increase in the level of cADPR (Fig. 1A) represents a signature for cADPR in response to ß-adrenergic stimulation in cardiac cells. This cADPR signature is similar to that reported during stimulation of pancreatic acinar cells with cholecystokinin [20], a T-cell line with antigen [21], urchin eggs with sperm [22,23] and pancreatic ß-cells with glucose [24], and thus may be a universal signature for agonist-induced increases in cADPR. The sustained increase in cADPR (Fig. 1A) is consistent with one of the dual effects of cADPR in which there is an increase in the Ca^{2+} load of the sarcoplasmic reticulum which takes minutes to develop [4]. At the 3-min time point, when cADPR levels had plateaued (Fig. 1A), the cADPR response to isoprenaline was concentration-dependent with an EC_{50} of about 30 nM (Fig. 1B). These increases in cADPR levels are support results reported previously showing ß-adrenergic receptor stimulation increasing ADP-ribosyl cyclase activity and signaling through cADPR and ryanodine receptors [8,9,25,26]. To validate our results with the radioreceptor assay, we also employed the cycling assay [17] to measure cADPR. The absolute cADPR levels per mg protein are within 30% of one another in resting tissue, and, most importantly, the increase in cADPR upon isoprenaline stimulation is replicated (Fig. 1C and D). On an absolute basis, the resting cADPR levels of about 10 pmol/mg protein (Fig. 1) are on the high side of those reported for most tissues of about 1–2 pmol/mg protein [17] including heart from which ranges from about 0.1–20 pmol/mg protein [16,17,26–29]. This range may represent a species difference or different physiolgoical states as suggested previously [27]. ß-Adrenergic receptor stimulation increased cADPR by about 2–3-fold, which, as the first report, can only be compared to increases reported for other agonists of 2–5-fold [20,22–24,30–33].

3.2. ß-Adrenergic receptor stimulation increases NAADP

ß-Adrenergic receptor signaling has been linked to NAADP signaling and we reported previously an increase in NAADP levels

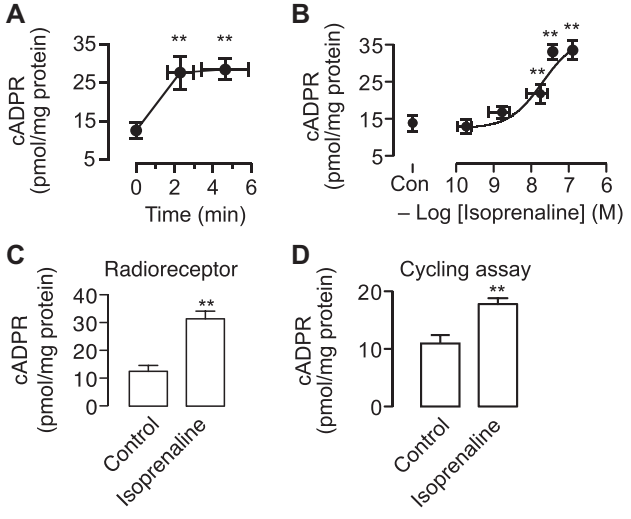

Fig. 1. Isoprenaline increases cADPR levels in intact Langendorff-perfused heart from guinea pig. (A) Isoprenaline (100 nM) increases cADPR levels over time. Values are presented as mean ± standard error of the mean. Circles marked with asterisks are significantly different from the time zero based on Dunnett's Multiple Comparison Test ($p < 0.01$) after a significant one-way analysis of variance ($p = 0.0004$). (B) Isoprenaline-mediated increases in cADPR levels are dependent on concentration. Samples were taken 3 min after isoprenaline addition. Values are the mean ± standard error of the mean, $n = 3$–5. Data were compared statistically with a one-way analysis of variance ($p < 0.001$) followed by Dunnett's Multiple Comparison Test against the control (Con). Circles marked with asterisks indicate $p < 0.05$ compared to control. (C, D) Summary histograms showing that 100 nM isoprenaline increases cADPR levels at 3 min. Amounts of cADPR were determined with either a (C) radioreceptor binding assay or an (D) enzymatic cycling assay. Values are the mean ± standard error of the mean, $n = 4$–5, $p < 0.05$, one-tailed *t*-test.

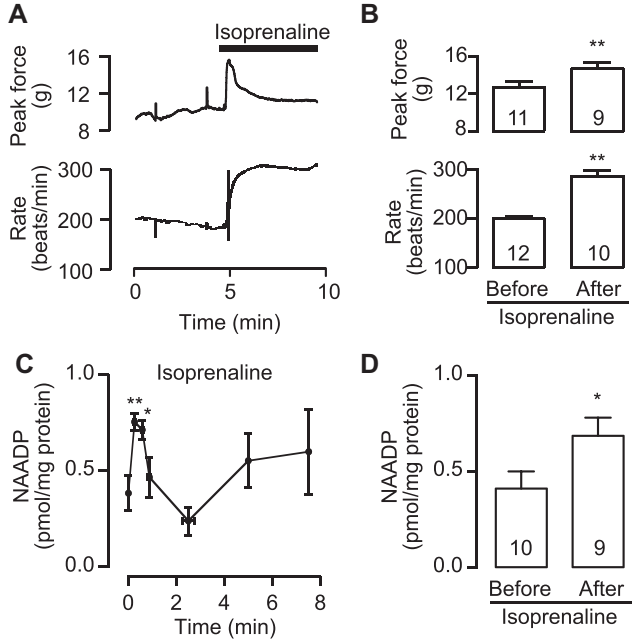

Fig. 2. Isoprenaline increases NAADP levels in intact Langendorff-perfused heart from guinea pig. (A) The effect of 100 nM isoprenaline on the rate and force of contraction illustrated by (A) a trace from an individual heart and (B) the pooled experimental data with *n* shown on the bars. (C) Isoprenaline (100 nM) increases NAADP levels over time. Values are presented as mean ± standard error of the mean. Circles marked with asterisks are significantly different from the time zero based on Dunnett's Multiple Comparison Test ($p < 0.01$) after a significant one-way analysis of variance ($p = 0.0004$). (D) Summary histograms showing that peak NAADP levels are higher after isoprenaline (100 nM). Values are the mean ± standard error of the mean, $n = 4$–5, $^{*}p < 0.05$, one-tailed *t*-test.

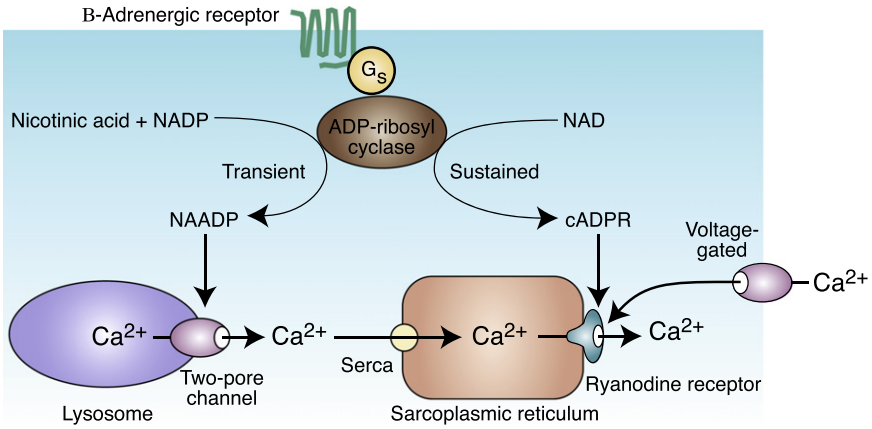

Fig. 3. Model depicting the possible effects of ß-adrenergic stimulation on the Ca^{2+}-releasing messengers cADPR and NAADP. ß-Adrenergic receptors are shown to link to ADP-ribosyl cyclase directly, however, this is done for simplicity in the diagram and this may require cyclic AMP and protein kinase A. ADP-ribosyl cyclase synthesis of NAADP is transient. The Ca^{2+} released from the lysosome is taken up by the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (serca) pump which increases the Ca^{2+} load of the sarcoplasmic reticulum. ADP-ribosyl cyclase synthesis of cADPR is sustained. This results in an increase in cADPR that sensitizes the ryanodine receptors to Ca^{2+}-induced Ca^{2+} release initiated by an influx of Ca^{2+} through voltage-gated Ca^{2+} channels in the plasma membrane upon depolarization. Both NAADP and cADPR serve to increase the amount of Ca^{2+} released during each depolarization resulting in increased inotropy and contractile force.

measured 5 min after isoprenaline stimulation of Langendorff-perfused hearts [5]. Given that NAADP increases are rapid and transient in response to other hormones and neurotransmitters in other cell types [20,21,34–36], we investigated the time course for NAADP changes in response to ß-adrenergic receptor signaling. Isoprenaline perfusion increased both heart rate and force (Fig. 2A and B), confirming that isoprenaline was having the expected effect on the heart physiology [1]. Isoprenaline induced a rapid and transient increase in NAADP levels that peaked at 15 s and returned to baseline by about 2 min (Fig. 2C). This reflects the NAADP signature commonly reported in other cell types [20,21,34–36]. On an absolute basis, the resting NAADP levels of about 0.4 pmol/mg protein (Fig. 2) are within the published range, albeit broad, for other tissues of about 0.1–10 pmol/mg protein [5,18–20,24,29,34–42]. Although our resting NAADP level value is much lower than a previous determination in the guinea pig heart using a bioassay [5], the relative increases upon agonist stimulation are similar of about 2-fold. This relative increase in NAADP is on the lower side of the range, as NAADP increases in response to agonists other than beta-adrenergic of 2–80-fold [5,20,24,29,34–42].

3.3. Roles of cADPR and NAADP in heart

Previous reports have demonstrated in the heart effects of cADPR [2–4,7–9,19,25,26,43,44] and NAADP [5,43]. We have now demonstrated that ß-adrenergic receptor stimulation results in increases in both cADPR and NAADP, which provides strong direct evidence for the physiological relevance of these messengers in the heart. Moreover, these finding add weight to the emerging concept that ß-adrenergic-mediated increases in rate and force arise from several redundant mechanisms including not just the classical protein kinase A phosphorylation of phospholamban and L-type Ca^{2+} channels [1]. The canonical ß-adrenergic receptor signaling pathway using cyclic AMP has provided numeours validated drug targets. As this pathway also couples to NAADP and cADPR, these pathways provide several alternative drug targets. Indeed, inhibitors of the ADP-ribosyl cyclase are anti-arrhythmics [12].

In this paper we present evidence that the agonist-induced temporal profiles of cADPR and NAADP are remarkably similar to those in nonexcitable cells. In contrast to the role of NAADP in nonexcitable cell types, in which it functions as a trigger to initiate the Ca^{2+}-induced Ca^{2+} release, the data support a model whereby NAADP increases the Ca^{2+} load of the sarcoplasmic reticulum (Fig. 3), as reported previously [5]. The transient increase in NAADP leads to an initial pulse of Ca^{2+} being released through two-pore channels [45–47] on lysosome-related organelles [48,49]. Such Ca^{2+} is rapidly sequestered by Ca^{2+} pumps on the sarcoplasmic reticulum leading to an increase in Ca^{2+} load and more Ca^{2+} release during ryanodine receptor activation [5], which is driven by plasma membrane depolarization. An increase in sarcoplasmic reticulum Ca^{2+} loading increases inotropy.

Acknowledgments

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