INTRODUCTION

NAADP (nicotinic acid–adenine dinucleotide phosphate) is a potent mobilizer of intracellular Ca\(^{2+}\) stores [1,2]. The actions of NAADP were first described in sea urchin eggs where it targets a unique Ca\(^{2+}\) channel located on Ca\(^{2+}\) stores insensitive to IP\(_3\) (inositol 1,4,5-trisphosphate) and/or ryanodine receptors [3–5]. The NAADP-sensitive Ca\(^{2+}\) channel has yet to be unequivocally defined at the molecular level [6], but the stores have been identified as acidic, lysosome-like organelles in most preparations studied [7]. Indeed, in a variety of cells, NAADP-induced Ca\(^{2+}\) release is inhibited by agents that interfere with acidic compartments, such as bafilomycin A1, a highly selective inhibitor of V-type H\(^{+}\)-ATPases [8]. These proteins are expressed on several acidic organelles and are responsible for generation of proton gradients thought to drive Ca\(^{2+}\) uptake [9–15]. One remarkable feature of the release process is that activation of NAADP receptors is invariably associated with the recruitment of IP\(_3\), and/or ryanodine receptors via Ca\(^{2+}\)-induced Ca\(^{2+}\) release [11,16–20]. Thus NAADP may serve to co-ordinate the activity of Ca\(^{2+}\)-sensitive ER-based Ca\(^{2+}\) channels [2,21,22]. That several extracellular stimuli have now been demonstrated to increase cellular NAADP levels [9,14,20,23–27] establishes NAADP as a bona fide Ca\(^{2+}\)-mobilizing messenger critical for the generation of agonist-specific Ca\(^{2+}\) signals.

Although the mechanism by which NAADP mediates release of Ca\(^{2+}\) mobilization has been intensively investigated, much less explored are the possible effects of NAADP on plasma membrane channels. In both mature starfish oocytes [28] and sea urchin eggs [23], NAADP responses initiate in the cell peripherally. In starfish, NAADP-mediated Ca\(^{2+}\) signals require functional IP\(_3\) or ryanodine receptors for propagation; however, they are heavily reliant on extracellular Ca\(^{2+}\) [19]. These results suggest that NAADP-mediated Ca\(^{2+}\) influx is the primary trigger. In the sea urchin, the first detectable NAADP-mediated event is a small, highly localized and rapid cortical ‘flash’ that is followed by a Ca\(^{2+}\) wave which then traverses the cell entirety [23]. Removing extracellular Ca\(^{2+}\) from these cells blocks the former response only [23], again suggesting that NAADP mediates Ca\(^{2+}\) influx. A dependence for extracellular Ca\(^{2+}\) in NAADP-mediated Ca\(^{2+}\) signalling has also been noted in T-lymphocytes [29], and NAADP has been demonstrated to activate TRPM2 (transient receptor potential cation channel, subfamily M member 2) channels [30], although relatively high concentrations are required to achieve the latter. In all cases, however, whether the observed influx is due to activation of a plasmalemmal NAADP-sensitive Ca\(^{2+}\) channel or mediated indirectly through prior store release is not clear.

There is increasing evidence that NAADP is an important second messenger in the nervous system [22,31]. NAADP triggers Ca\(^{2+}\) release from brain microsomes [32] and cultured cortical neurons [11], and has been implicated in important neuronal functions, such as neurosecretion [33,34], neurite outgrowth [11] and neuronal differentiation [12]. NAADP might also function as an extracellular messenger in several neural cell types [35]. Our previous autoradiographic analysis revealed wide and heterogeneous distribution of NAADP binding sites throughout the mammalian brain with particularly high levels noted in the

NAADP (nicotinic acid–adenine dinucleotide phosphate) is a potent Ca\(^{2+}\)-mobilizing messenger that stimulates Ca\(^{2+}\) release in a variety of cells. NAADP-sensitive Ca\(^{2+}\) channels are thought to reside on acidic Ca\(^{2+}\) stores and to be functionally coupled to IP\(_3\), (inositol 1,4,5-trisphosphate) and/or ryanodine receptors located on the endoplasmic reticulum. Whether NAADP-sensitive Ca\(^{2+}\) channels ‘chatter’ to other channels, however, is not clear. In the present study, we have used a cell-permeant NAADP analogue to probe NAADP-mediated responses in rat medulla oblongata neurons. NAADP-AM (NAADP-acetoxyethyl ester) evoked global cytosolic Ca\(^{2+}\) signals in isolated neurons that were reduced in amplitude by removal of external Ca\(^{2+}\), abolished by disruption of acidic compartments and substantially inhibited by blockade of ryanodine receptors. In rat medullary slices, NAADP-AM depolarized neurons from the nucleus ambiguus in the presence of intracellular EGTA, but not of the faster Ca\(^{2+}\) chelator BAPTA [1,2-bis(\(\rho\)-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid]. Depolarization was also dependent upon extracellular Ca\(^{2+}\), acidic stores and ryanodine receptors. In voltage-clamp mode, NAADP-AM induced an inward current with a reversal potential of approx. 0 mV. The results of the present study reveal the presence of acidic NAADP-sensitive Ca\(^{2+}\) stores in medulla neurons, the mobilization of which results not only in global Ca\(^{2+}\) signals but also in local signals that activate non-selective cation channels on the cell surface resulting in depolarization. Thus NAADP is capable of co-ordinating channels both within the cell interior and at the cell membrane representing a novel mechanism for excitation of central neurons.

Key words: calcium, nicotinic acid–adenine dinucleotide phosphate (NAADP), non-selective cation channel, patch-clamp.
medulla [36]. This region is crucial for the regulation of autonomic functions. Parasympathetic regulation of heart rate, for example, is mediated by cardiac vagal neurons located within the nucleus ambiguus [37].

In the present study, we used a newly described cell-permeant analogue of NAADP [20,38] to probe the role of NAADP in medulla neurons using both dissociated and slice preparations. We provide evidence for the presence of functional NAADP receptors located on acidic Ca\(^{2+}\) stores and for coupling of these channels to channels on the plasma membrane. This newly described form of ‘chatter’ [21] may provide a novel means of excitation of autonomic neurons.

EXPERIMENTAL

NAADP-AM synthesis

NAADP (0.030 g, 0.040 mmol) was evaporated 2-3 times with 0.5 ml of DIEA (di-isopropylethylamine) to form the diisopropylethylammonium salt. The salt was then dissolved in 2 ml of acetonitrile. To this was added DIEA (0.026 g, 0.201 mmol) and the mixture was stirred at room temperature (20 ± 1°C) for 15 min under an argon atmosphere. Acetoxyethylene bromide (0.031, 0.201 mmol) was then slowly added to the above stirred solution and the resulting mixture was stirred at room temperature for 24 h. After the completion of the reaction, the solids were filtered off and the solution was evaporated to dryness to provide the acetoxymethyl derivative of NAADP as a light-yellow solid.

Neuronal culture

Neurons from medulla oblongata were dissociated from neonatal (1–3-day-old) Sprague–Dawley rats, by enzymatic digestion with papain followed by mechanical trituration, as previously described [11,39]. Experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee. After centrifugation at 500 g, fractions enriched in neurons were collected and re-suspended in Neurobasal-A medium (Invitrogen) containing 2 mM glutamine, 100 units/ml penicillin G, 100 μg/ml streptomycin and 10% (v/v) fetal bovine serum. Cells were plated on round glass coverslips in 24-well plates. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO\(_2\).

Localization of acidic compartments and ER in medulla oblongata neurons

Cultured neurons were incubated with LysoTracker Red (1 μM), ER-Tracker Green (1 μM), or a combination of both, for 30-60 min. In another series of experiments, neurons were incubated with LysoTracker Red (1 μM) following a 1 h pretreatment with either bafilomycin A1 (1 μM) or the vehicle DMSO (0.1%). Cells were fixed in 4% (w/v) paraformaldehyde, mounted with Citifluor and examined under a confocal laser-scanning microscope (Leica TCS SL) with excitation/emission wavelengths set to 488/520 nm and examined under a confocal laser-scanning microscope (Leica TCS SL) with excitation/emission wavelengths set to 488/520 nm and examined under a confocal laser-scanning microscope (Leica TCS SL) with excitation/emission wavelengths set to 488/520 nm and examined under a confocal laser-scanning microscope (Leica TCS SL) with excitation/emission wavelengths set to 488/520 nm. ".." was a yellow solid.

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[Ca\(^{2+}\)], was measured by the Ca\(^{2+}\)-imaging technique, as previously described [11,12]. Cultured neurons were loaded with the fluorescent Ca\(^{2+}\) indicator fura 2 (3 μM) by incubation of the cells in HBSS (Hanks balanced salt solution) plus fura 2/AM (fura 2 acetoxyethyl ester) for 45 min and HBSS alone for an additional 15–60 min to allow de-esterification of the dye. Coverslips were mounted in a 500 μl bath on the stage of a

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patch-clamp technique was similar to that described previously [39]. The hindbrain was dissected and placed in ice-cold Krebs solution (127 mM NaCl, 1.9 mM KCl, 1.2 mM KH2PO4, 2.4 mM CaCl2, 1.3 mM MgCl2, 26 mM NaHCO3 and 10 mM glucose) gassed with 95% O2 and 5% CO2. Brainstem slices of 200 μm thickness were prepared using a vibratome. In experiments where Ca2+-free Krebs solution was used, Ca2+ was omitted and EGTA (2.5 mM) was added. In some experiments, low external Na+ solution containing 26 mM NaCl and 127 mM NMDG (N-methyl-D-glucamine) was used. Recordings were conducted at room temperature from neurons in the ventral perimeter of nucleus ambiguus, previously identified as cardiac vagal neurons [42]. Patch electrodes pulled from thin-walled borosilicate glass capillaries were filled with a solution containing 130 mM potassium gluconate, 1 mM MgCl2, 2 mM CaCl2, 4 mM ATP, 0.3 mM GTP, 10 mM EGTA and 10 mM Hepes, and had a resistance of 2–5 MΩ; the pH of the solution was adjusted to 7.3. In some experiments EGTA was replaced with 10 mM BAPTA [1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetra-acetic acid]. In other experiments, K+ was replaced with Cs+ in intracellular solution. Signals were recorded using an Axopatch 1C amplifier (Axon Instruments/Molecular Devices) and a Digidata 1320 digitizer in voltage- or current-clamp mode, filtered at 2 kHz, displayed on a two-channel Gould chart recorder RS 3200. Experimental protocols were controlled and data were acquired by a personal computer using Clampex 8.0 software (Axon Instruments). Steady-state I–V (current–voltage) relationships of NAADP-AM-induced currents were investigated in rat nucleus ambiguus neurons voltage-clamped to −60 mV. The I–V relationships were obtained by applying a series of 400 ms voltage command steps every 5 s from a holding potential of −60 mV to potentials varying from −120 to +60 mV, with 10 mV increments, before and during the superfusion of NAADP-AM. Currents elicited by each voltage command step in control medium were subtracted from their counterparts in the presence of NAADP-AM to yield a steady-state I–V curve of NAADP-AM-induced currents.

**Chemicals**

ER-Tracker Green, LysoTracker Red and fura 2/AM were from Molecular Probes; ryanodine, bafilomycin A1, xestospongin C, charybdotoxin and apamin were from Calbiochem and NMDG was from Sigma–Aldrich.

**Statistical analysis**

A paired Student’s t test followed by one-way ANOVA was used to evaluate significant differences between controls and NAADP-AM-treated neurons. *P < 0.05* was considered statistically significant.

**RESULTS AND DISCUSSION**

We first measured [Ca2+], in isolated medulla oblongata neurons loaded with the fluorescent Ca2+ indicator, fura 2. Basal [Ca2+]i, was 86 ± 2.6 nM (n = 126). To test for the presence of NAADP-sensitive Ca2+ channels, cells were perfused with NAADP-AM, a cell-permeant analogue of NAADP that has recently been shown to induce Ca2+ signals in cortical neurons, cardiomyocytes and lymphoblasts [20,38,43]. In medulla oblongata neurons, NAADP-AM (500 nM) increased [Ca2+]i in 84 out of the 126 cells examined (Figure 1). Two profiles of [Ca2+]i increase were identified: (i) [Ca2+]i oscillations with a mean amplitude increase of 417 ± 7.3 nM (n = 63, Figure 1A) and (ii) slow and sustained increase in [Ca2+]i, with a mean amplitude of 483 ± 9.4 nM (n = 21, Figure 1B). These results provide direct evidence for the presence of functional NAADP receptors in the medulla neurons consistent with our previous autoradiography analysis [36]. In Ca2+-free saline, application of NAADP-AM (500 nM) produced either [Ca2+]i oscillations (Figure 1C, solid line; n = 26/35) or rapid transient responses (Figure 1C, broken line; n = 9/35). Thus NAADP mobilizes intracellular Ca2+ stores as shown previously [9,16]. The mean amplitude of both responses in Ca2+-free medium (302 ± 4.6 nM, n = 26 and 327 ± 3.4 nM, n = 9) was,
however, reduced relative to those in Ca\textsuperscript{2+}-containing medium (see above). These results indicate that NAADP stimulates both Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} influx.

In many intact cells, NAADP-mediated Ca\textsuperscript{2+} signals are sensitive to inhibitors of IP\textsubscript{3}/ryanodine receptors or depletion of ER Ca\textsuperscript{2+}-store release [11,16–20]. These results are consistent with functional coupling of NAADP receptors to ER Ca\textsuperscript{2+} channels, but evidence for a more direct effect of NAADP on ryanodine receptors has also been proposed [44]. That NAADP-mediated Ca\textsuperscript{2+} signals in several cell types are also inhibited by interfering with acidic compartments [7,9–15], however, is inconsistent with the latter mechanism. To determine the mechanism of action of NAADP in medulla neurons, we examined the effects of the V-type H\textsuperscript{+}-ATPase inhibitor bafilomycin A1 on NAADP responses. As shown in Figure 1(D), pretreatment with bafilomycin A1 (1 \textmu M, 60 min) completely abrogated the response to NAADP-AM (\(n = 114\)). These results suggest that NAADP targets acidic Ca\textsuperscript{2+} stores. To define the contribution of ER Ca\textsuperscript{2+} channels to NAADP responses, we examined the effect of pre-treating cells with the ryanodine receptor antagonist, ryanodine (1 \textmu M, 10 min) or the IP\textsubscript{3} receptor blocker, xestospongin C (10 \textmu M, 15 min) on the responses to NAADP. As shown in Figure 1(E), only small amplitude (168 ± 3.4 nM; \(n = 76/107\)) transient responses were observed in response to NAADP-AM in the presence of ryanodine (Figure 1E). In marked contrast, NAADP responses in Ca\textsuperscript{2+}-free saline were unaffected by xestospongin C; both Ca\textsuperscript{2+} oscillations (\(n = 25/33\); Figure 1F, solid line) and transient responses (\(n = 8/33\); Figure 1F, broken line) were observed that were similar in magnitude (308 ± 3.7 nM, \(n = 25\) and 319 ± 4.2 nM, \(n = 8\)) to those in the absence of the drug (Figure 1C). Xestospongin C inhibited Ca\textsuperscript{2+} responses to microinjected IP\textsubscript{3} by 72% (\(n = 6\); Supplementary Figure 1 at http://www.BiochemJ.org/bj/419/bj4190091add.htm). Taken together, the above results suggest that NAADP receptors in medulla neurons are localized on acidic Ca\textsuperscript{2+} stores, the activation of which results in selective recruitment of ryanodine receptors on the ER in a manner similar to that reported in smooth muscle cells [9].

To determine the subcellular localization of acidic compartments in medulla neurons, we performed confocal microscopy of cells loaded with fluorescent weak base LysoTracker Red. As shown in Figure 2, small discrete vesicles arranged in either a perinuclear fashion (Figure 2A) or distributed more sparsely throughout the cytoplasm (Figures 2B and 2E) were observed. Pretreatment with bafilomycin A1 (1 \textmu M, 60 min) substantially reduced staining, as expected (Figure 2C). In order to compare the distribution of acidic compartments with the ER, we imaged cells co-loaded with LysoTracker and ER-Tracker. As shown in Figures 2(D)–2(F), the distribution of the two organelles was distinct with individual acidic vesicles surrounded by the dense ER.

The reduction of NAADP-mediated Ca\textsuperscript{2+} signals upon removal of extracellular Ca\textsuperscript{2+} (Figure 1C) prompted us to probe for possible functional coupling of NAADP receptors to plasma membrane channels. We therefore measured electrical responses in nucleus ambiguous neurons in brain stem slices by the whole-cell patch-clamp technique. The mean resting membrane potential and input resistance of nucleus ambiguous neurons were −55.9 ± 1.3 M\Omega and 715.4 ± 45.9 M\Omega respectively (\(n = 58\)). Nearly all neurons were silent; and injection of depolarizing currents (100–500 pA, 300 ms) elicited repetitive firings followed by a hyperpolarization (results not shown) as previously reported [45]. In the presence of tetrodotoxin (1 \textmu M), NAADP-AM (500 nM) application depolarized 11 out of 19 neurons studied. The response of an exemplar neuron is shown in Figure 3(A), where NAADP-AM caused a depolarization of 10 mV. NAADP-mediated depolarization was reduced in amplitude in Ca\textsuperscript{2+}-free saline (Figure 3B), consistent with possible Ca\textsuperscript{2+} entry. Because all of the above experiments were performed in the presence of intracellular EGTA, the effects of NAADP-AM on membrane potential are unlikely to be mediated by global changes in [Ca\textsuperscript{2+}], since these would be effectively buffered by the chelator [46]. We therefore considered the possibility that the effects of NAADP may be due to local [Ca\textsuperscript{2+}], changes. To test this, we performed patch-clamp experiments using an intracellular solution containing the fast Ca\textsuperscript{2+} chelator BAPTA in place of EGTA [46]. Under these conditions, cells failed to depolarize in response to NAADP-AM (Figures 3C and 3F). These results suggest that the effects of NAADP on membrane potential are not direct, but instead...
NAADP activates rat medulla oblongata neurons

Figure 4 Effect of NAADP-AM on whole-cell currents recorded in nucleus ambiguus neurons

(A and B) Typical whole-cell currents before (-NAADP-AM) and during (+NAADP-AM) administration of NAADP-AM, in response to voltage command steps from a holding potential of −60 mV to potentials varying from −120 mV to +60 mV (D). Steady-state I–V relationships before (C) and during (+NAADP-AM) superfusion are shown in (D) and the difference in currents in (E). (F–I) Pooled difference currents (means ± S.E.M.) from four to six neurons perfused with NAADP-AM in control Krebs solution (F), Ca²⁺-free Krebs solution and BAPTA-containing intracellular solution (G), control Krebs solution supplemented with ryanodine (1 μM) 10 min prior to recording (H) and modified Krebs solution in which NaCl was replaced with NMDG (I).

Secondary, to local changes in cytosolic Ca²⁺. To probe the role of acidic Ca²⁺ stores in this process, cells were pretreated with bafilomycin A1 (1 μM, 60 min) prior to NAADP-AM challenge. As shown in Figure 3(D), depolarization in response to NAADP-AM was abolished. The effects of NAADP-AM on membrane potential were also inhibited by pretreatment with ryanodine (1 μM, 10 min; Figure 3E). Thus the pharmacology of NAADP-mediated depolarization (summarized in Figure 3F) and NAADP-mediated changes in global cytosolic Ca²⁺ (Figure 1) are similar. Combined with the sensitivity of NAADP-mediated depolarization to slow and fast Ca²⁺ chelators, we conclude that the effects of NAADP on membrane potential are due to coupled activation of subplasmalemmal NAADP-sensitive Ca²⁺ channels and ryanodine receptors. Thus Ca²⁺ entry through this pathway might contribute to global cytosolic Ca²⁺ changes to NAADP, perhaps in conjunction with store-operated Ca²⁺ entry (Figure 1). Although our localization studies suggested that acidic compartments in these cells are predominantly cytoplasmic (Figure 2) it is possible that a small subpopulation of peripheral acidic vesicles might not be resolvable using LysoTracker. Indeed, even in the large eggs of the sea urchin, acidic vesicles are relatively uniformly distributed, yet imaging of luminal pH clearly demonstrates heterogeneity in the spatial organization of NAADP-sensitive Ca²⁺ stores [47].

To further investigate the mechanism of NAADP-mediated depolarization, we examined current–voltage relationships. Subtraction of the two I–V curves obtained before and during perfusion with NAADP-AM revealed the presence of an inward current activated by NAADP (Figures 4A–4F). The mean reversal potential was ~0 mV (n = 6). As with depolarization,
the NAADP-induced currents were markedly reduced in the presence of intracellular BAPTA in Ca\textsuperscript{2+}-free saline (Figure 4G) and following pretreatment with ryanodine (Figure 4H). Since in our experimental conditions, the equilibrium potential for K\textsuperscript{+} is −98 mV and for Na\textsuperscript{+} is +56 mV, the reversal potential of the NAADP-AM-induced current suggests an involvement of a non-selective cationic conductance. Indeed, the inward component of the NAADP-AM-induced current was abolished and the reversal potential shifted to more negative values (−40 mV) when extracellular Na\textsuperscript{+} was replaced with NMDG (Figure 4I). Additionally, inward NAADP-mediated currents were readily detectable (but slightly reduced) when K\textsuperscript{+} in the intracellular solution was replaced with Cs\textsuperscript{+} to block voltage-gated K\textsuperscript{+} channels, and the slices were pretreated with apamin and charybdotoxin to block Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (see Supplementary Figure 2 at http://www.BiochemJ.org/bj/419/bj4190091add.htm). Although depolarization was clearly reduced upon removing extracellular Ca\textsuperscript{2+} (Figure 3B), we were unable to record an inward Ca\textsuperscript{2+} current in the presence of NMDG (Figure 4I). These results might suggest block of the channel by NMDG as reported for some non-selective cation channels [48] and/or the presence of an extracellular facilitatory Ca\textsuperscript{2+}-binding site. Taken together, these results demonstrate functional coupling of NAADP-sensitive Ca\textsuperscript{2+} channels to Ca\textsuperscript{2+}-activated non-selective cation channels.

NAADP-induced depolarization has previously been observed in starfish oocytes [49]; however, in those cells, the response was abolished upon removal of extracellular Ca\textsuperscript{2+} and the reversal potential of recorded currents was +50 mV suggesting activation of a Ca\textsuperscript{2+} current [50]. It is possible that the currents we observe are due to activation of TRPM2 channels which are Ca\textsuperscript{2+}-permeable non-selective cation channels which show little permeability in the presence of NMDG and which are regulated by both cytosolic and extracellular Ca\textsuperscript{2+} [51], consistent with the properties we describe (Figure 4). Indeed, TRPM2 channels are expressed in the medulla [52]. Although previous studies have proposed direct modulation of TRPM2 by NAADP [51], we note that whole-cell recordings were performed in intracellular solutions in which [Ca\textsuperscript{2+}]\textsubscript{i} was unbuffered, raising the possibility that the observed effects might be secondary store release as reported in the present study. Intriguingly, such a mechanism offers an alternative explanation for the sensitivity of NAADP-mediated currents to a cyclic ADP-ribose antagonist [51] if mobilization of NAADP-sensitive stores is coupled to activation of ryanodine receptors.

In summary, in the present study we provide evidence for the presence of acidic NAADP-sensitive Ca\textsuperscript{2+} stores in medulla neurons. We show that activation of NAADP receptors in these cells using a novel cell-permeant analogue is intimately associated with selective recruitment of an ER Ca\textsuperscript{2+} channel to mediate global cytosolic Ca\textsuperscript{2+} changes. Additionally, we provide new evidence for recruitment of non-selective cation channels on the cell surface by NAADP resulting in depolarization. Based on differential sensitivity of the latter to slow and fast Ca\textsuperscript{2+} chelators, we infer that this ‘chatter’ is due to local sub-membrane Ca\textsuperscript{2+} fluxes again involving co-ordinated mobilization of acidic and ER Ca\textsuperscript{2+} stores. Indeed, it is notable that such a cortical NAADP-sensitive store has been recently visualized in sea urchin eggs [47]. The dependence of the cortical flash in sea urchin eggs on both NAADP and L-type Ca\textsuperscript{2+} channels is consistent with NAADP acting indirectly, possibly through prior mobilization of cortical Ca\textsuperscript{2+} stores and subsequent depolarization. NAADP-mediated currents in starfish oocytes might also involve a cortical (ER) Ca\textsuperscript{2+} store based on their sensitivity to thapsigargin [50]. Thus strategic placing of NAADP-sensitive Ca\textsuperscript{2+} stores may allow co-ordination of signalling events not only in the cell interior, but also at the cell surface (Figure 5). NAADP-mediated depolarization described in the present study could provide a novel mechanism for excitation of cardiac vagal neurons possibly through NAADP synthesis via excitatory glutamatergic and/or cholinergic inputs [53].

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References


SUPPLEMENTARY ONLINE DATA

NAADP-mediated channel ‘chatter’ in neurons of the rat medulla oblongata

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Figure S1 Effect of xestospongin C on IP$_3$-mediated Ca$^{2+}$ responses

Cytosolic Ca$^{2+}$ responses (expressed as mean ratios of 340/380 nm fluorescence) of medullary neurons to intracellular injection of control solution (A; n = 4), or control solution supplemented with 10 μM IP$_3$ (B and C; n = 6). The neurons in (C) were pretreated with xestospongin C (10 μM) 15 min prior to injection (n = 6).

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Figure S2  Effect of K⁺-channel blockade on NAADP-mediated currents

(A and B) Typical whole-cell currents before (− NAADP-AM) and during (+ NAADP-AM) administration of NAADP-AM, in response to voltage command steps from a holding potential of −60 mV to potentials varying from −120 mV to +60 mV. Steady-state I–V relationships before (○) and during (●) NAADP-AM superfusion are shown in (C) and the difference in currents is shown in (D). (E) Pooled difference currents (mean ± S.E.M. from four neurons). K⁺ in the intracellular solution was replaced with Cs⁺ and slices were pretreated with apamin (APA, 5 μM) and charybdotoxin (ChTX, 50 nM).