

# Vas deferens – A model used to establish sympathetic cotransmission

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**The vas deferens has been used as a model for many diverse studies of different aspects of autonomic neurotransmission since its introduction including, in particular, sympathetic cotransmission involving release of ATP and neuropeptide Y together with noradrenaline and prejunctional and postjunctional neuromodulation. It has also been used to study sympathetic reinnervation following vasectomy and castration, as well as the deleterious effects of diabetes, hypertension and chronic alcohol.**

## Introduction – The innervated vas deferens model preparation

The vas deferens, as first introduced by Huković in 1961, is densely innervated by sympathetic nerve fibres mostly arising from the hypogastric ganglion and from neurones in the hypogastric nerve trunk, together with some cholinergic nerve fibres (Figure 1) [1]. Subpopulations of sympathetic nerve fibres in the human vas deferens contain somatostatin and galanin as well as neuropeptide Y (NPY) [2]. Nerve fibres containing vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) have also been described in the guinea-pig vas deferens, probably colocalised in acetylcholine (ACh)-containing parasympathetic nerves [3]. Evidence for release of calcitonin gene-related peptide (CGRP) from capsaicin-sensitive sensory nerves in the rat and pig vas deferens has been presented [4]. Nerve fibres originating from pelvic neurones in a discreet location in the pelvic ganglia supply the rat vas deferens [5]. A population of varicose nerves has been identified in the mouse vas deferens after destruction of sympathetic nerves by 6-hydroxydopamine (6-OHDA) [6]. These nerves might be parasympathetic and/or sensory afferent fibres arising from L1, L2, L6 and S1 dorsal root ganglia travelling to the rat vas deferens in both hypogastric and pelvic nerves.

It is of interest that when Burn and Rand [7] first proposed that ACh and noradrenaline (NA) were cotransmitters in sympathetic nerves, one of their arguments was that hexamethonium significantly reduced the contractile responses of the vas deferens to stimulation of the hypogastric nerve. Unfortunately, they did not realise that the hypogastric nerve contains many sympathetic neurone cell bodies at intervals along its length, and thus they were blocking preganglionic cholinergic transmission rather than neuromuscular transmission from sympathetic terminals.

In addition to studies of sympathetic cotransmission, the vas deferens has also been used to explore the function

of capsaicin-sensitive primary afferent neurones. Capsazepine was shown to antagonise the action of capsaicin via a vanilloid receptor, whereas non-competitive antagonism by ruthenium red involved a more complex mechanism [8]. Some neurones on pelvic ganglia containing VIP immunoreactivity also showed NADPH-diaphorase reactivity [9]. This was taken to suggest that NO could be a neurotransmitter in guinea-pig vas deferens, particularly in nerves supplying the circular muscle layer. Functional evidence for the participation of NO in the excitatory neurotransmission in the rat vas deferens has also been presented [10]. Some of the NO synthase (NOS)-containing neurones of the pig inferior mesenteric ganglion supplying the vas deferens are probably involved in regulation of local blood flow and muscular tone [11]. NOS is present in subpopulations of both sympathetic and non-sympathetic nerves in the human vas deferens [12]. Histamine-containing neurones, distinct from NA-containing neurones, have been claimed to be present in the rat vas deferens [13].

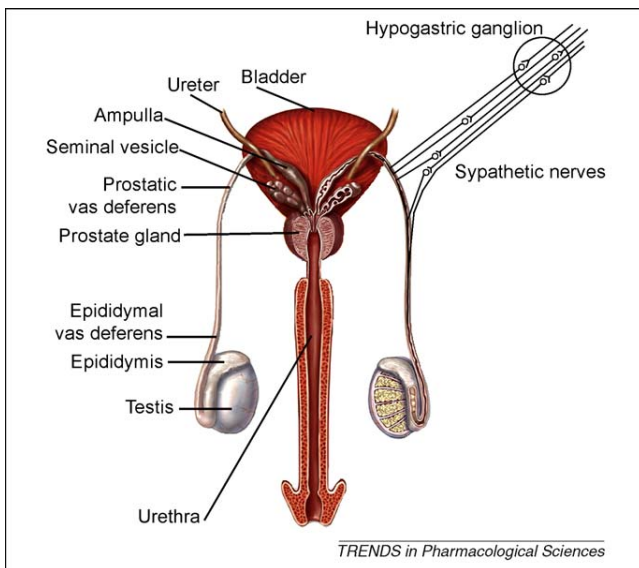
An early electronmicroscopic serial section study by Merrillees [14] of the innervation of the guinea-pig vas deferens supported and extended the early proposal of Hillarp in 1946 for an autonomic grand plexus consisting of varicose nerve fibres. Merrillees showed that axon varicosities containing vesicles were frequently within 20–100 nM of a muscle fibre, usually with no intervening Schwann cell processes. Later studies showed that the transmitter was released *en passage* from varicosities during conduction of an impulse, although excitatory junction potentials (EJPs) [15] are probably only elicited at close junctions. Neuroeffector junctions do not have a permanent geometry with postjunctional specialisations, suggesting that the varicosities might be continuously moving and that their close relation with muscle cell membranes changes with time, including dispersal and reformation of receptor clusters [16,17]. These and the studies of the rat vas deferens have provided the data to establish non-synaptic transmission at autonomic neuroeffector junctions ([17], Figure 2).

The classical work of Sir Henry Dale and Ulf Von Euler established NA as the transmitter released from sympathetic nerves [18,19]. Here, we review studies using the sympathetically innervated vas deferens preparation to show that ATP is a cotransmitter with NA for sympathetic neurotransmission.

## Sympathetic cotransmission

Burnstock and Holman [15,20] carried out several studies of the electrophysiology of sympathetic neurotransmission,

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**Figure 1.** The anatomy of the vas deferens–sympathetic nerve preparation is shown.

showing that EJPs in smooth muscle in response to single nerve pulses summed and facilitated, until at a critical depolarisation threshold, spikes were initiated associated with contraction. They were puzzled, however, that adrenoceptor antagonists did not abolish the EJPs, because NA was established as the sympathetic neurotransmitter at that time. It was not until over 20 years later that it was recognised that ATP acting as a cotransmitter with NA was responsible for the EJPs [21]. The David Westfall group were the first to demonstrate that ATP produced fast contraction of the vas deferens as a cotransmitter with NA released from sympathetic nerves producing slower contractions (Figure 3b) [22]. The non-adrenergic contractile component of the responses to sympathetic stimulation has been shown to be antagonised by arylazido aminopropionyl ATP, suramin, pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid, NF023 [23] and desensitised by  $\alpha,\beta$ -methylene ATP ( $\alpha,\beta$ -meATP) both *in vitro* and *in vivo*

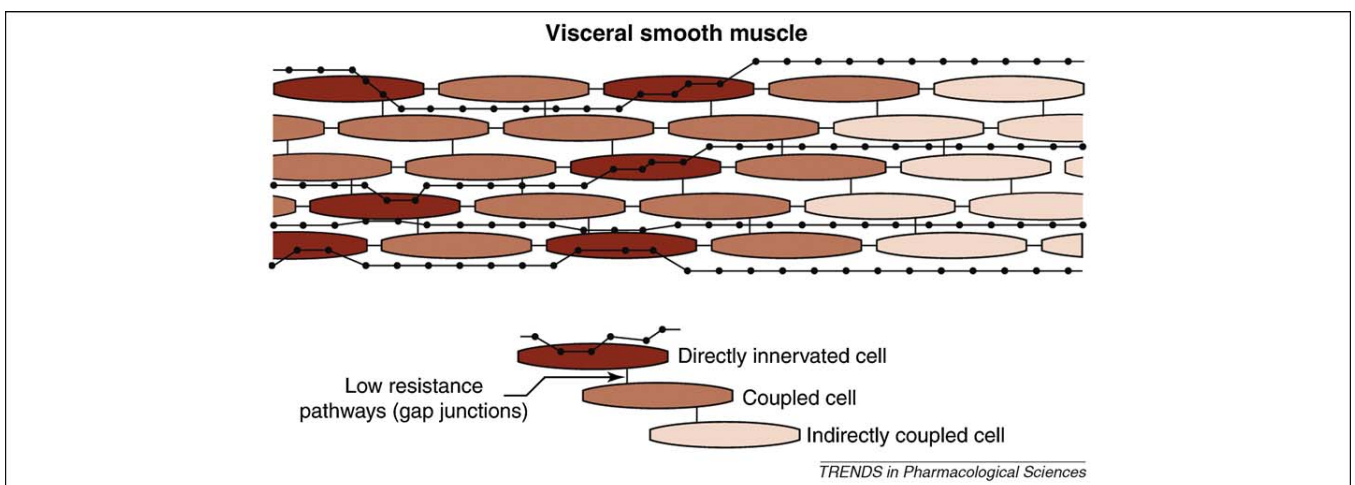
[24,25]. 6-OHDA blocked both adrenergic and purinergic components, supporting the view that they were cotransmitters in sympathetic nerves [26]. During transmural stimulation of nerves in the vas deferens, ATP and its breakdown products ADP, AMP and adenosine were detected in the superfusate [27].

In the mid-1980s Neild and Hirst proposed that EJPs were as a result of NA acting on hypothetical  $\gamma$ -adrenoceptors [28]. This was much debated at the time. However, when it was shown that NA, unlike ATP, did not mimic the EJP [29] and when reserpine, which depleted neuronal NA, but not ATP, failed to affect the rapid component of sympathetic nerve-modulated responses [30], the  $\gamma$ -hypothesis was abandoned. Direct evidence for concomitant release of NA, ATP and NPY from sympathetic nerves supplying the guinea-pig vas deferens was presented in 1998 [31]. A more recent paper has described a purinergic component of sympathetic nerves control of the *human* vas deferens [32].

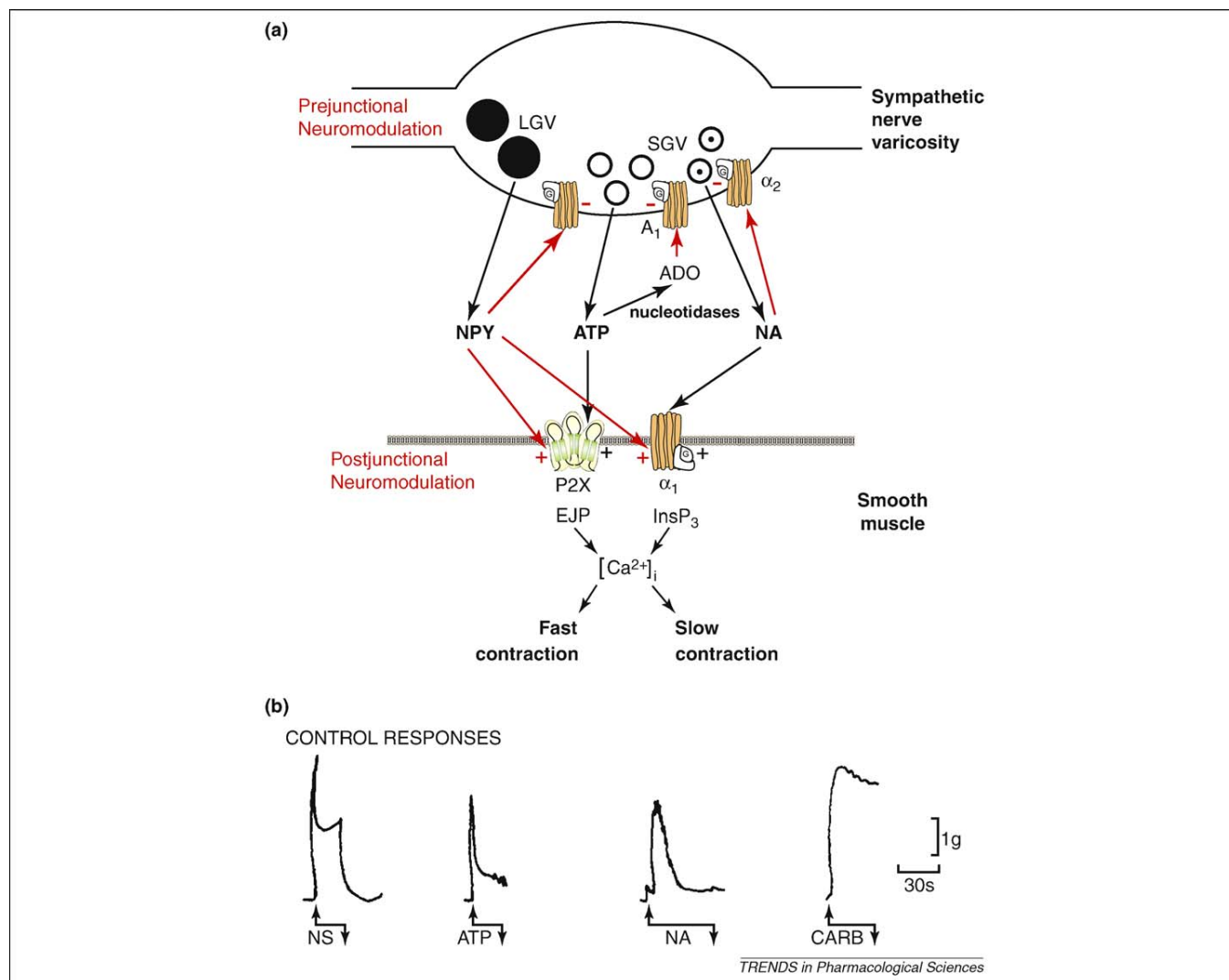
There is postjunctional synergism by the sympathetic cotransmitters NA and ATP [33]. It was proposed that NA potentiates the contractile responses of the vas deferens to ATP via a protein kinase C (PKC) mechanism that might involve the inhibition of myosin light chain phosphatase and subsequent calcium sensitisation [34].

Sophisticated electrophysiological studies have been carried out on the vas deferens to study packaged release of ATP from sympathetic nerve varicosities [35–40] showing that:

- Secretion of transmitters from a single varicosity is highly intermittent, i.e. only a small percentage of varicosities release transmitters during sympathetic nerve stimulation. Intermittence is caused by a low probability of release from varicosities, rather than by failure of the action potential to invade the varicosities.
- A higher number of varicosities release transmitters with increasing frequency of nerve stimulation.
- Fast transmitter release from a varicosity is quantal, whereas slow excitatory junction currents appear to be non-quantal release.
- Many, but not all, varicosities secrete ATP.



**Figure 2.** Schematic representation of the innervation of visceral smooth muscle. “Directly innervated” cells are those that are directly activated by neurotransmitter released from nerve varicosities; “coupled cells” are those where junction potentials spread from “directly innervated” cells. When a sufficient area of the muscle effector bundle is depolarised, a propagated action potential will activate the “indirectly coupled” cells. (Modified, with permission from Springer Science and Business Media, from Ref. [119].)



**Figure 3.** (a) Schematic representation of excitatory cotransmission in the vas deferens. NA, ATP and NPY are stored in and released from postganglionic sympathetic nerves, most likely from separate neurotransmitter vesicles. NA acts on postjunctional  $\alpha_1$ -adrenoceptors, which are coupled to the intracellular generation of inositol trisphosphate (InsP<sub>3</sub>). InsP<sub>3</sub> in turn triggers the release of Ca<sup>2+</sup> from the endoplasmic reticulum. NA can also act on prejunctional  $\alpha_2$ -adrenoceptors, which modulate neurotransmitter release. NPY is released from LGV to modulate cotransmitter release via prejunctional NPY receptors and to potentiate the action of the cotransmitters via their postjunctional receptors. ATP acts on postjunctional P2X<sub>1</sub> receptors, which leads to the opening of non-selective cation channels through which Ca<sup>2+</sup> influx can occur. Synaptic ATP is metabolised by soluble nucleotidases to adenosine (ADO). The nucleotide and adenosine can act on prejunctional A<sub>1</sub> receptors to modulate transmitter release. NPY is released from LGV to modulate cotransmitter release via prejunctional NPY receptors and to potentiate the action of the cotransmitters via their postjunctional receptors. LGV, large granular vesicle; SGV, small granular vesicle. (b) Biphasic contractile responses of the guinea-pig vas deferens to sympathetic nerve stimulation (NS) (8 Hz, 0.3 ms, supramaximal voltage for 20 s) and to exogenous ATP (10<sup>-4</sup> M) (fast twitch contraction, after short latency), NA (10<sup>-4</sup> M) and carbachol (CARB, 10<sup>-4</sup> M) (slower sustained contraction, after longer latency). (Reproduced, with permission from Elsevier, from Ref. [120].)

- PKC plays a fundamental role in ATP release from sympathetic nerves and in particular on the mechanisms underlying facilitation.
- Single release sites show dependency on external Ca<sup>2+</sup>.

A schematic summarising sympathetic cotransmission is shown in Figure 3a.

### Neuromodulation

The vas deferens preparation has been used to establish the concept of neuromodulatory *inhibition* of transmitter release via *prejunctional* receptors for a wide spectrum of agents, including NA via  $\alpha_2$ -adrenoceptors, dopamine, ACh via M<sub>1</sub> muscarinic receptors, adenosine via A<sub>1</sub> receptors,  $\gamma$ -aminobutyric acid via GABA<sub>B</sub> receptors, CGRP, NPY, prostaglandins, histamine via H<sub>2</sub> receptors, angiotensin, opioids, 5-hydroxytryptamine (5-HT<sub>1</sub>) and cannabinoids

[41–43]. The electrically stimulated mouse vas deferens has been used as a sensitive preparation for studying the pharmacology of  $\mu$ -opioid,  $\delta$ -opioid [44] and nociceptin [45].

Prejunctional P2Y receptors have been shown to inhibit, whereas prejunctional P2X receptors facilitate transmitter release [46]. Prejunctional nicotinic receptors also facilitate neurotransmitter release [47].  $\beta_2$ -Adrenoceptor-mediated *prejunctional facilitation* and postjunctional inhibition of sympathetic neuroeffector transmission has been shown in the vas deferens [48]. There is facilitation of NA release via A<sub>2A</sub> receptors in the epididymal portion and via A<sub>2B</sub> receptors in the prostatic portion of the rat vas deferens [49]. Prejunctional facilitation of nerve-mediated responses has also been reported in vas deferens via NK tachykinin receptors and receptors to capsaicin [50].

Postjunctional potentiation of responses to both NA and ATP via dopamine D<sub>4</sub> receptors and endothelin has been

reported [51]. Purinergic neurogenic contractions and responses to ATP are also potentiated by carbachol via  $M_3$  muscarinic receptors, but responses to NA were not [52]. Postjunctional facilitation of contractile responses of the vas deferens with high concentrations of 5-HT have been reported [53].

#### Are ATP and NA stored and released from the same vesicles?

Evidence that suggests that NA and ATP are largely stored in separate vesicles in the sympathetic nerve terminals comes from experiments showing differential prejunctional modulatory effects of various agents. These include the actions of angiotensin II, prostaglandins (Figure 4), CGRP, atrial natriuretic peptide, endothelin-3 and NA via  $\beta$ -adrenoceptors [54,55] on noradrenergic and purinergic responses and on NA and ATP release. Temporal dissociation of the release of ATP and NA also supported the view that these sympathetic cotransmitters occur largely from two different populations of vesicles [56]. It has been suggested that sympathetic axon varicosities in the mouse vas deferens recycle vesicle membrane through the plasma membrane in a manner similar to that described for cholinergic nerve terminals. NPY and NA are coreleased from large dense core vesicles in sympathetic nerves of the bovine vas deferens [57].

#### Inactivation of ATP and NA

Ectonucleotidase activity has been shown in smooth muscle membranes of the vas deferens, including 5'-nucleotidase. The ecto-ATPase inhibitor, ARL67156, enhanced sympathetic purinergic neurotransmission in the guinea-pig vas deferens [58].

The vas deferens was also utilised to show for the first time that soluble nucleotidases are released together with transmitters from sympathetic nerves as a novel mechanism for neurotransmitter inactivation [59]. The releasable ATPase exhibits some similarities to known ectonucleoside

triphosphate/diphosphohydrolases, whereas the releasable AMPase exhibits some similarities to ecto-5'-nucleotidases [60].

In contrast, inactivation of NA released by sympathetic nerves is largely via reuptake into nerve terminals, where it is either re-injected into vesicular stores or degraded by monoamine oxidase (MAO); some NA is taken up by smooth muscle cells and inactivated by MAO or catechol-*O*-methyl transferase [61].

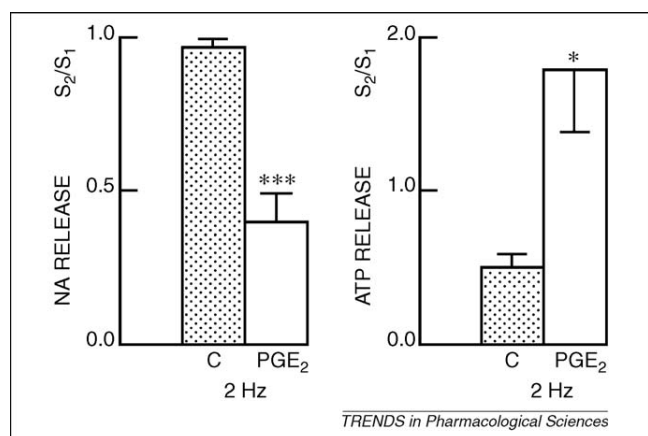
#### Regional variation in purinergic and adrenergic responses to vas deferens

An early paper revealed a predominance of  $\alpha$ -adrenoceptors in the testicular (epididymal) segment of the vas deferens, compared with the urethral (prostatic) segment [62]. Later, regional variation showing dominance of purinergic signalling at the prostatic segment was reported, whereas NA was significantly more potent in the epididymal segment [63]. The ectonucleotidase system was shown to differ between epididymal and prostatic portions with the epididymal portion presenting a different and higher capacity to form adenosine [64]. Most varicosities at the epididymal end of the vas deferens release an insufficient amount of ATP to evoke EJPs [65]. It seems likely that the regional variation is as a result of different subpopulations of sympathetic nerves containing a predominance of NA or of ATP in the epididymal and prostatic regions, respectively.

#### Receptors on smooth muscle of vas deferens

The nerve-stimulation evoked postjunctional receptor for NA is the  $\alpha_{1A}$ -adrenoceptor, acting via inositol triphosphate leading to increase in intracellular  $Ca^{2+}$  and the slow component of nerve-mediated contraction [66]. However, the possibility that there are differences between neurogenic and exogenously applied NA has been raised [67]. They showed that contractions to exogenous NA involved both  $\alpha_{1A}$  and  $\alpha_{2A/D}$  adrenoceptors.

The main postjunctional receptor to ATP is the P2X<sub>1</sub> ion channel receptor, leading to increase in intracellular calcium and the fast component of contraction. The presence of P2Y<sub>2</sub> receptors mediating contraction of the rat vas deferens has also been claimed [68], whereas P2Y<sub>1</sub> receptors mediate a minor relaxing effect of ATP [69]. It was reported that nifedipine blocked P2X-mediated responses to ATP but not to NA [70], although NA responses have also been claimed to be sensitive to nifedipine [71,72]. The calcium agonist Bay K 8644 enhanced the non-adrenergic (purinergic) response, whereas the calcium antagonist nifedipine attenuated this response but not the NA response [70]. Nifedipine preferentially blocks nerve-mediated contractions of the prostatic portion of the vas deferens (demonstrated by  $\alpha, \beta$ -meATP-mediated responses) leaving the epididymal end largely unaffected [73]. N-type  $Ca^{2+}$  channels predominate in the central sympathetic transmission in the vas deferens, although P- and Q-type channels also mediate  $Ca^{2+}$  influx at high stimulation frequencies [74]. Antagonist affinities at P2X receptors in rat vas deferens have been described previously [75]. Antagonism of P2X<sub>1</sub>, but not P2Y receptors, in guinea-pig vas deferens by diinosine pentaphosphate was observed [76].



**Figure 4.** Effect of PGE<sub>2</sub> (100 nM) on the overflow of [<sup>3</sup>H]-NA and ATP from the guinea-pig vas deferens induced by electrical field stimulation (2 Hz). Alterations in the overflow/release of [<sup>3</sup>H]-NA and ATP are shown by comparing the control S<sub>2</sub>/S<sub>1</sub> ratio when no drug is present throughout the experiment (stippled column) to the S<sub>2</sub>/S<sub>1</sub> ratio obtained when PGE<sub>2</sub> is added to the superfusate 15 min prior to the second stimulation (open column). Significance of drug effect was made by using the Student t-test for unpaired observations. \*\*\**P* < 0.001. (Reproduced, with permission from John Wiley and Sons, from Ref. [121].).

An important advance was made when clusters of P2X<sub>1</sub> receptors on smooth muscle opposite close sympathetic terminal varicosities was described in the mouse vas deferens [16]. However, a later paper questioned this finding [77]. In P2X<sub>1</sub> receptor-deficient mice, contraction of the vas deferens to sympathetic nerve stimulation was reduced by up to 60% [78]. P2X<sub>2</sub> receptor expression by interstitial cells of Cajal in vas deferens have been claimed to be involved in semen emission [79]. P2X<sub>1</sub> receptors have been shown to be internalised after exposure to the agonist  $\alpha,\beta$ -meATP [80], perhaps underlying the mechanism of desensitisation. Perinuclear P2X<sub>7</sub> receptor-like immunoreactivity has been described in smooth muscle cells of the guinea-pig vas deferens [81].

### Development and aging

Responses to NA, ACh, histamine and 5-HT did not produce full contractility until 3–4 weeks postnatal in rat vas deferens [82]. Autonomic nerves containing dopamine- $\beta$ -hydroxylase, NPY and enkephalin first appeared at 3 weeks gestation in the human foetal vas deferens [12]. Changes in adrenergic and purinergic signalling in the vas deferens might be expected to occur later than in the gut, because rats are not sexually active until approximately 10 weeks, although the morphology of the vas deferens appears mature by day 35 [83]. Furness *et al.* [84] showed that EJPs, produced by ATP, in response to nerve stimulation of the vas deferens, were not observed in mice of less than 18 days postnatal. Another early study of postnatal development of functional neurotransmission in the rat vas deferens showed that at 3 weeks postnatal (the earliest time studied) the responses to field stimulation with single or trains of pulses lacked the adrenergic component, although the non-adrenergic component was present [85]. Responses to ATP first appeared at day 15 and increased with age [86].

Examination of the ontogeny of P1 purinergic receptors showed that adenosine, acting via prejunctional A<sub>1</sub> receptors, inhibited neurotransmission when nerve-mediated contractions of the rat vas deferens were first observed at day 15, but its potency decreased with age [87]. In a later study, this group claimed that inhibitory postjunctional A<sub>2</sub>-like receptors and prejunctional A<sub>1</sub> receptors were present from days 10 and 15, respectively. In contrast, they identified postjunctional *excitatory* A<sub>1</sub> receptors that did not appear until after day 20 [87].

In 2-week-old guinea-pigs, stimulation of the hypogastric nerve produced monophasic contractions which were only partially blocked by the combination of prazosin and  $\alpha,\beta$ -meATP, suggesting the involvement of an unknown transmitter; however, in 10–15-week-old animals, stimulation produced a biphasic contraction, which was almost completely inhibited by both blockers [88].

Studies of developmental changes on sympathetic nerve-evoked contractions of the *circular* muscle layer of the guinea-pig vas deferens showed that the contractions produced a significant decrease with increasing age, apparently as a result of postjunctional rather than prejunctional mechanisms, responses to  $\alpha,\beta$ -meATP decreasing in parallel [89]. An increase in P2X<sub>1</sub> receptor mRNA expression has been demonstrated between postnatal days 10

and 42 [90]. An early study showed that nerve-mediated contractions in newborn rat vas deferens were susceptible to  $\alpha$ -adrenoceptor antagonism by up to 10 days but resistant thereafter [91], suggesting an increase in input of non-adrenergic responses.

Both prejunctional and postjunctional mechanisms cause the maturation of fast purinergic junctional transmission of the longitudinal muscle of the mouse vas deferens between 21 and 42 days postnatal [90]. Postnatal androgen deprivation dissociates the development of smooth muscle innervation from functional neurotransmission in mouse vas deferens [92].

### Regulation of smooth muscle contractility by the epithelium

By analogy with evidence for the release of transmitters from endothelial cells lining blood vessels [93] and urothelial cells in bladder and ureter [94,95], it has been recently shown that prostaglandin E<sub>2</sub> is released from epithelial cells of the rat vas deferens in response to neurally released ATP acting via P2Y receptors to participate in neurogenic contractions [96]. The contractile responses to NA and ATP were not modified by removal of the epithelium from the rat vas deferens [97].

### Effects of disease on vas deferens

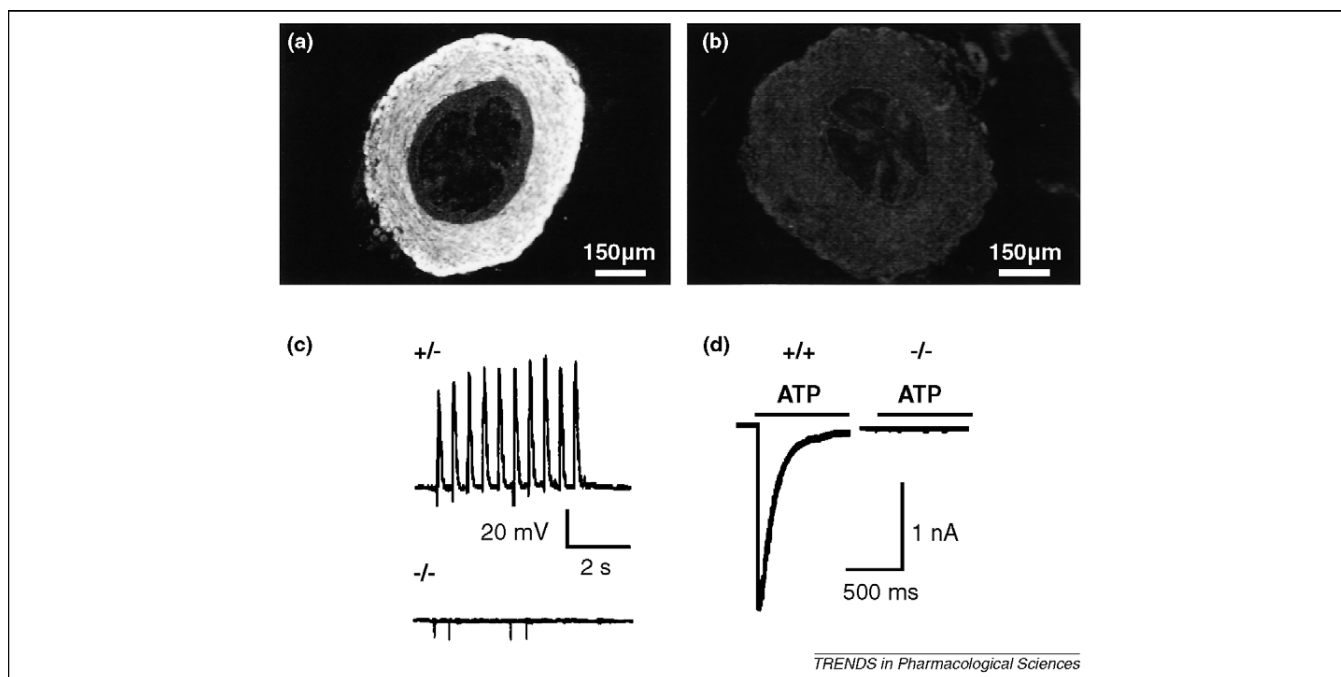
An enhanced initial fast component of sympathetic nerve-mediated responses from spontaneously hypertensive rats (SHRs) was noted as far back as 1985 [98]. However, neurogenic responses were found to be significantly enhanced in the epididymal but not in the prostatic portion of the vas deferens from SHRs compared with Wistar Kyoto rats [99], implying a greater potentiation of adrenergic compared with purinergic components in the vas deferens of SHRs. This appears to be in contrast to reports of a significant increase in the purinergic component of cotransmission from sympathetic nerves supplying blood vessels of SHRs [100]. A reduction in the prejunctional neuromodulatory role of adenosine via A<sub>1</sub> receptors in SHRs has also been described [101].

Studies of streptozotocin diabetic rats showed that after 8 and 12 weeks there was an increase in the purinergic component of the responses to sympathetic nerve stimulation in the vas deferens [102]. An earlier paper concluded that a sympathetic neuropathy occurred in the vas deferens of the streptozotocin diabetic mouse [103]. Treatment of streptozotocin diabetic rats with testosterone for 8 weeks prevented the loss of weight of the vas deferens and the supersensitivity to NA associated with diabetes [104].

Chronic alcohol treatment has been shown to differentially affect noradrenergic and purinergic responses in the rat vas deferens, perhaps altering male reproductive tract function [105].

### Reinnervation of vas deferens following vasectomy and castration

Vasectomy, cutting the vas deferens, is a contraceptive procedure designed to block the passage of sperm. There have been several studies of the effect of this procedure over time on the innervation of the vas deferens in animals [106] and man [107]. Innervation of the epididymal (distal)



**Figure 5.** (a,b) Confocal images of transverse sections of vas deferens show immunohistochemical detection of the P2X<sub>1</sub> receptor protein in the smooth muscle layers of the wild type vas deferens (a); no immunoreactivity was present in the  $-/-$  mutant animal (b). (c,d) Response to ATP and nerve stimulation of wild type and P2X<sub>1</sub> receptor-deficient mouse vas deferens. (c) Intracellular membrane potential recordings from vas deferens smooth muscle cells. Sympathetic nerve stimulation (10 pulses at 10 Hz, 0.5 ms pulse width) evoked excitatory junction potentials in  $+/-$  but not  $-/-$  vas deferens (resting membrane potential  $-87.7 \pm 1$  mV and  $87.6 \pm 1.7$  mV for  $+/-$  and  $-/-$  mice, respectively,  $n = 16$  for each). (d) Whole-cell patch-clamp recordings from acutely dissociated vas deferens smooth muscle cells. ATP evoked a rapidly inactivating inward current in  $+/+$  but had no effect on  $-/-$  acutely dissociated vas deferens smooth muscle cells (holding potential  $-60$  mV). (Agonist applications indicated by bar.) (Reproduced, with permission from the Nature Publishing Group, from Ref. [78]).

portion of the vas deferens was severely compromised even 15 years after the operation [108]. The epididymal segment of the vas deferens shows prolonged supersensitivity to NA [109]. Anastomosis (vasovasostomy) of epididymal and prostatic halves of the vas deferens was carried out in rats in which unilateral or bilateral medial transection (vasectomy) had been performed 4 weeks previously [110]. It was shown that 8 weeks after anastomosis NA levels were back to 40% of the controls and fertility restored.

Following castration, vas deferens exhibited spontaneous contractions, the adrenergic component of the nerve-mediated contractile response was lost, whereas the non-adrenergic (purinergic) component remained and prejunctional inhibition of contractions was significantly reduced [111]. Treatment of castrated rats with testosterone for 8 weeks prevented the decreased vas deferens weight and contractile changes associated with castration [104].

#### P2X<sub>1</sub> receptor antagonists as potential contraceptives

The fast purinergic component of the contraction is required to coordinate the rapid emission of sperm into the urethra prior to ejaculation, whereas the sustained noradrenergic contraction probably prevents any reflux into the vas deferens during ejaculation. The concept of a P2X<sub>1</sub> receptor antagonist acting as a non-hormonal male contraceptive is attractive [78,112], but the effectiveness of such drugs in man is not yet clear because of species differences in the components of purinergic cotransmission. A recent paper has shown the presence of a purinergic cotransmitter pathway in man [32], although in another study a twitch component was claimed to be missing [113].

In P2X<sub>1</sub> receptor-deficient mice, contractions of the vas deferens to sympathetic nerve stimulation is reduced by up to 60%, and there is a 90% decrease in male fertility (Figure 5) [78]. An investigation of neurotransmission in the vas deferens from  $\alpha_{2A/D}$ -adrenoceptor knockout mice led to the conclusion that there is a major loss of prejunctional  $\alpha_2$ -adrenoceptor activity [114].

#### Concluding remarks

Data from the vas deferens preparation has produced substantial support for sympathetic cotransmission involving NA and ATP as cotransmitters and this has been supported by further studies showing sympathetic cotransmission to many different blood vessels [115]. It has produced a very convenient model for studies of prejunctional and postjunctional neuromodulation and has also been used to examine changes in sympathetic neurotransmission in pathophysiology. After all these years, it would seem likely that all is known about this preparation but amazingly new discoveries continue to be made [116–118].

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