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Sympathetic Neurogenic Ca^{2+} Signaling in Arteries: ATP, Noradrenaline and NPY

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Abstract

The sympathetic nervous system (SNS) plays an essential role in the control of total peripheral vascular resistance by controlling the contraction of small arteries. The SNS also exerts long-term trophic influences in health and disease; SNS hyperactivity accompanies most forms of human essential hypertension, obesity, and heart failure. At their junctions with smooth muscle cells, the peri-arterial sympathetic nerves release ATP, noradrenaline (NA) and neuropeptide Y (NPY) onto smooth muscle cells. Confocal Ca^{2+} imaging studies reveal that ATP and NA each produce unique types of post-junctional Ca^{2+} signals, and consequent smooth muscle cell contractions. Neurally released ATP activates post-junctional $P2X_1$ receptors to produce local, non-propagating Ca^{2+} transients, termed 'junctional Ca^{2+} transients', or jCaTs. Neurally released NA binds to α_1-adrenoceptors and can activate Ca^{2+} waves or more uniform global changes in $[Ca^{2+}]$. Neurally released NPY does not appear to produce Ca^{2+} transients directly, but significantly modulates NA-induced Ca^{2+} signaling. The neural release of ATP and NA, as judged by post-junctional Ca^{2+} signals, electrical recording of excitatory junction potentials (EJPs), and carbon fiber amperometry to measure NA, varies markedly with the pattern of nerve activity. This probably reflects both pre- and post-junctional mechanisms, which are not yet fully understood. These phenomena, together with different temporal patterns of sympathetic nerve activity in different regional circulations, are probably an important mechanistic basis of the important selective regulation of regional vascular resistance and blood flow by the sympathetic nervous system.

Keywords

sympathetic nerve; Ca^{2+} Signaling; arterial smooth muscle; receptors; junctional Ca^{2+} transients (jCaTs); confocal microscope

Introduction

All three sympathetic co-transmitters, ATP, NA, and NPY contribute to sympathetically mediated vasoconstriction of small arteries (Bradley *et al*., 2003). Abundant evidence supports the concept that, in arteries, neurally released ATP can activate a rapid, transient, component of smooth muscle contraction whilst neurally released NA activates slower, sustained and stronger contraction. These components of contraction are often referred to as the 'purinergic' and the 'adrenergic' component of neurogenic contraction, respectively. The actions and mechanisms of neurally released NPY remain more obscure; although NPY is a weak vasoconstrictor, it is likely that its primary (post-junctional) role is to modulate the actions of NA, and possibly, ATP.

The actions and relative contributions of each transmitter to sympathetic neuromuscular transmission vary markedly throughout the vascular system, and with the pattern of sympathetic nerve activity. For example, in the mesenteric vascular bed, the purinergic component of the contraction is relatively larger in the very small mesenteric arteries, compared to the larger ones, and the purinergic component predominates during brief bursts of sympathetic nerve fiber activity (Gitterman & Evans, 2001). In these arteries also, the relative importance of ATP as an activator of contraction may depend on arterial pressure (Rummery *et al*., 2007). Contraction of distal small arteries supplying skeletal muscle however does not seem to involve ATP at all (Tarasova *et al*., 2003). The varying contributions of the three sympathetic co-transmitters in different conditions and in different arteries is undoubtedly the result of many factors, including; 1) the unique frequency-dependence of release of each transmitter, 2) the identity, location and intracellular mechanisms of pre-and post-junctional receptors for each, and 3) the activity of mechanisms for terminating the actions of each transmitter.

Here, we focus on the Ca^{2+} signaling that is elicited in arterial smooth muscle cells by neurally released sympathetic neurotransmitters. As we have pointed out recently (Zang *et al*., 2006) Ca^{2+} signaling during neurogenic contractions activated by trains of sympathetic nerve fiber action potentials is, in fact, significantly different from that elicited by the simple application of exogenous neurotransmitters (both ATP and NA) to isolated arteries (or single isolated smooth muscle cells). Neurogenic Ca^{2+} signaling in some other types of smooth muscles, such as vas deferens (Brain *et al*., 2003) and urinary bladder (Heppner *et al*., 2005) has also been studied recently.

Results

jCaTs: The post-junctional Ca^{2+} transient elicited by neurally released ATP and $P2X_1$

Initial studies on neurogenic Ca^{2+} signaling in vascular smooth muscle utilized confocal imaging of Ca^{2+}-activated fluo-4 fluorescence in pressurized (70 mmHg) rat mesenteric small arteries subjected to electrical field stimulation (EFS). To facilitate imaging, low frequency (0.67 Hz), low voltage EFS was used to excite nerve fibers without causing an appreciable contraction. This was referred to as ‘sub-threshold’ EFS, as it was sub-threshold for muscle contraction. Thus, in these experiments, motion did not occur and the characteristics of neurogenic Ca^{2+} signals could be studied in detail. A novel type of Ca^{2+} transient, arising near nerve fibers, was observed (Fig. 1). These were called ‘junctional Ca^{2+} transients’ or ‘jCaTs’, as they appeared to represent the post-junctional response to release of sympathetic neurotransmitter. Nerve fiber Ca^{2+} transients were also observed. The results showed that 1) nerve fibers are excited by each EFS pulse; 2) jCaTs occur nearly simultaneously with an EFS pulse, 3) jCaTs occur near nerve fibers, and 4) jCaTs are events of very low probability. JCaTs are larger in spatial spread and last longer than spontaneous Ca^{2+} sparks (Jaggar *et al*., 2000). JCaTs always occurred with brief latency to the EFS pulse. The spatial full-width-at-half-maximum (FWHM) for jCaTs was 4.8μm, and the time taken to fall to half-amplitude, $t_{1/2}$, (from the peak) is 145ms. Unequivocal identification of the receptor(s) and ion channels that underlie jCaTs has been accomplished recently through the use of the $P2X_1$–receptor deficient mouse (Lamont *et al*., 2006). In the arteries of these animals, the P2X receptor agonist, α, β-methylene ATP elicited no response, and jCaTs were completely absent during nerve stimulation, confirming the absolute requirement for $P2X_1$ receptors in the post-junctional responses to ATP.

Neurogenic Ca^{2+} Signals in Contracting Arteries

As mentioned, some of the studies described above were obtained in pressurized small arteries that did not contract because the electrical stimulation was ‘sub-threshold’ for

contraction. However, neurogenic Ca^{2+} signals have also been observed during isometric neurogenic contractions. For these studies, segments of arteries were mounted on a confocal myograph (Danish Myo Technology A/S Aarhus, Denmark) between two 40μm wires, one of which was fixed the other of which was attached to a force transducer and peri-vascular nerve fibers were stimulated with EFS. Studies utilizing a myograph permitted simultaneous i) high-speed confocal imaging of fluorescence from individual smooth muscle cells, ii) electrical stimulation of perivascular nerves, and iii) recording of isometric tension. As shown in Fig. 2, during the first 20s of EFS, force rose to a small peak, then declined, similar to that recorded previously (Nilsson *et al*., 1986). During this time, jCaTs were present at relatively high frequency. Propagating asynchronous Ca^{2+} waves, previously associated with bath-applied α_1-adrenoceptor agonists (Zang *et al*., 2001), were not initially present. During the next 2.5 minutes of EFS, force rose slowly, and asynchronous propagating Ca^{2+} waves appeared. The selective α_1-adrenoceptor antagonist, prazosin, abolished both the slowly developing contraction and the Ca^{2+} waves, but reduced the initial transient contraction by only ~25%. These results suggested that neurogenic contractions under these conditions consisted of an early, transient purinergic component (the jCaTs) and a later developing adrenergic component.

Purinergic component—In order to study selectively the Ca^{2+} signals and contractions generated by neurally released ATP, arteries were exposed to prazosin (1-10μM) to block α_1-adrenergic receptors (Lamont *et al*., 2003). Others (Gitterman & Evans, 2001) have shown that purinergic receptor antagonists, such as suramin, abolish the small contractions that remain after prazosin. After prazosin treatment 73.7±14.0% (n=7) of the initial transient contraction remained and 5.00± 0.98% of the maintained contraction (Lamont *et al*., 2003). The changes in frequency and amplitude of jCaTs that might occur during the EFS were also characterized. Confocal imaging of fluo-4 fluorescence at 30 images s^{-1} was performed for 3 periods of 20s in the beginning (0 to 20s), middle (80 to 100s), and end (160 to 180s) of 3 minutes of EFS (Lamont *et al*., 2003). In arteries exposed to prazosin and stimulated with EFS, the frequency of jCaTs declined markedly during the 3 minutes of EFS. In contrast to the frequency, the peak amplitude of the jCaTs changed little during 3 minutes of EFS. Propagating Ca^{2+} waves were not observed during EFS in the presence of prazosin. JCaTs occurred in sufficient numbers during the first 20s of EFS to produce a detectable elevation of average $[Ca^{2+}]$ (fluorescence ratio), which paralleled the transient contraction that occurred during this time. On the other hand, jCaTs occurred at a very low frequency later in the EFS, when contractile force fell to very low levels (Lamont *et al*., 2003). Thus, it seems reasonable to attribute the contractile activation to jCaTs, despite their limited spatial extent and frequency.

Adrenergic component—During the subsequent neurogenic contractions (Fig. 2B), asynchronous Ca^{2+} waves propagated within individual smooth muscle cells of the arterial wall. Ca^{2+} signal was obtained as the average fluorescence within a single smooth muscle cell. Images were obtained at $2s^{-1}$, a rate, which is too slow to resolve the jCaTs generated during the initial purinergic component (Lamont & Wier, 2002). In this study 809 Ca^{2+} waves were detected in 74 cells, and the time of onset and peak amplitude of each was determined.

NPY's effect on the adrenergic component of neurogenic contraction—Adrenergic contractions were induced with PE (2μM) in the absence and presence of 10nM NPY (Fig. 3). Calcium signals within the arterial smooth muscle cells were also observed using confocal wire myography. 10nM NPY increased the frequency of asynchronous calcium waves induced by 2μM PE from 1.38±0.28 to 3.95±0.54 waves/cell/min (120 cells, 3 arteries) and increased the PE-induced force by up to 3-fold (Fig. 3).

Discussion

We have previously advanced a scheme to explain the Ca^{2+} signals and isometric contraction elicited by electrical field stimulation of perivascular sympathetic nerves of a rat mesenteric small artery (Fig. 4). Early during a train of nerve fiber action potentials, smooth muscle contraction is activated mainly by jCaTs induced by neurally released ATP. JCaTs are localized to the post-junctional region, and arise from Ca^{2+} that has entered via P2X receptors. At this time, sympathetic varicosities may release mainly small vesicles that contain a relatively high concentration of ATP (*viz*. the relatively few 'big' quanta proposed by Stjarne (Stjarne, 2001). Later during a train of nerve fiber action potentials, jCaTs are rare, and contraction is activated by Ca^{2+} waves that arise from sarcoplasmic reticulum (SR). Ca^{2+} release from SR is activated by $InsP_3$, produced after binding of NA to α_1-adrenoceptors. At this time, sympathetic varicosities may release small synaptic vesicles that contain a relatively high concentration of NA.

JCaTs are distinct from Ca^{2+} transients activated in isolated venous myocytes by exogenously applied ATP (Mironneau *et al*., 2001). Previous studies of the effects of ATP on small arteries utilized spatially averaged measurements of Ca^{2+} (Lagaud *et al*., 1996; Mironneau *et al*., 2001) and we cannot determine therefore whether jCaTs might be produced by bath-applied ATP or not. In rat mesenteric small arteries similar to those used here, low concentrations of exogenous ATP (*viz*. 0.01-1mM) caused 'global' Ca^{2+} transients that seemed to involve Ca^{2+} influx through channels sensitive to nifedipine and the putative blocker of receptor operated channels, SKF 96365 (Putney *et al*., 2001), whereas higher concentrations of ATP (1-3mM) caused a release of Ca^{2+} from intracellular stores (Ca^{2+} transients were elicited by high [ATP] in the absence of external Ca^{2+}). When applied to isolated venous myocytes, low concentrations of ATP (0.1μM) induced 'rather uniform' increases in Ca^{2+} (which started from the edges of the cell), and at higher concentrations (1μM), propagating Ca^{2+} waves (Mironneau *et al*., 2001).

In the arteries studied in the presence of prazosin, no propagating Ca^{2+} waves were ever observed during EFS. This can be interpreted as neurally released ATP not evoking significant release of Ca^{2+} from intracellular stores, a result in agreement with previous pharmacological studies on rat mesenteric small arteries (Gitterman & Evans, 2001). It can be thus speculated that the differences between the effects of bath-applied ATP and neurally-released ATP are due to a markedly different spatio-temporal pattern of [ATP] on the smooth muscle cell in the two cases.

Both the contraction and the underlying Ca^{2+} signals during the adrenergic component of the neurogenic isometric contraction are also distinctly different from those occurring during externally applied α_1-adrenoceptor agonist (typically phenylephrine). After the initial purinergic component, the adrenergic component of the neurogenic contractions, even at maximally effective EFS, rises much more slowly than does the contraction in response to bath-applied α_1-adrenoceptor agonist. Maximally effective concentrations of exogenous PE elicit an initial synchronous release of Ca^{2+}, followed by asynchronous propagating Ca^{2+} waves, both in veins (Ruehlmann *et al*., 2000) and in arteries (Mauban *et al*., 2001; Zang *et al*., 2001). Thus, the initial rapid rise in force in response to externally applied PE appears to be generated by a synchronous release of Ca^{2+} from intracellular stores. This does not occur during neurogenic contractions, possibly because neuronally released NA does not initially reach the uniformly high levels that are achieved rapidly after external application.

It has been suggested previously (Prieto *et al*., 1997) that NPY activates mesenteric small arteries through two different mechanisms; 1) activation of non-selective cation channels, with consequent Ca^{2+} entry, and 2) inhibition of cAMP-mediated hyperpolarization

(consequent to a decrease in [cAMP]). The latter is consistent with the fact that some types of NPY receptors are coupled to pertussistoxin-sensitive G proteins and are able to mediate decreases in [cAMP]. Consistenst with this, neuronally released NPY potently inhibits the ability of forskolin to relax agonist induced contraction of rat mesenteric arteries (Prieto *et al*., 2000), an effect likely due to stimulation of G_i with subsequent inhibition of Adenylyl cyclase (AC) and decrease in [cAMP]. A cAMP mediated mechanism to explain the present results (increasing the frequency of NA-induced Ca^{2+} waves) seems unlikely. Such a mechanism would require that basal levels of cAMP (presumably already low) be significantly reduced by NPY and, further, that this decrease of cAMP would have a significant effect on membrane potential. To explain the action of NPY to increase the frequency of NA induced Ca^{2+} waves in arteries, the mechanism described recently for cardiac cells may be more relevant. In those cells, NPY, acting on Y1 receptors, has a positive inotropic effect that is not pertussis-toxin sensitive but that involves stimulation of phospholipase C (PLC) and increased Ca^{2+} release (Heredia Mdel *et al*., 2005). Such an effect would be expected to increase the frequency of Ca^{2+} waves.

Acknowledgments

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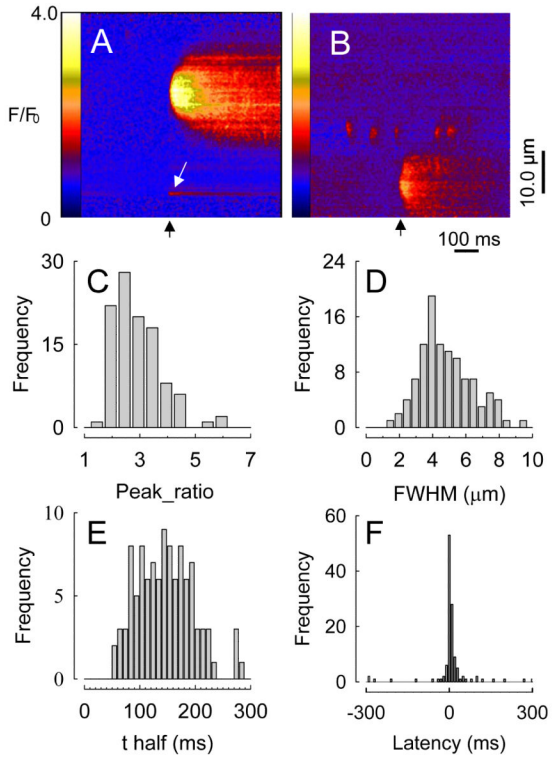

Figure 1. Junctional Ca^{2+} transients in line-scan images, expressed as fluorescence pseudo-ratios (F/F_0)

A, Black arrow indicates time of stimulus pulse. White arrow indicates (unrelated) nerve fiber Ca^{2+} transient. B, jCaT with spontaneous Ca^{2+} sparks. C through E, Probability density histograms of peak F/F_0, FWHM, and $t_{1/2}$ for all jCaTs (F). Probability density histogram of latencies of all jCaTs, showing a few spontaneous jCaTs (Lamont & Wier, 2002).

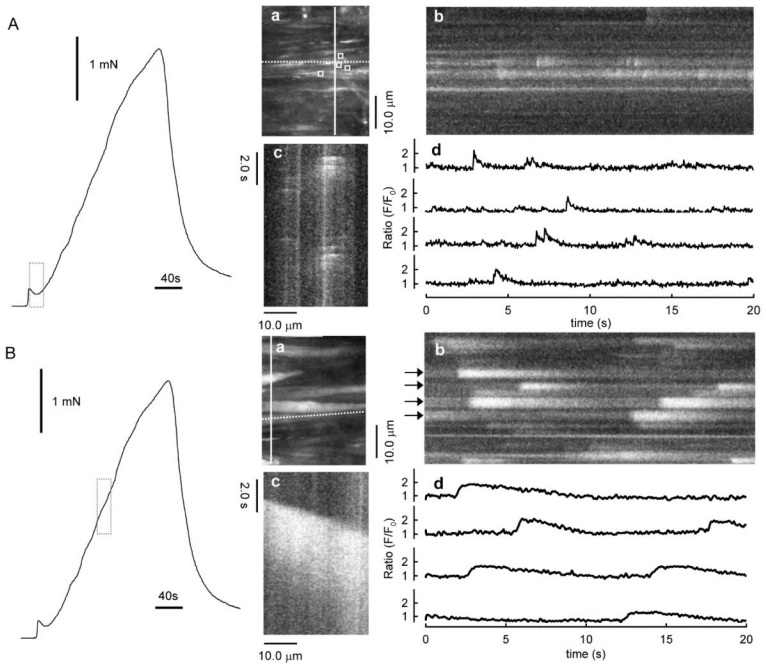

Figure 2. Occurrence of jCaTs during the predominantly purinergic component (A) and Ca^{2+} waves during the predominantly adrenergic component (B) of neurogenic contraction

Records at left of both (A) and (B) are the force produced by EFS for 3 minutes. The dotted vertical bars indicate the times during which images in (a)–(c) and traces in (d) were obtained. (Aa and Ba) Image obtained by averaging 30 frames (1s) during the period indicated. Ab and Bb are virtual line-scan images derived from a selected single vertical line of pixels in images Aa and Bb, respectively (solid white line). Ac and Bc are virtual line-scan images derived from a single horizontal line of pixels in images Aa and Ba, respectively (dotted white line). Traces in (Ad) are fluorescence pseudo-ratios (F/F_0) derived from the average fluorescence within selected areas-of-interest (AOIs) in the images in Aa (white boxes). Traces in (Bd) are derived from the line-scan image in Bb, at the lines indicated by the black arrows. All the data illustrated here were obtained at 30 images s^{-1} (Lamont *et al*., 2003).

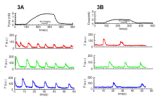

Figure 3. Mechanism of NPY's effect on the adrenergic component of sympathetic neurogenic contraction

Adrenergic contractions were induced with PE (2μM) in the absence (Fig. 3A) and presence (Fig. 3B) of 10nM NPY. The top most trace shows force, the bottom traces are calcium signals within three different arterial smooth muscle cells using confocal wire myography. 10nM NPY increases the frequency of asynchronous calcium waves induced by 2μM PE from 1.38±0.28 to 3.95±0.54 waves/cell/min (120 cells, 3 arteries) and increased the force 3-fold, n=3.

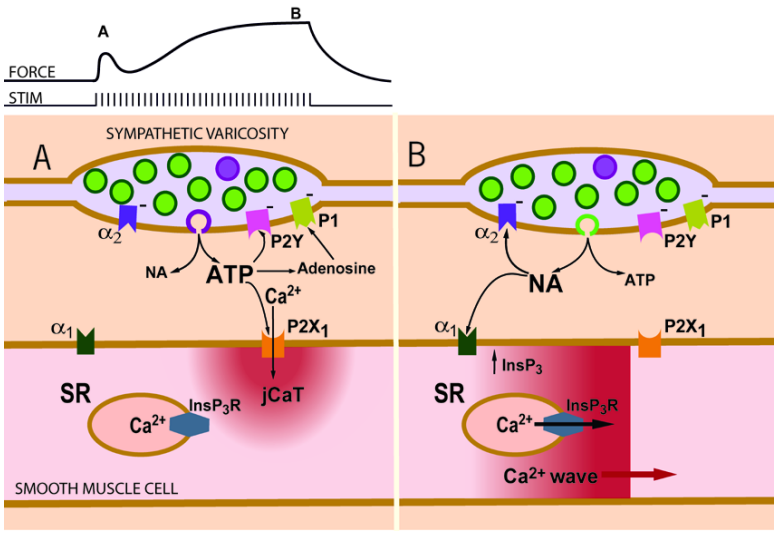

Figure 4. Scheme of Ca^{2+} signaling induced by sympathetic neuromuscular transmission in a small artery

(A) Early during a train of nerve fiber action potentials, smooth muscle contraction is activated mainly by post-junctional Ca^{2+} transients (jCaTs) induced by neurally released ATP. JCaTs are localized to the post-junctional region, and arise from Ca^{2+} that has entered via P2X receptors. At this time, sympathetic varicosities may release mainly small vesicles that contain a relatively high concentration of ATP (*viz*. the relatively few 'big' quanta proposed by Stjarne, 2001) (B) Later during a train of nerve fibre action potentials, jCaTs are rare, and contraction is activated by Ca^{2+} waves that arise from sarcoplasmic reticulum (SR). Ca^{2+} release from SR is activated by $InsP_3$, produced after binding of NA to α_1-adrenoceptors. At this time, sympathetic varicosities may release small synaptic vesicles (the more numerous 'small' quanta, green) that contain a relatively high concentration of NA. An effect not shown in the scheme is the action of neurally released NPY to increase the frequency of NA induced Ca^{2+} waves. Figure reproduced from (Wier, 2003)).