Sympathetic innervation of the kidney in health and disease: emphasis on the role of purinergic cotransmission

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Abstract

There is introductory information about non-synaptic transmission at sympathetic neuroeffector junctions and sympathetic nerve cotransmission utilizing noradrenaline and ATP as cotransmitters. Then the organzation and location of sympathetic nerves in different sites in the kidney are described, including renal arteries, juxtaglomerular arterioles and renal tubules. Sympathetic nervous control of glomerular filtration rate and of renin secretion are discussed. Evidence, obtained largely from experiments on animals, for sympathetic nerve modulation of the transport of water, sodium and other ions in the collecting duct of the nephron is described. Finally, there is coverage of the roles of sympathetic nerves in renal diseases, including hypertension, diabetes, hypothyroidism and ischaemia.

Keywords: ATP; noradrenaline; renin; collecting duct; hypertension; diabetes

1. Introduction

We start with an anecdote concerning the lack of early attention to the roles of sympathetic nerves in the kidney. GB was present at a lecture by a leading kidney physiologist. During the discussion after the talk, the speaker was asked why he did not consider the influence of nerves on the various mechanisms he had discussed. 'That is because the nerves in the kidney are not important', he replied, 'because the kidney functions perfectly well after denervation'. Clearly an unacceptable response. Influenced by the beautiful early studies of synaptic transmission in skeletal muscle, ganglia and in the CNS, peopled looked, but did not find, specialised neuroeffector synapses (boutons) on smooth muscle and non-excitable cells in the peripheral system. However, it is clear that non-synaptic transmission is characteristic of neuroeffector transmission in the periphery. Innervation involves release of neurotransmitters from autonomic nerve varicosities making transient contact with effector cells, which possess the receptors to mediate junctional transmission (see Burnstock, 2008). This is described in Section 2.

Another major advance was made when cotransmission involving two or more transmitters was recognised (see Burnstock, 1976). This was in conflict with what was known as Dale's Principle that one nerve only released one transmitter, with the belief that sympathetic nerves release noradrenaline (NA) and parasympathetic nerves acetylcholine. In particular it is now well established that sympathetic nerves release adenosine 5'-triphosphate (ATP) and NA as cotransmitters to both visceral and vascular targets, together with neuropeptide Y (NPY), although this usually acts as a prejunctional modulator after release

rather than as a cotransmitter (Burnstock, 2007). The evidence for sympathetic cotransmission will be discussed in Section 3.

Sympathetic nerves in the kidney are involved in a number of physiological processes, including control of renal blood flow, glomerular filtration rate, reabsorption of water, sodium and other ions, release of renin and production of prostanoids. These roles will be explored in the following sections. Finally, the roles of sympathetic nerves in pathophysiological conditions, such as hypertension, diabetes and hypoglycaemia will be discussed. Reviews concerned with purinergic signalling and kidney function are available (Bailey et al., 2012; Leipziger, 2016; Praetorius and Leipziger, 2010).

2. Non-synaptic transmission at sympathetic neuroeffector junctions

Non-synaptic sympathetic nerve transmission to smooth muscle and non-muscular cells has been recognised (see Burnstock, 1986, 2004; Burnstock & Iwayama, 1971; Gabella, 1995). Transmission is 'en passage', where transmitter is released from varicosities that come close to effector cells. Sympathetic nerve fibres become varicose in the vicinity of the effector tissue (Fig 1; Fig 2a and b). The width of the junctional cleft is variable; prejunctional thickening on varicosities represent the sites of release of transmitter, but no post-junctional specialisations are present (Fig 2c). The vascular smooth muscle effector is a muscle bundle rather than a single muscle cell, which are connected by low resistance pathways that allow electrical spreading of activity within the effector bundle (Fig 3a). Epithelial and immune cells are also transiently innervated when varicosities come close enough to release transmitter to reach the receptors on the cells (Fig 3b).

Fig 1, 2

Fig 3

3. Sympathetic nerve cotransmission

The concept of purinergic neurotransmission (i.e. ATP as an extracellular signalling molecule) was proposed in 1972 and ATP was claimed to be a non-adrenergic, non-cholinergic (NANC) transmitter in the gut and urinary bladder (Burnstock, 1972). ATP was shown to be released from sympathetic nerves supplying the guinea pig taenia coli as well as from the intrinsic NANC inhibitory nerves (Su et al., 1971). This was suggestive that ATP may be a cotransmitter with NA in sympathetic nerves and, together with some other studies (e.g. Langer and Pinto, 1976; Nakanishi and Takeda, 1973), Dale's Principle was questioned that one nerve utilizes one transmitter and the concept of cotransmission proposed and in particular that ATP and NA were cotransmitters in sympathetic nerves (Burnstock, 1976). Electrophysiological experiments in the 1960's concerned with sympathetic

neurotransmission in the vas deferens showed excitatory junction potentials (ejps) that summed and facilitated and at a critical threshold depolarisation, a spike was initiated and associated with contraction (Burnstock and Holman, 1961; Fig 4a). It was puzzling at that time, when NA was regarded as the sole transmitter in sympathetic nerves, that adrenoceptor antagonists did not block the ejps. However, about 20 years later, α,β-methylene ATP (α,β-meATP) that desensitizes the ATP receptor (Kasakov and Burnstock, 1983), was shown to block the ejps (Sneddon and Burnstock, 1984a; Fig 4b) and responses to ATP mimicked the ejps (Fig 4c). Thus it became clear that ATP and NA were cotransmitters in the sympathetic nerves. The release of ATP during stimulation of sympathetic nerves to the vas deferens was blocked by tetrodotoxin (showing that it was released from nerves), by guanethidine, an antagonist that acts by preventing release of sympathetic neurotransmitters and by 6-hydroxydopamine, that destroys sympathetic nerves. Sympathetic cotransmission to blood vessels was also shown (Burnstock 1990, 1995; Katsuragi and Su, 1982; Sneddon and Burnstock, 1984b; see Fig 5a and b). The currently accepted model of sympathetic cotransmission is shown in Figure 6. NPY is also contained and released by most sympathetic nerves, but it rarely acts as a cotransmitter, rather as a prejunctional neuromodulator inhibiting the release of NA and ATP and/or as a postjunctional neuromodulator enhancing the actions of the cotransmitters (Cheung, 1991; Ellis and Burnstock, 1990). There is considerable variation in the proportions of ATP and NA in sympathetic nerves in different organs and species and during development and ageing (see Burnstock, 1995, 2014a). It was interesting that while NA was the dominant neurotransmitter in renal arteries, ATP was the sole neurotransmitter in sympathetic nerves supplying the rabbit jejunal artery and intestinal arterioles, with released NA acting as a neuromodulator of ATP release (Evans and Surprenant, 1992; Ramme et al., 1987).

Fig 4

Fig 5

Fig 6

4. Sympathetic innervation of kidney: general

It is well established that the kidneys and ureters are innervated by sympathetic, parasympathetic and sensory nerves (Ansell and Gee, 1990; Segura et al., 1996). Anatomically, most of the renal sympathetic innervation derives from prevertebral and paravertebral ganglia including the renal plexus located around the renal artery (Ferguson et al., 1986). The postganglionic sympathetic nerve fibres (axons) from these ganglia project along the renal arteries and intrarenal vascular system, and they also reach the proximal (PT) and distal (DT) renal tubules. Therefore the sympathetic innervation of renal tubules and the loop of Henle frequently occurs as an associate of the innervation of the intrarenal

vasculature (Barajas and Powers, 1990; DiBona., 1989; Gandhi, 2007; McLachlan and Luff, 1992). There were pioneering ultrastructural studies of the innervation of juxtaglomerular apparatus and/or arterioles in renal cortex of monkey, rat and sheep kidney (Barajas, 1964; Barajas and Müller, 1973; Müller and Barajas, 1972; Simpson and Devine, 1966).

An example of a close relationship between the vascular, tubular and collecting duct sympathetic innervation in the rat renal cortex is shown in Figure 7, from a study of immunolabelling for tyrosine hydroxylase (TH), the rate limiting enzyme in NA synthesis (Levitt et al., 1965; Pickel et al., 1975) and also of aquaporin 2 (AQP2), a vasopressin-regulated water channel (Christensen et al., 1998; Christensen et al., 2003). Application of pre-embedding immunocytochemistry of TH antibody in conjunction with transmission electron microscope methods allowed observation at the ultrastructural level of sympathetic nerve fibres in the cortex and to some extent in the outer medulla of rat kidney, where these nerves could be seen at renal vasculature and collecting ducts, in addition to PTs and DTs (not shown). Examples of the relationships of these nerves to vasculature and especially to cortical collecting ducts are shown in Figures 8-10. Usually the TH-positive sympathetic nerve fibres appear in a nerve bundle accompanied by Schwann cells and/or their processes (Fig. 8, 9a-b). However, single TH-positive nerve fibre profiles being partially or completely free of Schwann cells are also present at the adventitial vascular smooth muscle (Fig. 8) as well as at the interstitial basolateral sites of collecting duct epithelial cells (Fig. 9c-d, 10a-b). In fact, adjoining TH-positive and TH-negative nerve fibres, which both can be observed in individual nerve bundles, suggest that these bundles are heterogeneous, e.g. they might consist of a mixture of parasympathetic and/or sensory nerves fibres along with the sympathetic ones. The main function of the sympathetic nerves in the kidney is generally associated with (i) renal vasoconstriction and reduction of renal blood flow via α_{1A} adrenoceptors on vascular smooth muscle; (ii) the regulation of secretion of renin from juxtaglomerular apparatus granular cells via β_1 adrenoceptors, and also (iii) stimulation of renal tubular sodium reabsorption via α_{1B} adrenoceptors on tubular epithelium (DiBona, 2000; Johns et al., 2011; Kobayashi and Takei, 1996).

Fig 7

Figs 8-10

Most of the studies on innervation of the kidney have focussed on innervation of intrarenal vasculature (Barajas et al., 1984; Ferguson and Bell, 1988; Liu et al., 1996; Luff et al., 1991; Müller and Barajas, 1972). Evidence has been presented that the efferent arterioles are also innervated by sympathetic nerves, although less densely than the afferent arterioles (Luff et al., 1992). However, it has been reported that, on the basis of ultrastructural studies, two distinct sympathetic nerve types are differentially distributed to afferent and efferent

arterioles (Luff et al., 1992; Denton et al., 2004). Apparently, these nerve fibres also differed structurally from the sympathetic nerve fibres supplying other intrarenal arteries. It seems that in general renal sympathetic innervation is structurally heterogeneous. Though this might not be surprising as the autonomic nerves, including perivascular nerves, are generally very plastic and their structure may vary both in physiological and pathophysiological conditions (Burnstock, 2009a; Cowen and Burnstock, 1986). It is interesting that while P2X1 receptors are abundantly expressed by afferent arterioles, they were not found on the post glomerular efferent arterioles (Chan et al., 1998), suggesting that the proportion of ATP as a cotransmitter with NA is higher in the sympathetic nerves supplying the afferent arterioles compared to those supplying the efferent arterioles.

The density of sympathetic innervation in the kidney is diverse. A quantitative autoradiography study of rat kidney, for example, showed a moderate sympathetic input in all portions of the cortical nephron, while the innervation of the renal vasculature was substantially greater; with the densest innervation of the afferent arterioles followed by that of efferent arterioles and cortical capillaries (Barajas et al., 1984). The innervation of capillaries implies a nervous influence on the activities of capillary endothelial cells. However, there are also reports suggesting rather marked innervation of the PTs and DTs, and the ascending limb of Henle's loop in the kidney of rat and rabbit (Barajas and Powers, 1990; DiBona, 1989; McLachlan and Luff, 1992). According to Barajas et al. (1992) the efferent sympathetic innervation is supplied to all the segments of the renal vasculature but to a much lesser extent to the tubular nephron. Details of histological structures and their innervation by sympathetic nerves in the human and animal kidney can be found in a number of elegant articles and monographs (e.g. Burnstock, 2014b; Burnstock et al., 2014; DiBona, 2000; DiBona and Kopp, 1997; Gorgas, 1978a,b; Johns et al., 2011; Kobayashi and Takei, 1996; Kopp, 2011; Taugner et al., 1978).

Studies performed by DiBona and colleagues (DiBona, 2000, 2005; DiBona and Kopp, 1997; DiBona et al., 1996) revealed complex functional relations between sympathetic nerves and the intrarenal effectors including blood vessels, renal tubules and the juxtaglomerular granular cells, where functional coordination between sympathetic nerves and these intrarenal effectors are probably essential for overall healthy renal function (DiBona, 2005; Johns et al., 2011). There is background evidence for close interactions between sympathetic and sensory nerves (see Burnstock, 2009b), so it is likely that this also occurs in the kidney (see Kopp et al., 2008; Mulder et al., 2013).

Early papers did not recognise that ATP was released as a cotransmitter from sympathetic nerves supplying the kidney. However, release of ^{3}H-purines from [^{3}H]-adenine labelled rabbit kidney was reported following sympathetic nerve stimulation (Fredholm and Hedqvist, 1978). Intravenous injection of ATP had renal vasoconstrictor actions (Hashimoto et al., 1988). Renal periarterial sympathetic nerve stimulation at low frequencies produced vasoconstriction mainly due to the release of the purinergic transmitter component (Schwartz and Malik, 1989). ATP released from sympathetic nerves produced vasoconstriction of rat juxtamedullary afferent arterioles via P2X receptors, followed by further vasoconstriction via P1 receptors after extracellular breakdown of ATP to adenosine (Inscho et al., 1991; Weihprecht et al., 1992), but not postglomerular juxtamedullary microvessels (Inscho et al., 1992).

Since α,β-meATP contracted vessels in the kidney, this suggested that ATP released by sympathetic nerves caused vasoconstriction via P2X1 receptors (Churchill and Ellis, 1993; Eltze and Ullrich, 1996). Figure 11 shows the distribution of P2X1 receptors in the intrarenal vasculature. ATP-mediated constriction of juxtamedullary afferent arterioles was shown to be dependent on influx of extracellular calcium and that the sustained constriction was dependent primarily on calcium entry via voltage-gated L-type calcium channels (Inscho et al., 1995). Release of NA from renal sympathetic nerves was inhibited by prejunctional P1 and P2 receptors activated by ATP co-released from sympathetic nerves and its breakdown product adenosine (Bohmann et al., 1997). Endogenous nitric oxide (NO) was shown to be an inhibitory modulator of renal vasoconstrictor responses to NA, but not to the responses of the sympathetic cotransmitter ATP (Malmström et al., 2001). It was concluded from a study of rat renal artery that NA was the dominant cotransmitter relative to ATP and that P2X1, $P2Y_1$, and $P2Y_2$ receptors all mediated vasoconstriction of the vessel (Knight et al., 2003). NA and ATP sympathetic cotransmission involved activation of vasoconstrictor P2X1, P2X3 and $P2Y_6$-like receptors in mouse perfused kidney (Vonend et al., 2005). P2X1 receptor blockade inhibited whole kidney regulation of renal blood flow *in vivo* (Osmond and Inscho, 2010). P2X1 receptors mediate vasoconstriction evoked by the sympathetic cotransmitter ATP, so it is not surprising that P2X1 receptor antagonists have this effect. Evidence was presented that two purinergic receptors are activated in renal vascular smooth muscle cells by ATP released from sympathetic nerves, namely P2X1 homomultimer and P2X1/4 heteromultimer receptors (Harhun et al., 2010).

Fig 11

Adenosine A_1 receptors mediate vasoconstriction of all preglomerular vessels, whereas A_2 receptors mediate pre- and postglomerular vasodilation. In particular, adenosine

mediates vasoconstriction of afferent arterioles (Hansen et al., 2007). Infusion of adenosine into the renal artery of pigs, rabbits, rats and dogs, induced vasoconstriction in the cortex (Osswald, 1975; Sakai et al., 1981) followed by vasodilation (Hansen and Schnermann, 2003; Macias et al., 1983), probably mediated via different P1 purinoceptor subtypes.

Reviews concerned with the roles of ATP in the regulation of microvascular function in the kidney are available (Burnstock and Ralevic, 2014; Guan et al., 2007; Inscho, 2001, 2009; Jankowski, 2008).

5. Sympathetic nervous control of glomerular filtration rate

The macula densa cells are the sensing component of the tubuloglomerular feedback (TGF) mechanism and respond to changes in tubular fluid composition by transmitting signals to the afferent arterioles concerned with the regulation of preglomerular vascular resistance and filtered load to the tubules (Nishiyama and Navar, 2002). ATP released from both sympathetic nerves and following paracrine release from macula densa cells, is involved in the mechanism of TGF (Nishiyama and Navar, 2002). Data was presented indicating that TGF signals are coupled to preglomerular vasoconstriction by ATP-mediated activation via P2X1 receptors (Inscho et al., 2003). Later studies of P2X1 receptor knockout mice led to the conclusion that P2X1 receptors play an essential role in TGF-mediated afferent arteriolar vasoconstriction (Inscho et al., 2004). ATP increased the reactivity of the mouse afferent arteriole to low concentrations of NA (Hultström et al., 2007).

Adenosine, after breakdown of released ATP, affected glomerular filtration rate in sodium restricted rats (Osswald et al., 1978). A_1 receptors were identified to modulate this effect. However, in a later study an A_1 receptor antagonist was shown to inhibit TGF (Franco et al., 1989). Ectonucleotidases play a major role in the regulation of glomerular filtration barrier (Bakker et al., 1993). TGF was abolished in A_1 receptor-deficient mice (Brown et al., 2001; Sun et al., 2001). NTPDase1/CD39 ectonucleotidase deficient mice showed compromised TGF regulation of glomerular filtration rate (Oppermann et al., 2008). Enhanced TGF was shown in mice with vascular over-expression of A_1 receptors (Oppermann et al., 2009). More recently, A_2 receptors were claimed to modulate the TGF response by counteracting the effects of A_1 receptors (Carlström et al., 2010). Maintained TGF responses during acute inhibition of P2 purinergic receptors in mice have been reported (Schnermann, 2011).

The roles of ATP and adenosine as mediators of TGF regulation of glomerular filtration have been reviewed (Castrop, 2007).

6. Sympathetic nervous control of renin secretion

The renin-secreting juxtaglomerular cells in the kidney are modified vascular smooth muscle cells that are innervated by sympathetic nerves. ATP increases renin release from juxtaglomerular cells (Gaál et al., 1976). Sympathetic junctional cotransmission mediated by NA and ATP releases renin from epithelioid juxtaglomerular cells and vascular smooth muscle cells of the mouse kidney afferent arterioles (Bührle et al., 1986). A contribution of $P2Y_{11}$ receptor-mediated effects to sympathetic stimulation of renin release has been suggested (van der Weyden et al., 2000).

Adenosine, the breakdown product of ATP released from sympathetic nerves, was shown to suppress renin secretion in sodium restricted rats (Osswald et al., 1978). Theophylline, an adenosine receptor antagonist, prevented the adenosine-induced decrease in renin release (Spielman, 1984). A paper utilizing A_1 receptor knockout mice identified the role of A_1 receptors in regulating renin release (Brown et al., 2006). The renin-secreting juxtaglomerular cells express both A_1 and A_2 receptors, A_1 receptors mediate inhibition, while A_2 receptors stimulate renin secretion (Churchill and Churchill, 1988; Ortiz-Capisano et al., 2013).

7. Sympathetic modulation of resorption of water, sodium and other ions

One function of the sympathetic nerves in the kidney is generally associated with stimulation of renal tubular sodium reabsorption and decreased urinary sodium excretion via $\alpha 1_B$ adrenoceptors on tubular epithelium (DiBona, 2000; Johns et al., 2011; Kobayashi and Takei, 1996).

There is evidence for the involvement of ATP co-transmission with NA in sympathetic nerves supplying renal collecting ducts, since P2X and P2Y ATP-receptors have been identified on nephron epithelium (Birch et al., 2013; Leipziger, 2016; Schwiebert, 2001; Schwiebert and Kishore, 2001). Basolaterally located P2X and P2Y receptors on collecting duct epithelial cells can be activated by ATP released from adjacent nerve varicosities to mediate an increase in sodium and water transmembrane transport (Bailey et al., 2012; Schwiebert and Kishore, 2001). Immunohistochemical studies have already shown the existence of P2X and P2Y receptors on collecting duct epithelial cells (Bailey et al., 2012; Turner et al., 2003; Wildman et al., 2008). In our study of rat renal collecting duct innervation at the electron microscope level, immunocytochemistry of TH was used to reveal sympathetic nerves (Loesch et al., 2009). It was shown that the sympathetic nerve varicosities immunolabelled for TH were anatomically present not only on intrarenal vasculature but also

on cortical collecting ducts, establishing neuroeffector junctions with collecting duct epithelial cells (Fig 7, 8, 9c, d). It was concluded that alongside sympathetic nerves affecting the physiology of intrarenal vasculature via neuromuscular junctions, these nerves may, via the neuroepithelial junctions, influence physiology of collecting duct epithelial cells. It is known that collecting duct epithelium expresses ATP-gated P2 receptors, and we demonstrated the expression of P2X5 receptors in collecting duct epithelium as observed at the electron-immunocytochemical level (Fig 12a, b). This agrees with the immunofluorescence observation of P2X5 receptors on cortical and medullary collecting ducts, where in fact the distribution of this receptor increases along the collecting duct from the outer cortex to outer and inner medulla (Turner et al., 2003). As collecting duct epithelium also displays the presence of AQP2 water channel (see Fig. 7), it is therefore highly likely that both P2X (e.g. P2X5) and AQP2 receptors are co-localised in the same collecting duct epithelial cell(s) to influence the transmembrane water and sodium transport. However, in addition to ATP action from sympathetic nerves it has also been suggested that ATP can be released from nephron luminal epithelium. There is abundant evidence that gentle mechanical stimulation causes release of ATP from a wide range of cells (especially induced following shear stress) including glomerular endothelial cells (see Burnstock, 1999; Lazarowski, 2012). Therefore it seems likely that this is also the case for nephron epithelium. The ATP then acts on P2X and P2Y receptors at luminal locations to inhibit sodium and water transport (Wildman et al., 2008). This can be partially supported by the presence of an apical location of P2X5 receptor-immunoreactivity in some collecting duct epithelial cells (Fig. 12b). However, there might be confusion as to the precise classification of the collecting duct epithelial cell type involved based simply on their ultrastructural features alone (e.g. see Stanton et al., 1985; Verlander, 1998; Verlander et al., 1987, 1994). It has been observed, for instance, that the AQP2-negative principal cells might be $[H^+]$ATP-positive, which classifies these cells as intercalated ones (Christensen et al., 2003). This can be further complicated by the evidence that some AQP2-positive principal cells possibly possess properties of progenitor cells, as has been observed in mice collecting ducts, which may differentiate into intercalated cells or even "null cells" in response to K^+ depletion (Kim et al., 2015). It is apparent that signalling involving renal collecting duct epithelium is a complex phenomenon with the possible involvement of sympathetic nerves with catecholaminergic and purinergic signalling together with a contribution of other non-neuronal signalling.

It is recognised that sympathetic and other nerves can influence renal physiology

(Burnstock, 1993; Ferguson and Bell, 1988; Liu et al., 1996). This may be partially supported by our own observation of non-sympathetic TH-negative nerve fibres present close to the sympathetic TH-positive ones at neuroeffector junctions involving intrarenal vasculature and collecting ducts (e.g. Fig. 8 and 9b). We have previously suggested (Loesch et al., 2009) a few possibilities as to the chemical nature of the TH-negative nerve fibres observed on renal vasculature and at collecting ducts. Hence it is possible that TH-negative axons are in fact: (i) sensory afferent axons containing substance P and calcitonin gene related peptide (Ferguson and Bell, 1988; Johns et al., 2011), also (ii) comprise ATP involved in 'axon reflex' activity (see Burnstock, 1993), or (iii) they may be NO producing axons of renal intrinsic innervation modulating the activities in renal sympathetic nerves (Liu et al., 1996) and subsequently modulating cortical and medullary blood flow (Walkowska et al., 2004).

8. Roles of sympathetic nerves in renal diseases

8.1 Hypertension

Hypertension is associated with sympathetic hyperactivity and chronic kidney disease (see Amann and Veelken, 2003). It was suggested that ATP-induced hypotension is associated with attenuation of sympathetic efferent nerve activity mediated through vagal afferent pathways, via reflex activation, and vagal afferent impulses may be one of the mechanisms that inhibit reflex sympathetic activities, such as rebound hypertension after ATP-induced hypotension (Taneyama et al., 1991). The purinergic component of sympathetic cotransmission has been shown to be much increased in spontaneously hypertensive rats (Bulloch and McGrath, 1992; Vidal et al., 1986). Hypertension is due to a large part by sympathetic overactivity which can be triggered by afferent signals emanating from the kidney and resetting sympathetic tone by stimulation of hypothalamic centres. The effects of sympathetic overactivity also lead to accelerated progression of renal failure (Orth et al., 2001). It has been suggested that ATP receptors contribute to renal vessel hypertrophy during angiotensin II-induced hypertension (Graciano et al., 2008). There is evidence that hypertension reduces renal vascular reactivity to P2 receptor stimulation (Inscho, 2009). P2X1 receptor-mediated sympathetic vasoconstriction of afferent arterioles in angiotensin II-infused hypertensive rats fed a high salt diet was reported (Inscho et al., 2011). A physiological and potential pathophysiological role for P2X7 receptors in controlling renal vascular function, resulting in the susceptibility to hypertension-related kidney damage, has been suggested (Menzies et al., 2013). P2X7 receptor antagonism attenuated hypertension and renal injury in Dahl salt-sensitive rats (Ji et al., 2012). Angiotensin induced rapid release

of ATP, an effect enhanced in Dahl salt-sensitive rats (Palygin et al., 2013). The authors suggest that this shows that ATP can contribute in the development of salt-sensitive hypertension. Angiotensin has been shown to cause release of ATP from sympathetic nerves, acting prejunctionally (Ellis and Burnstock, 1989).

Chronic activation of renal sympathetic nerves, in both animal and human studies, is critical in the pathogenesis and perpetuation of treatment-resistant hypertension and evidence has been presented that renal denervation may be a therapeutic option in disease states characterized by central sympathetic overactivity (Nishi et al., 2015; Thorp and Schlaich, 2015). It is claimed that renal sympathetic nerves contribute to dendritic cell activation, subsequent T-cell infiltration and end-organ damage in the kidney in the development of hypertension (Xiao et al., 2015). Renal sympathetic nerves contribute to hypertension via activities in the kidney that enhance sodium retention and renin secretion and by increases in CNS dictated sympathetic activity; therefore, renal denervation is suggested for the treatment of hypertension (Weber et al., 2015). However, the results of clinical trials have shown that renal sympathetic denervation against hypertension were disappointing (Bhatt et al., 2014; Briasoulis and Bakris, 2015; Laffin and Bakris, 2015). The efficacy of renal denervation for reducing blood pressure in drug-resistant hypertension is currently inconclusive, although it appears to work in some patients (see Hart et al. 2013). A review discussing the role of ATP in regulating renal microvascular function and in hypertension is available (Guan and Inscho, 2011).

Both essential hypertensive patients and patients with renal artery stenosis showed a dose-dependent vasodilation following adenosine infusion (Wierema et al., 1998). The increase in ecto-5'-nucleotidase activity suggests a rise in renal adenosine levels that protected the kidney against hypertension (Fürstenau et al., 2010).

8.2 Diabetes

It has been reported that renal glucose release during hypoglycaemia is partly controlled by sympathetic nerves (Bischoff et al., 2015). Therefore, the authors suggested that the effects of sympathetic denervation of the kidney on renal glucose metabolism should be taken into account when considering renal denervation as a therapy for diabetic patients. $P2Y_{12}$ receptors may play a role in renal handling of water in health and in nephrogenic diabetes insipidus (Zhang et al., 2015).

8.3 Heart Failure

In heart failure there is an increase in renal sympathetic nerve activity resulting in renal vasoconstriction, increased renin release and sodium retention (Booth et al., 2012; Ramchandra and Barrett, 2015).

8.4 Hypothyroidism

ATP released from sympathetic nerves mediates renal vasoconstriction, which is increased in hyperthyroid kidneys and attenuated in kidneys from hypothyroid rats (Vargas et al., 1996).

8.5 Ischaemia

Renal ischaemic-reperfusion is a major cause of kidney injury. Post-ischaemic infusion of ATP was shown to have a beneficial effect in renal failure (Gaudio et al., 1982; Glazier et al., 1978; Osias et al., 1977; Paller et al., 1998; Siegel et al., 1983; Sumpio et al., 1985). Over-expression of the ectonucleotidase, CD39, protects against renal ischaemia-reperfusion and transplant vascular injury (Crikis et al., 2010), while inhibition of the ecto-5'-nucleotidase (CD73) protects against ischaemia-reperfusion injury (Rajakumar et al., 2010). Renal sympathetic denervation suppresses atrial fibrillation induced by atrial ischaemia (Zhou et al., 2015).

There are reviews about P2 receptors in renal pathophysiology (Amann and Veelken, 2003; Booth et al., 2012; Persson and Carlström, 2015; Turner et al., 2009).

9. Conclusions

In summary, the present review has focused on the roles of sympathetic nerves at different sites in the kidney. Emphasis is placed on the roles played by ATP released as a cotransmitter with NA from sympathetic nerves. Their influence on control of glomerular filtration rate and renin secretion is described. In addition emphasis is made of the neglected role of sympathetic nerves in modulating the transport of water, sodium and other ions in the collecting duct of the nephron. This information will contribute to the possible therapeutic developments for the treatment of renal diseases, such as hypertension, diabetes and ischaemia. Ultrastructural and immunocytochemical descriptions of the innervation show TH-positive axon varicosities on the renal cortical collecting duct. Immunocytochemical expression of ATP-gated P2X5 receptors by collecting duct epithelial cells is also illustrated. P2X1 and P2X7 receptor antagonists show promise for the treatment of hypertension associated with sympathetic hyperactivity and chronic kidney disease. $P2Y_{12}$ receptors appear

to be involved in nephrogenic diabetes, while inhibition of ecto-5-nucleotidase protects against kidney ischaemia-reprofusion injury.

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Figure Legends

Figure 1

A scanning electron micrograph of a single terminal varicose nerve fiber lying over smooth muscle of the small intestine of the rat. The intestine was pre-treated to remove connective tissue components by digestion with trypsin and hydrolysis with HCl. Scale bar = 3μm. (Reproduced from Burnstock (1988) with permission from Marcel Dekker).

Figure 2

(a) A medium-sized intramuscular bundle of axons within a single Schwann cell (S) in the guinea pig vas deferens. Varicosity A1 containing many vesicles, perhaps related to the proximity (80 nm) to the muscle cell (M). The small profiles (N), less than 0.25 μ in diameter, are probably sections through intervaricosities and contain neurofilaments. e.r. = endoplasmic reticulum. (Reproduced from Merrillees et al. (1963) with permission from Rockefeller University Press).

(b) Whole mount preparation of the sheep mesenteric vein at the level of the inner surface of the adventitia, showing innervation of the medial muscle coat by an autonomic ground plexus consisting of bundles of fine varicose nerves containing noradrenaline. Incubated in formaldehyde vapour for 1 hr. Scale bar = 50μ. (Reproduced from Burnstock (1970) with permission from Edward Arnold).

(c) An autonomic varicosity in guinea pig vas deferens showing dense prejunctional thickenings and bunching of vesicles, probably representing transmitter release sites (*arrows)*, but there is no postjunctional specialization. (Reproduced from Burnstock (2004) with permission from Elsevier).

Figure 3

(a) Schematic representation of control of vascular smooth muscle by perivascular varicose nerves in the adventitia (-●-●-) and endothelial factors (*arrows*). (Modified from Burnstock and Costa (1975) with permission from CRC Press).

(b) Close apposition between rat mast cell protease 1 immunoreactive and calcitonin gene-related peptide immunoreactive nerve fibres observed by confocal microscopy. (Reproduced from Dimitriadou et al. (1997) with permission from Elsevier).

Figure 4

(a) Excitatory junction potentials (ejp's) in response to repetitive stimulation of sympathetic nerves at 1 Hz in the guinea-pig vas deferens. The upper trace records the tension, the lower trace the electrical activity of the muscle recorded extracellularly by the sucrose gap method. Note both summation and facilitation of successive junction potentials. At a critical depolarization threshold an action potential is initiated which results in contraction. (Reproduced from Burnstock and Costa (1975) with permission of Chapman and Hall.)
(b) The effect of various concentrations of α,β-methylene ATP on ejp's recorded from the guinea-pig vas deferens (intracellular recording). Top panel: The control responses to stimulation of the motor nerves at 0.5 Hz are shown on the left. After at least 10 trains in the continuous presence of the indicated concentration of α,β-methylene ATP, ejp's were recorded using the same stimulation parameters. The ejp's are clearly reduced in magnitude in the presence of α,β-methylene ATP. Notice also that in control cells several large spontaneous ejp's were seen, whereas after α,β-methylene ATP no spontaneous ejp's were recorded. (Reproduced from Sneddon and Burnstock (1984a) with permission from Elsevier).
(c) Spritzed ATP, but not NA, mimicked the ejp recorded in the vas deferens. (Reproduced from Burnstock and Verkhratsky (2012) with permission from Springer.)

Figure 5

(a) Contractions produced in the isolated saphenous artery of the rabbit following neurogenic transmural stimulation (0.08–0.10 ms; supramaximal voltage) for 1 s at the frequencies indicated (triangles). Nerve stimulations were repeated in the presence of 10 μM prazosin (a_1-adrenoceptor antagonist) added before desensitization of the P2 purinoceptors with 10 μM α,β-methylene ATP as indicated by the arrows. (Reproduced from Burnstock and Warland (1987) with permission from Elsevier.)
(b) Intracellular recording of the electrical responses of single smooth muscle cells to field stimulation of the sympathetic motor nerves (the pulse width was 0.1 ms at 0.5 Hz, indicated by ●). Top panel (i): Control response of the muscle. Note that to each individual stimulus there was a rapid depolarisation, and as the train of pulses progressed, a slow depolarisation developed. Similar responses were obtained in bottom panel i, which are also control responses in Krebs solution. Top panel ii and iii show the effect of phentolamine (2×10^{-6} M, added to the bathing solution). The fast depolarisations produced by each stimulus were not reduced, but there was a progressive reduction in the size of the slow depolarisation, which was almost abolished after 6 min. Bottom panel ii shows the effect of a higher concentration of α,β-methylene ATP. Here the fast depolarisation was totally abolished, whilst the slow

depolarisation persisted, although reduced to some extent. Subsequent addition of phentolamine (2 x 10^{-6} M), together with α,β-methylene ATP, abolished the neurogenic response completely (iii). (Reproduced from Sneddon and Burnstock (1984b) with permission from Elsevier.)

Figure 6

Schematic of sympathetic cotransmission. ATP and NA released from small granular vesicles (SGV) act on P2X and α_1 receptors on smooth muscle, respectively. ATP acting on inotropic P2X receptors evokes excitatory junction potentials (EJPs), increase in intracellular calcium ($[Ca^{2+}]_i$) and fast contraction; while occupation of metabotropic α_1 adrenoceptors leads to production of inositol triphosphate (IP_3), increase in $[Ca^{2+}]_i$ and slow contraction. Neuropeptide Y (NPY) stored in large granular vesicles (LGV) acts after release both as a prejunctional inhibitory modulator of release of ATP and NA and as a postjunctional modulatory potentiator of the actions of ATP and NA. Soluble nucleotidases are released from nerve varicosities, and are also present as ectonucleotidases. (Reproduced from Burnstock and Verkhratsky (2010) with permission from Elsevier.)

Figure 7 Fluorescence confocal laser microscopy of an adult male Sprague-Dawley rat kidney cortex (20 µm cryostat transverse-sections) co-immunolabelled for TH and AQP2. Sympathetic TH-positive perivascular nerve fibres (*red*) with visible varicosities (arrows) innervate a small arteriole (Bv); the TH-positive nerve fibres are also present at collecting duct (CD), proximal tubules (PT), and project to distal tubule (DT). It is likely that this pattern of innervation is a part of a sequential '*en passant*' innervation (DiBona, 2005), at least of the CD by the same nerve fibre shared with the arteriole perivascular innervation. Also note the AQP2-positive labelling (*light green*) of CD epithelium. G - Glomeruli. Bar: 25 µm. [Note the main steps of TH-labelling involved: a rabbit polyclonal anti TH antibody (1:200; Biomol Int., Exeter, UK), biotin-conjugated donkey anti-rabbit polyclonal IgG (1:500; Vector Labs Ltd, Peterborough, UK), and Avidin-D-Texas Red (1:200; Vector), while the AQP2-labelling involved: a goat polyclonal anti-AQP2 antibody (1:1000, Santa Cruz Biotech Inc., USA), a donkey anti-goat FITC (1:1000; Santa Cruz). Confocal laser microscope: BioRad Radiance 2100, Hercules, CA]. (Reproduced and modified from Fig. 5A of Gandhi (2007).)

Figure 8

Electron microscopy of perivascular region of an arteriole from the renal cortex of the adult male Sprague-Dawley rat, immunolabelled for tyrosine hydroxylase (TH). Note TH-positive (TH; 'black' immuneprecipitate) perivascular nerve fibres, including axon varicosities, innervating a small arteriole. In the TH-positive varicosities numerous vesicles and mitochondria are present. Also note a TH-negative nerve fibre (Ax), Schwann cell profile (SC), arteriole vascular smooth muscle (sm) and endothelium (En); lu-lumen. Bar: 0.5 µm. (Reproduced from Loesch et al. (2009) with permission from S. Karger AG.)

Figure 9

Electron microscopy of collecting ducts of the renal cortex of the adult male Sprague-Dawley rat, immunolabelled for tyrosine hydroxylase (TH).

(a) A general view of a fragment of collecting duct with surrounding interstitium (inter) shows location of TH-positive nerve fibres (arrows). Collecting ducts principal epithelial cell (Ep), nucleus (N), mitochondria (m) and duct's lumen (lu) can be seen. Bar: 1 µm.

(b) At high magnification, the TH-positive fibres (arrows) display numerous microtubules (mt), which are densely packed in the axon profile indicated by short arrow; perhaps one or two vesicle-like structures are present. Also note a TH-negative axon profile (Ax) and Schwann cell (SC). Bar: 0.5 µm.

(c) A low magnification of a fragment of collecting duct with surrounding interstitium shows close apposition of a TH-positive nerve profile (arrow). Note the numerous small microprojections present on the luminal surface (intercalated epithelial cells). Bar: 1 µm.

(d) At high magnification, more details of the TH-positive nerve varicosity (size: about 0.5 µm by 1.1 µm) can be seen, despite the obscuring effect of immunoprecipitate. Note the small agranular (av) and granular (gv) vesicles of about 40 and 70 nm in diameter, respectively; at least 1 large granular vesicle (Lgv) of about 100 nm diameter can be seen. Also note that there is no Schwann cell presence in this section and the width of the gap between the varicosity and the basal membrane of the epithelium (Ep) is about 100 nm; the gap is mostly occupied by the basement membrane (bm). Bar: 200 nm. (Reproduced from Loesch et al. (2009) with permission from S. Karger AG.)

Figure 10

Electron microscopy of collecting ducts of the renal cortex of the adult male Sprague-Dawley rat, immunolabelled for tyrosine hydroxylase (TH) (a, b) and unlabelled control preparation (c, d).

(a) At low magnification; note TH-positive axon varicosity (arrow) located at the base of collecting duct displaying intercalated epithelial cells (Ep); note that luminal membrane of intercalated cells is scarce in microprojections. Nucleus (N) of epithelial cell and duct's lumen (lu) can be seen. Bar: 0.5 µm.
(b) TH-positive axon varicosity from (a) at higher magnification displays immunoprecipitate obscuring numerous microtubules and possibly vesicles (ve). Note the close proximity (~100 nm) of the varicosity to the epithelium basolateral membrane, which forms folds joined by junctional complexes including desmosomes (de). Also note that the varicosity is naked, i.e. its surface is free of Schwann cells. Bar: 100 nm.
(c) A fragment of a collecting duct with surrounding interstitium including fibroblasts (F) at low magnification; note the presence of an axon varicosity (arrow) close to the epithelium of the collecting duct containing numerous mitochondria (m). Bar: 0.5 µm.
(d) At high magnification note structural details of naked (free of Schwann cells) axon varicosity showed in (c) displaying numerous, mostly small, agranular vesicles (av; ~ 30-40 nm) and 2 mitochondria (m). The distance between the varicosity and the epithelium basolateral membrane (making numerous folds) is about 100 – 150 nm; the basement membrane (bm) can also be seen. Bar: 100 nm. (Reproduced from Loesch et al. (2009) with permission from S. Karger AG.)

Figure 11

Immunohistochemical staining (reddish brown) for P2X1 purinoceptors along the microdissected intrarenal vasculature. a: Low magnification (x35) showing arcuate artery (ArA), interlobular artery (IA), and afferent arteriole (AA). b: Higher magnification (x280) of the area *B* of a showing P2X1 immunoreactivity on the vascular smooth muscle cells on IA and AA. c: High magnification (x280) light micrograph showing positive P2X1 immunoreactivity on vascular smooth cells of the microdissected IA and AA. No significant immunostaining was detected in the glomerulus (G). (Reproduced from Chan et al. (1998) with permission from the American Physiological Society.)

Figure 12

Electron microscopy of collecting ducts of the kidney cortico-medullary region of the adult male Wistar-Kyoto rat, immunolabelled for ATP-gated P2X5 receptors.
(a) Note a fragment of tangentially sectioned collecting duct showing P2X5-positive epithelial cell with visible nucleus (N), and displaying 'black' immunoprecipitate finely

dispersed in the cytoplasm. Also note one immune-negative epithelial cell, possibly intercalated (IC) type A, containing numerous mitochondria (m), a few lysosomes (ly) and dispersed in the cytoplasm numerous small vesicular and tubular structures, which are not obvious at this magnification. Collecting duct lumen (lu), basolateral short infolds of cell surface (bl) and basement membrane (bm) can also be seen. Bar: 2 µm.
(b) Note an axon-like profile (asterisk) enclosed between basolateral regions of P2X5-positive and P2X5-negative epithelial cells; the apical cytoplasm of P2X5-positive cell displays clustered immunoprecipitate (arrow) adjacent to mitochondria and cytoplasmic vesicles (NB: the vesicles might be involved in the water channels, see: Wade et al., 1981; Christensen, 1988; Christensen et al., 1998; Nielsen et al., 2002). Bar: 1 µm. [Note the main steps of pre-embedding P2X5-immunolabelling involved paraformaldehyde-glutaraldehyde fixation; vibratome cutting (at 60 µm), and then exposure of vibratome sections to: hydrogen peroxide-sodium azide; normal goat serum (Nordic Immunology, Tilburg, The Netherlands); a rabbit polyclonal anti P2X5 receptor antibody (at 1.5 - 5.0 µg/ml; Roche Bioscience, Palo Alto, USA & Research Genetics, Huntsville, AL, USA; also see: Oglesby et al., 1999); a goat anti-rabbit IgG serum (DAKO, Glostrup, Denmark); a rabbit PAP complex (DAKO); 3,3'-diaminobenzidine (Sigma, Poole, UK); osmium tetroxide; ethanol-propylene oxide; and then embedment in Araldite. Ultrathin sections (80 nm thick) were stained with uranyl acetate and lead citrate and subsequently examined with a JEM-1010 transmission electron microscope]. (From A. Loesch and G. Burnstock unpublished study performed in July 1998).