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# Neuroeffector $Ca^{2+}$ transients for the direct measurement of purine release and indirect measurement of cotransmitters in rodents

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**Determining whether ATP and noradrenaline are released from the same vesicle at mature autonomic neuroeffector junctions is challenging because of the difficulty of simultaneously detecting the packeted release of these neurotransmitters. Contraction, overflow and electrophysiology experiments all show that both ATP and noradrenaline are released following field stimulation (although the ratio might vary) from autonomic nerves in tissues including the vas deferens, rat tail artery and mesenteric artery. The occurrence of purinergic neuroeffector $Ca^{2+}$ transients (NCTs) has been used to detect the packeted release of the neurotransmitter ATP acting on postjunctional P2X receptors to cause $Ca^{2+}$ influx. Neuroeffector $Ca^{2+}$ transients can also be used to detect the local effects of noradrenaline through its $\alpha_2$-adrenoceptor-mediated prejunctional autoinhibitory effects on nerve terminal $Ca^{2+}$ concentration and the probability of exocytosis (measured by counting NCTs). Evidence is presented that exocytosis from sympathetic varicosities does not occur in a manner independent of the history of that varicosity, but rather that the release of a packet of ATP transiently suppresses (or predicts the transient suppression of) subsequent release. This could arise by autoinhibition (by the prejunctional action of noradrenaline or purines) or due to a transient shortage of vesicles readily available for release. In summary, two high-resolution approaches are proposed to measure the intermittent release of packets of neurotransmitter: (1) local transient suppression of nerve terminal $Ca^{2+}$ transients; and (2) the local and transient inhibition of NCTs to infer local autoinhibition, hence transmitter release. Such approaches may allow the packeted corelease of ATP and noradrenaline to be investigated without the need to measure both neurotransmitters directly.**

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It is now widely accepted that more than one neurotransmitter is released from sympathetic postganglionic nerve terminals, with the particular mix of neurotransmitters varying with the species and with the tissue innervated. The most prevalent neurotransmitters [noradrenaline, ATP and neuropeptide Y (NPY)] are accompanied by a mix of other peptides, which perhaps have more target-specific importance. With the acceptance of multiple neurotransmitters come the fundamental questions of whether they are also co-stored, released in consistent packet sizes, or engage in coregulation at the level of the single junction, single nerve terminal, or in a paracrine or even endocrine manner.

Perhaps the most startling example of cotransmission without co-storage comes from the parasympathetic nervous system, where terminals staining for the vesicular acetylcholine transporter can also contain nitric oxide synthase (Hedlund *et al.* 1999), suggesting that they release NO as a gaseous neurotransmitter. Regarding sympathetic terminals, the differential responses of noradrenaline and purine overflow or transmission in response to a range of interventions, including angiotensin (Trachte, 1988; Ellis & Burnstock, 1989), prostaglandin $E_2$ (Ellis & Burnstock, 1990) and a change in the stimulation frequency (Todorov *et al.* 1999), has led many to suggest that noradrenaline and ATP release are differentially


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controlled, although these transmitters still might be co-stored (Stjärne, 2001). Biochemical evidence for co-storage comes from studies of the chemical composition of synaptic vesicle fragments separated by density gradient centrifugation from the rat vas deferens (Fredholm *et al.* 1982). Recently, a vesicular ATP transporter has been identified (the gene product of *SLC17A9*; Sawada *et al.* 2008), and co-localization of this transporter with transporters for other neurotransmitters at the level of the vesicle would suggest co-storage. Detecting corelease, however, is difficult because of the challenge of monitoring the packeted release of cotransmitters with sufficient spatial resolution, sensitivity and molecular specificity.

Neurotransmitter release from sympathetic nerves to smooth muscle can be measured in several ways, including intracellular voltage-following [of excitatory junction potentials (EJPs); Burnstock & Holman, 1961, 1962], focal extracellular recording [of excitatory junction currents (EJCs)], by local amperometry for noradrenaline or by measuring the overflow of neurotransmitters from the smooth muscle. While all of these techniques are useful for monitoring bulk transmission, monitoring the release of single packets of evoked neurotransmitter is considerably harder. Spontaneous neurotransmitter release is readily monitored by detecting the spontaneous excitatory junction potential (sEJP), where the sympathetic innervation has close contact with purinergic P2X receptors on the smooth muscle. However, the large size of the almost electrically homogeneous smooth muscle cells (usually >100 $\mu$m in length) means that it is difficult to locate the site of neurotransmitter release. This is made even more difficult when the smooth muscle cells are electrically well coupled, in which case a given sEJP may be the muffled reporter of distant neurotransmitter release. Focal extracellular recording, using loose-patch electrodes, of the EJC spatially restricts the volume of tissue being monitored, but such measurements are hampered by the weak extracellular potential differences generated and by the confounding nature of the opposing electrical signals arising when neurotransmitter release occurs outside, but close to, the rim of the recording electrode.

Rapid EJPs and EJCs in most smooth muscles are generated by the action of ATP on P2X receptors, with most of the current through their channels being carried by $Na^+$, owing to the high permeability of the channel, the high extracellular concentration and the high electrochemical gradient for $Na^+$. However, the $Ca^{2+}$ permeability through these channels is also high (Benham & Tsien, 1987), leading to a significant $Ca^{2+}$ influx. Since the resting intracellular free $Ca^{2+}$ concentration is relatively low, this local $Ca^{2+}$ influx, while smaller in absolute value than the $Na^+$ influx, gives a much larger relative change in $Ca^{2+}$ concentration. Such a change in local $Ca^{2+}$ concentration, in some cells amplified by release from intracellular $Ca^{2+}$ stores, can be detected using fluorescent $Ca^{2+}$ indicators. Optical cross-sectioning by confocal microscopy has allowed several groups to use this approach to monitor the packeted release of ATP (Brain *et al.* 2001, 2002; Lamont & Wier, 2002; Heppner *et al.* 2005) within intact smooth muscle. Such $Ca^{2+}$ transients are significantly larger in amplitude, or of longer duration, than $Ca^{2+}$ sparks (Lamont & Wier, 2002; Heppner *et al.* 2005).

Validating the detection of focal purinergic $Ca^{2+}$ transients as a useful measure of neurotransmitter release is important before this technique can be used to make inferences about the fundamental properties of neurotransmission. It should be noted that this approach requires the presence of functional postjunctional P2X receptors in sufficiently close contact to the nerve terminals. This dependence on the function of the receptors means that it can be difficult to assess whether changes in the local $Ca^{2+}$ transient reflect changes in the amount of ATP released, or a change in the number of functional receptors located or clustered close to the varicosity. However, such a limitation also complicates the interpretation of sEJPs. There are interesting electrochemical approaches being developed to monitor the local ATP concentration (Llaudet *et al.* 2005), much as noradrenaline is measured, but the sensitivity of such approaches is fundamentally limited by the weak electrical signal generated per ATP molecule in the requisite chemical reactions. The reaction rate may also limit the sensitivity to brief local ATP transients.

To validate neuroeffector $Ca^{2+}$ transients (NCTs) as a measure of evoked neurotransmitter release it would be useful to compare the optical responses obtained with a more traditional high-sensitivity method for detecting ATP release; the measurement of either EJPs or EJCs. To measure EJCs requires the presence of a focal extracellular electrode directly over the site of neurotransmitter release. The presence of the electrode tip interferes with high-resolution optical measurements of the same region, although this might not be an impossible obstacle to overcome. Measuring the EJP simultaneously with optical recording is technically much easier because it requires a sharp intracellular electrode that can be located some distance from the site of optical recording (still within the same cells recorded from optically). The problem with the EJP is that it reports the action of ATP at any location on the smooth muscle cell impaled and, in the presence of sufficiently good electrical coupling, from nearby cells. Furthermore, field stimulation of the nerves activates a large number (if not all) of the terminals within the tissue, leading to a large local quantal content, even in the presence of highly intermittent release. However, single packets of ATP are released spontaneously and asynchronously from nerve terminal varicosities, producing the sEJPs. The sEJPs are of relatively low frequency, making the correlation of optically recorded

$Ca^{2+}$ signals with them difficult because the recording time is limited by photobleaching of fluorescent $Ca^{2+}$ probes. One way to resolve this problem is to use the black widow spider venom toxin component, latrotoxin, to increase the rate of sEJPs to a point where spontaneous NCTs can be detected within a practical duration (Young *et al.* 2007*a*). We showed a good correlation between the timing of sEJPs and spontaneous NCTs, helping to validate this optical approach with the more traditional electrophysiological method. Additionally, the added spatial resolution provided by optical monitoring led us to conclude that the broad amplitude distribution of sEJPs, previously attributed to the damped signal from distant, electrically coupled smooth muscle cells, could be explained entirely by spontaneous neurotransmitter release onto the cell from which recordings were made.

The measurement of 'discrete events' can reduce the spatial range over which neurotransmitter release is detected (Blakeley & Cunnane, 1979). Using standard intracellular recording with a sharp microelectrode, but differentiating the electrical signal with respect to time ($dV/dt$), intermittent transient peaks in $dV/dt$ could be detected, and these were attributed to the action of local neurotransmitter release only (although the range implied by 'local' had not been established). By using the occurrence of NCTs to locate the site of neurotransmitter release, we (Young *et al.* 2007*b*) have been able to show that the discrete events represent transmitter release on the cell impaled, but do not seem to give very good information about the physical location (with respect to the electrode tip) within that cell. Therefore, discrete events can be used, even in a moderately well-electrically-coupled syncytium, to detect neurotransmitter release onto the smooth muscle cells impaled, but the virtual electrical homogeneity of each smooth muscle cell means that the spatial resolution obtained using 'discrete events' is limited. Transmitter release onto other cells causes only a slow change in the membrane potential (hence no early peak in $dV/dt$), presumably because the electrical impendence of the intervening gap junctions slows the rate at which the potential equilibrates amongst the cells.

Of the preparations in which focal purinergic $Ca^{2+}$ transients have been observed, the mouse vas deferens has the experimental advantages that there is a relatively high density of close-contact varicosities (Taxi, 1965; Jones & Spriggs, 1975) and a well-established technique for monitoring the nerve terminal $Ca^{2+}$ concentration, which can be performed while imaging the smooth muscle (Fig. 1; Brain & Bennett, 1997). The principal cotransmitters released from the sympathetic nerves in the vas deferens are noradrenaline and ATP (Burnstock, 1976; Morris & Gibbins, 1992). In the rat tail artery, local detection of noradrenaline with amperometry and ATP release using EJCs, two high-resolution techniques, has shown a similar regulation of ATP and noradrenaline release in response to two-pulse stimulation (under control conditions and in presence of $K^+$ channel blockers; Msghina *et al.* 1998), but this approach was not sufficient to demonstrate that packets of ATP are released synchronously with packets of noradrenaline. While EJCs are readily measured in the mouse vas deferens, it has proved very much more difficult to use amperometry to detect packeted noradrenaline release in this organ, possibly because of the distributed nature of the innervation and the slow diffusion from release sites located within the muscle (J. Brock, personal communication).

The local detection of the action of noradrenaline has been possible, at high resolution, using the nerve terminal itself as a biological sensor. Both noradrenaline, acting at prejunctional $\alpha_2$-adrenoceptors (Illes & Starke, 1983), and purines, acting mainly at P2Y receptors (von Kügelgen *et al.* 1989, 1994), inhibit neurotransmitter release. By monitoring nerve terminal $Ca^{2+}$ concentration in the mouse vas deferens using brief trains of field stimulation at a frequency of 4.63 Hz, we were able to show that the $\alpha_2$-adrenoceptor antagonist yohimbine increased the amplitude of the $Ca^{2+}$ transient following five-impulse trains (Brain & Bennett, 1997). This suggests

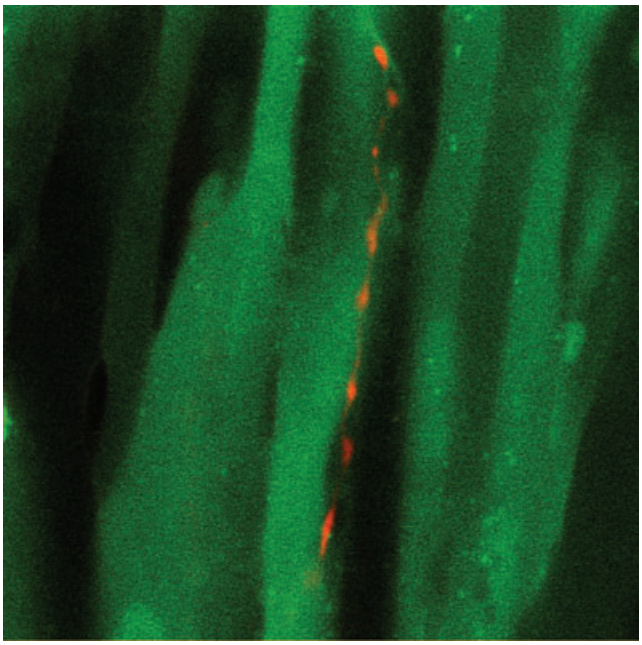

**Figure 1. Confocal imaging of smooth muscle and a nerve terminal**
Smooth muscle cells of the isolated mouse vas deferens were loaded with the $Ca^{2+}$ indicator Oregon Green 488 BAPTA-1 AM (green), as previously described (Brain *et al.* 2003). The red $Ca^{2+}$ indicator, rhod-2, 10 kDa dextran, was loaded in an orthograde direction into a subset of nerve terminals, by adapting the approach used by Brain & Bennett (1997). In this confocal plane, only a single nerve terminal branch can be seen, although it is likely that there are other, unlabelled, nerve terminals nearby. The image field is 70 $\mu$m across.

that, during field stimulation, noradrenaline reaches the $\alpha_2$-adrenoceptors at an average varicosity, at a sufficient concentration to inhibit $Ca^{2+}$ influx, within 860 ms of the start of the stimulus train. This might seem surprising given that ATP release (hence noradrenaline release, if there is strict corelease) is highly intermittent at these junctions (Brain *et al.* 2002), with a probability that a given action potential will evoke the release from a given varicosity of only 0.019. If we consider that there might be '$n$' varicosities within the diffusion range of a particular varicosity, we can consider the number of such varicosities that might need to be present in order that, on average (using $P = 0.5$ to give the median value), neurotransmitter will be released locally. During a five-impulse train, assuming that the last impulse in the train cannot autoinhibit the $Ca^{2+}$ influx during the train, the expectation value of $n$ can be found by solving $[(1 - 0.019)^n]^4 = (1-0.5)$, i.e. the probability that there will be no local release, given $-n$ varicosities within the diffusion range. This is $n = [\ln(0.5)/\ln(0.981)]/4$, or $n \approx 9$. We know that the density of varicosities in this organ is around 2.2 varicosities per 1000 $\mu$m$^3$ (Karunanithi & Lavidis, 2001), so this number of varicosities (9) should occur within an average range (radius) of about 10 $\mu$m (noting that within such a radius there is a tissue volume of about 4200 $\mu$m$^3$). Therefore, even in the presence of highly intermittent noradrenaline release (probability of release of 0.019), one would expect the average varicosity in this organ to be within 10 $\mu$m of a released packet of noradrenaline at some time during a five-impulse stimulus train (excluding the last impulse). This range seems reasonable for the diffusion of noradrenaline within the restricted, although somewhat tortuous, extracellular space.

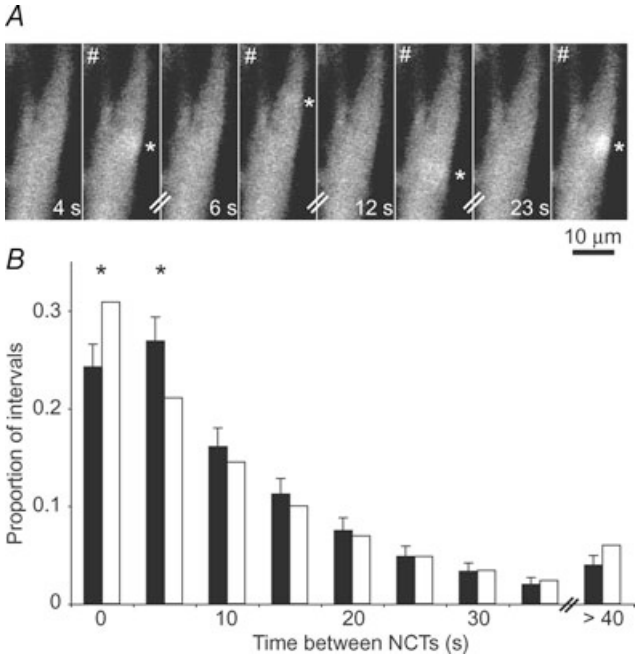


**Figure 2. Neurotransmitter release at a single junction depends on recent release from that junction**
*A* shows pairs of selected images (control, immediately followed by a field stimulus, #) acquired during trains of stimuli at 1 Hz. Each sterisk denotes a NCT. The second and final frames show NCTs occurring at the same site (due to neurotransmitter release at the same junction) occurring 19 s apart. The relative frequency of such durations across all preparations is given in *B*. This distribution of durations (filled columns) is compared with the distribution expected if NCTs occurred independently of previous events at the same junction (open columns). This expected distribution was calculated as follows. If NCTs occur independently, with a probability of $p$ on each trial, then the chance of events being precisely $k$ trials apart is $p(1-p)^{(k-1)}$ (a geometric progression). For '$m$' NCTs, the expected number of intervals is $mp(1-p)^{(k-1)}$. This expected count was calculated for each of the 16 junctions, using the mean probability of detecting an NCT at that junction ($p$). The data from all junctions (measured values and expected values) were then pooled. The measured distribution differed from that expected ($\chi^2$ test, $P = 0.016$); $^*P < 0.05$ with a post-$\chi^2$ paired Student's $t$ test.

A more direct method has now been investigated, attempting to detect the suppression of NCT probability during a train of low-frequency stimulation, following a NCT. To do so, smooth muscle cells of the mouse vas deferens were filled with the $Ca^{2+}$ indicator Oregon Green 488 BAPTA-1 AM, as previously described (Brain *et al.* 2003), and viewed under a confocal microscope. Low-frequency (1 Hz) field stimulation was applied, and the times of occurrence of NCTs (Fig. 2*A*) at each neuroeffector junction (identified by the spatial clustering of such NCTs over time) were recorded. If it is assumed that the release of packets of neurotransmitter (hence the occurrence of NCTs) occurs independently of the prior history of the muscle cell, then the distribution of time intervals between successive NCTs can be predicted (see the legend to Fig. 2). In order to analyse a sufficient number of NCTs, only junctions with a high release probability (where the probability of inducing an NCT following an

action potential, $P_{NCT}$, was >0.05) were considered. When stimulating at 1 Hz, the distribution of durations between consecutive NCTs differed from the distribution expected if NCTs are released independently (Fig. 2*B*; trials of more than 300 impulses; number of junctions, $n_j = 16$; number of preparations, $n_p = 8$; $P < 0.05$). There was a paucity of NCTs occurring during the 5 s following a NCT. This deficit was recouped during the period 5–10 s after an NCT.

The poverty of NCTs occurring within 5 s of one another indicates that exocytosis from a varicosity transiently suppresses the probability of release from that varicosity. This could arise by autoinhibition (for example, by the action of noradrenaline on prejunctional $\alpha_2$-adrenoceptors) or be due to a deficit in the number of vesicles available for release. Further investigation is required to determine whether this transient suppression represents $\alpha_2$-meditated autoinhibition, implying the corelease of noradrenaline and ATP from a single varicosity, with the noradrenaline transiently suppressing release probability from that varicosity.

On balance, while neurotransmitter overflow and contraction techniques show a differential regulation of the cotransmitters ATP and noradrenaline from sympathetic nerves, the techniques with the highest spatial resolution suggest the corelease of packets of these neurotransmitters during low-frequency nerve stimulation. It may be that during long stimulus trains the ratio of the amounts of the two neurotransmitters released may vary, perhaps reflecting the release of vesicle populations with different ATP/noradrenaline ratios. Further progress in this field can be made by detecting the packeted ATP release by monitoring NCTs, and combining this with the indirect local measurement of the autoinhibitory action of noradrenaline on prejunctional $\alpha_2$-adrenoceptors.

However, new or improved high-sensitivity techniques to directly measure the local release of noradrenaline and ATP are still needed to facilitate understanding of the differing regulation of these autonomic neurotransmitters. The study of cotransmission has progressed a long way since the doubting days of the mid-20th century; perhaps the coming decades will see the translation of our molecular understanding, to aid our understanding of cotransmission and its regulation at the level of the single neuroeffector junction in intact tissues.

## References


Benham CD & Tsien RW (1987). A novel receptor-operated $Ca^{2+}$-permeable channel activated by ATP in smooth muscle. *Nature* **328**, 275–278.

Blakeley AG & Cunnane TC (1979). The packeted release of transmitter from the sympathetic nerves of the guinea-pig vas deferens: an electrophysiological study. *J Physiol* **296**, 85–96.

Blakeley AG, Cunnane TC & Petersen SA (1982). Local regulation of transmitter release from rodent sympathetic nerve terminals? *J Physiol* **325**, 93–109.

Brain KL & Bennett MR (1997). Calcium in sympathetic varicosities of mouse vas deferens during facilitation, augmentation and autoinhibition. *J Physiol* **502**, 521–536.

Brain KL, Cuprian AM, Williams DJ & Cunnane TC (2003). The sources and sequestration of $Ca^{2+}$ contributing to neuroeffector $Ca^{2+}$ transients in the mouse vas deferens. *J Physiol* **553**, 627–635.

Brain KL, Jackson VM, Trout SJ & Cunnane TC (2001). A novel, high resolution method to study ATP release from single varicosities on an impulse-to-impulse basis in the mouse isolated vas deferens. *J Physiol* **536**, 26*P*.

Brain KL, Jackson VM, Trout SJ & Cunnane TC (2002). Intermittent ATP release from nerve terminals elicits focal smooth muscle $Ca^{2+}$ transients in mouse vas deferens. *J Physiol* **541**, 849–862.

Burnstock G (1976). Do some nerve cells release more than one neurotransmitter? *Neuroscience* **1**, 239–248.

Burnstock G & Holman ME (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J Physiol* **155**, 115–133.

Burnstock G & Holman ME (1962). Spontaneous potentials at sympathetic nerve endings in smooth muscle. *J Physiol* **160**, 446–460.

Cunnane TC & Stjärne L (1984). Transmitter secretion from individual varicosities of guinea-pig and mouse vas deferens: highly intermittent and monoquantal. *Neuroscience* **13**, 1–20.

Ellis JL & Burnstock G (1989). Angiotensin neuromodulation of adrenergic and purinergic co-transmission in the guinea-pig vas deferens. *Br J Pharmacol* **97**, 1157–1164.

Ellis JL & Burnstock G (1990). Modulation by prostaglandin $E_2$ of ATP and noradrenaline co-transmission in the guinea-pig vas deferens. *J Auton Pharmacol* **10**, 363–372.

Fredholm BB, Fried G & Hedqvist P (1982). Origin of adenosine released from rat vas deferens by nerve stimulation. *Eur J Pharmacol* **79**, 233–243.

Hedlund P, Alm P & Andersson KE (1999). NO synthase in cholinergic nerves and NO-induced relaxation in the rat isolated corpus cavernosum. *Br J Pharmacol* **127**, 349–360.

Heppner TJ, Bonev AD & Nelson MT (2005). Elementary purinergic $Ca^{2+}$ transients evoked by nerve stimulation in rat urinary bladder smooth muscle. *J Physiol* **564**, 201–212.

Illes P & Starke K (1983). An electrophysiological study of presynaptic $\alpha$-adrenoceptors in the vas deferens of the mouse. *Br J Pharmacol* **78**, 365–373.

Jones ME & Spriggs TL (1975). Noradrenaline and motor transmission in the vas deferens of the mouse. *Br J Pharmacol* **53**, 323–331.

Karunanithi S & Lavidis NA (2001). Effect of chronic morphine treatment on $\alpha_2$-adrenoceptor mediated autoinhibition of transmitter release from sympathetic varicosities of the mouse vas deferens. *Br J Pharmacol* **132**, 403–410.

Lamont C & Wier WG (2002). Evoked and spontaneous purinergic junctional $Ca^{2+}$ transients (jCaTs) in rat small arteries. *Circ Res* **91**, 454–456.

Llaudet E, Hatz S, Droniou M & Dale N (2005). Microelectrode biosensor for real-time measurement of ATP in biological tissue. *Anal Chem* **77**, 3267–3273.

Morris JL & Gibbins IL (1992). Co-transmission and neuromodulation. In *Autonomic Neuroeffector Mechanisms*, ed. Burnstock G & Hoyle CHV, pp. 33–119. Harwood Academic, Chur, Switzerland.

Msghina M, Gonon F & Stjärne L (1998). Paired pulse analysis of ATP and noradrenaline release from sympathetic nerves of rat tail artery and mouse vas deferens: effects of $K^+$ channel blockers. *Br J Pharmacol* **125**, 1669–1676.

Sawada K, Echigo N, Juge N, Miyaji T, Otsuka M, Omote H, Yamamoto A & Moriyama Y (2008). Identification of a vesicular nucleotide transporter. *Proc Natl Acad Sci USA* **105**, 5683–5686.

Stjärne L (2001). Novel dual 'small' vesicle model of ATP- and noradrenaline-mediated sympathetic neuromuscular transmission. *Auton Neurosci* **87**, 16–36.

Taxi J (1965). Contribution a l'étude des connexions des neurones moteurs du système nerveux autonomie. *Annales des sciences naturelles Zoologie et biologie animale* **7**, 413–674.

Todorov LD, Mihaylova-Todorova ST, Bjur RA & Westfall DP (1999). Differential cotransmission in sympathetic nerves: role of frequency of stimulation and prejunctional autoreceptors. *J Pharmacol Exp Ther* **290**, 241–246.

Trachte GJ (1988). Angiotensin effects on vas deferens adrenergic and purinergic neurotransmission. *Eur J Pharmacol* **146**, 261–269.

von Kügelgen I, Kurz K & Starke K (1994). P2-purinoceptor-mediated autoinhibition of sympathetic transmitter release in mouse and rat vas deferens. *Naunyn Schmiedebergs Arch Pharmacol* **349**, 125–132.

von Kügelgen I, Schoffel E & Starke K (1989). Inhibition by nucleotides acting at presynaptic P2-receptors of sympathetic neuro-effector transmission in the mouse isolated vas deferens. *Naunyn Schmiedebergs Arch Pharmacol* **340**, 522–532.

Young JS, Brain KL & Cunnane TC (2007*a*). The origin of the skewed amplitude distribution of spontaneous excitatory junction potentials in poorly coupled smooth muscle cells. *Neuroscience* **145**, 153–161.

Young JS, Brain KL & Cunnane TC (2007*b*). Electrical and optical study of nerve impulse-evoked ATP-induced, P2X-receptor-mediated sympathetic neurotransmission at single smooth muscle cells in mouse isolated vas deferens. *Neuroscience* **148**, 82–91.

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