Original Paper

nephron
Physiology

Nephron Physiol 2009;113:p15–p21
DOI: 10.1159/000235246

Published online: August 14, 2009

Sympathetic Nerve Varicosities in Close Apposition to Basolateral Membranes of Collecting Duct Epithelial Cells of Rat Kidney

Andrzej Loesch[a] Robert Unwin[b] Vanita Gandhi[a] Geoffrey Burnstock[c]

[a]Research Department of Inflammation, [b]Centre for Nephrology (London Epithelial Group), and
[c]Autonomic Neuroscience Centre, UCL Medical School, Royal Free Campus, London, UK

Key Words
Epithelial cell · Kidney · Nerve · Sympathetic · Varicosity

Abstract
Background: There are reports of sympathetic innervation of the nephron and of P2 purinergic receptors on epithelial cells. Since ATP is a cotransmitter with noradrenaline in sympathetic nerves, the objective of the present study was to re-investigate basolateral innervation of rat renal collecting duct epithelial cells by sympathetic nerves in the context of recent data on the effects of ATP on this nephron segment. ***Methods:*** Kidney sections were processed for electron immunocytochemistry, using tyrosine hydroxylase rabbit polyclonal antibody, with a second layer of biotinylated donkey anti-rabbit antibody and finally extravidin-horseradish peroxidase. Immunoreactivity was visualised with 3,3′-diaminobenzidine and examined with a Philips CM120 transmission electron microscope. ***Results:*** Electron microscopic evidence is presented for close apposition of sympathetic nerve varicosities immunolabelled with tyrosine hydroxylase to principal and intercalated type epithelial cells of the collecting duct of the cortical region. ***Conclusions:*** It is suggested that ATP is released as a cotransmitter from sympathetic nerve varicosities to act on basolateral P2 purinoceptors to influence sodium and water (and potentially acid-base) transport, in conjunction with the known (typically inhibitory) actions of autocrine and/or paracrine release of ATP from collecting duct epithelial cells acting via luminal P2 receptors. It is suggested that while luminal responses may dominate under normal physiological conditions, in pathophysiological states, such as stress and dehydration, sympathetic nerves might also be involved in modulating collecting duct fluid and electrolyte transport.

Introduction

It is increasingly accepted that ATP is released as an autocrine or paracrine agent to act on P2X and P2Y receptors on kidney nephron collecting duct epithelial cells to regulate sodium and water transport [1–6].

$P2Y_2$, $P2Y_4$, $P2X_4$ and $P2X_6$ receptors have been identified on both apical and basolateral surfaces of collecting duct epithelial cells in culture and in native collecting ducts [2, 6, 7]: depending on the receptor subtype and its distribution, apical receptors seem to mediate inhibition of sodium and water transport [8–12], while basolateral receptors can mediate not only inhibition of sodium and water transport [9, 13], but also potentially stimulation of sodium reabsorption [14].

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1660–2137/09/1133–0015$26.00/0

Accessible online at:
www.karger.com/nep

Geoffrey Burnstock
Autonomic Neuroscience Centre
UCL Medical School, Royal Free Campus
Rowland Hill Street, London NW3 2PF (UK)
Tel. +44 20 7830 2948, Fax +44 20 7830 2949, E-Mail g.burnstock@ucl.ac.uk

There are reports in the earlier literature of extensive sympathetic innervation of the nephron, particularly of the proximal and distal tubules, and the ascending limb of Henle's loop [15–17]. It is now well established that ATP is a cotransmitter with noradrenaline (NA) in sympathetic nerves supplying both visceral organs and blood vessels (see [18–21]). While the proportions of ATP to NA in sympathetic nerves vary in different situations and pathophysiological conditions, it is interesting that in the sympathetic nerves supplying mesenteric vessels [22] and intestinal arterioles [23], ATP is the sole transmitter, while NA released from the nerves acts only as a modulator of ATP release. The present study addresses the possibility that sympathetic nerves can influence the activities of collecting duct epithelial cells. It is now recognised that autonomic nerves do not form synapses with effector cells, but rather that varicosities in terminal nerve fibres come in close contact with effector cell membranes to form transient junctions, where released transmitters can act on receptors expressed by the effector cells and, unlike synapses, no post-junctional specialisations are present [21, 24, 25]. The possibility that such junctions are present on the collecting duct epithelial cells has been investigated with both immunohistochemical and electron microscopic techniques.

The objectives of this study were to re-investigate the basolateral innervation of the renal collecting duct (CD) epithelial cells by sympathetic nerves. A pre-embedding immunocytochemistry of tyrosine hydroxylase (TH) antibody to label sympathetic nerves was used in conjunction with electron microscopy of antigen detection.

Materials and Methods

Three adult male Sprague-Dawley rats (200–250 g) were used in this study according to UK regulations. Rats were anaesthetised by pentobarbitone (i.p. 60 mg/kg; Sigma Chemical Co., Poole, UK) and perfusion-fixed via the heart with fixative (~50 ml) consisting of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1% phosphate buffered saline (PBS). Kidneys were then dissected out and washed in PBS, and 100-μm-thick sections cut using a vibratome. Sections were processed for the standard pre-embedding electron immunocytochemistry, using the well-characterised TH rabbit polyclonal antibody (TZ1010 from Biomol International L.P., Exeter, UK). In brief, the TH antibody was used at 1:1,000 dilution in PBS containing 10% non-immune normal horse serum (Jackson ImmunoResearch Labs, West-Grave, Pa., USA); the second antibody was a biotinylated donkey anti-rabbit immunoglobulin G (IgG) (Jackson ImmunoResearch Labs) used at a dilution 1:500 in PBS containing 1% non-immune normal horse serum; as a third layer, extravidin-horseradish peroxidase (Sigma, St. Louis, Mo., USA) was used at a dilution 1:1,500. Immunoreactivity was visualised with 3,3′-diaminobenzidine (DAB, Sigma); specimens were post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol concentrations followed by propylene oxide, and then embedment in Araldite and polymerised. Ultrathin sections (~90 nm) were cut from the Araldite blocks, counterstained with uranyl acetate and lead citrate and examined with a Philips CM120 transmission electron microscope. Negative controls with omission of the TH antibody or biotinylated donkey anti-rabbit IgG resulted in lack of immunolabelling.

Results

Wide-ranging sympathetic innervation of the rat kidney was observed, with nerve fibres distribution predominantly associated with renal vasculature in the cortex and outer medulla. An example of a nerve plexus containing TH-positive nerve varicosities associated with a small intrarenal artery is shown in figure 1. TH-positive nerve fibres, including axon varicosities, were also seen in the vicinity of proximal and distal tubules, as well as in association with the CDs (fig. 2a–d, fig. 3a, b). An abundance of microtubules was apparent in some of the TH-positive axon profiles in the vicinity of CDs (fig. 2b), while other TH-positive axon profiles displayed numerous vesicles (fig. 2d), including small agranular and granular vesicles (~40 nm and 70 nm, respectively), and large granular vesicles (~100 nm). The TH-positive axon varicosities, free of Schwann cells, but rich in vesicles could be seen located as close as 100 nm to the basolateral surface of the CD's epithelial cells (fig. 2d). Small microprojections were present on the luminal surface of 1 intercalated epithelial cell (fig. 2c), while the surface of another intercalated epithelial cell (fig. 3a) was scarce of the luminal membrane microprojections. CDs were identified according to their anatomical localisation (cortex) and morphological/ultrastructural characteristics. (For more details on the ultrastructure of collecting ducts, see [26, 27].) Unlabelled control preparations showed more structural details of axon varicosities associated with CDs (fig. 3c, d). Small electron transparent agranular vesicles (~40 nm) and mitochondria could be seen in the varicosities.

Discussion

The present study demonstrates that TH-positive sympathetic nerve varicosities are associated with CDs in the cortex region of rat kidney. Earlier histological data showed nerve fibres in the renal cortex and medulla [28–

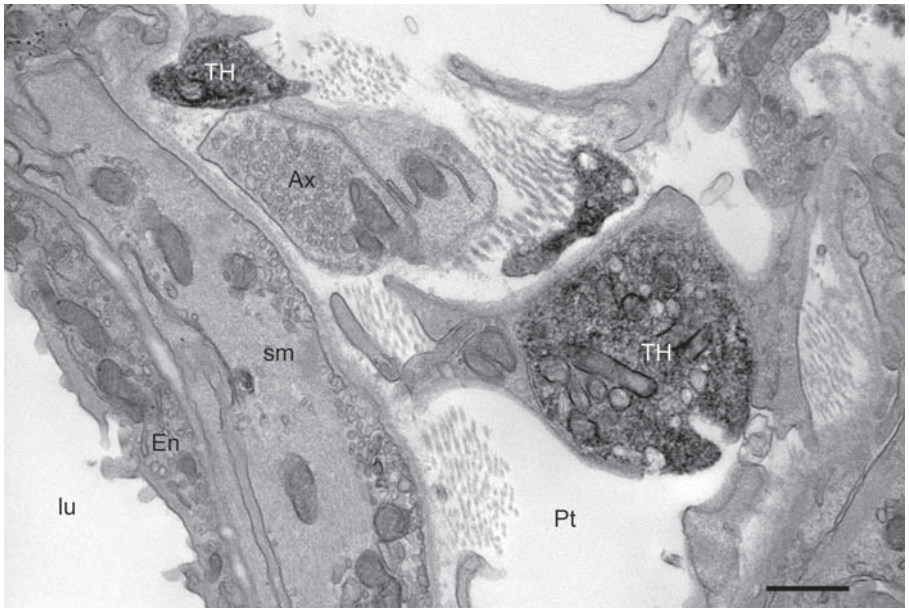

Fig. 1. Electron microscopy of perivascular region of an artery from the cortex region of the rat kidney, immunolabelled for TH. Note the TH-positive (TH; dark immunolabelling) perivascular nerve fibres, including axon varicosities, innervating a small arteriole. In the TH-positive varicosities, numerous vesicles and mitochondria are present. Also note a TH-negative nerve fibre (Ax), arterial vascular smooth muscle (sm) and endothelium (En); Pt = Proximal tubule; lu = lumen. Bar: 0.5 μm.

34]. However, most of these nerves were perivascular nerves, supplying intrarenal vessels that may receive as much as 5 times more innervation (e.g. afferent arterioles) than the renal tubules [29]. This is also in agreement with an unpublished immunofluorescence observation of TH-positive nerve fibres associated with intrarenal blood vessels in the cortico-medullary region, as well as with about 45% of neighbouring aquaporin-2-positive CDs (V.G., personal communication). In the present study, TH-positive varicosities were observed in the adventitia of intra-renal arteries and at CDs and other kidney structures. Barajas and Powers [17] made similar electron micrograph findings of nerve varicosities adjacent to CDs, but did not directly confirm their sympathetic nature using immunoreactivity.

Examination of TH-positive varicosities associated with CDs at higher magnification revealed a richness and diversity of intra-varicosity vesicular structures, such as small agranular and granular vesicles and large granular vesicles. These varicosities were located close to the membranes of the CD cells. The narrowest gap observed between a TH-positive varicosity and the basal membrane of a CD epithelial cell was about 100 nm. These findings strongly suggest that the sympathetic cotransmitters, NA and ATP, might be released from axon varicosities onto the basolateral membrane of CDs at this neuroeffector junction. It is well established that autonomic neuroeffector junctions do not form fixed synapses and that the 'synaptic' cleft width varies from 20 nm to 1–2 μm [24]. Thus, the observed apposition of both TH-positive varicosities and CD epithelium fulfil the structural criteria for the neuroeffector junction. Details about autonomic neuroeffector junctions and non-synaptic transmission have recently been highlighted [35].

In the present study, some varicosities were scarce in vesicles (or showed none), but were rich in microtubules. These varicosities were also present in the region of CDs, but they appeared to be not so close to the basal epithelium as those varicosities rich in vesicles. It is likely that varicosities containing numerous microtubules are the type I sympathetic axons, as previously observed on the juxtaglomerular arterioles of the rabbit and rat kidneys [33]. Two types of axons have been described on afferent and efferent intralobular arterioles: type I, with microtubules present continuously through intravaricosities and varicosities, and type II axons showing only synaptic vesicles; both types of axons also appeared to be catecholaminergic, since they were able to take up 6-hydroxydopamine [33]. In the present study, the axons were identified using the TH antibody. According to Luff et al. [36], both type of axons dominate in perivascular innervation, but the presence of the axons in apposition to proximal and distal tubules, macula densa, renin granular epithelial cells and Bowman's capsule were also observed, therefore suggesting significance of neural function for these renal structures. The differentiated functional relationships between sympathetic nerves and the intrarenal effectors such as blood vessels, renal tubules and the juxta-

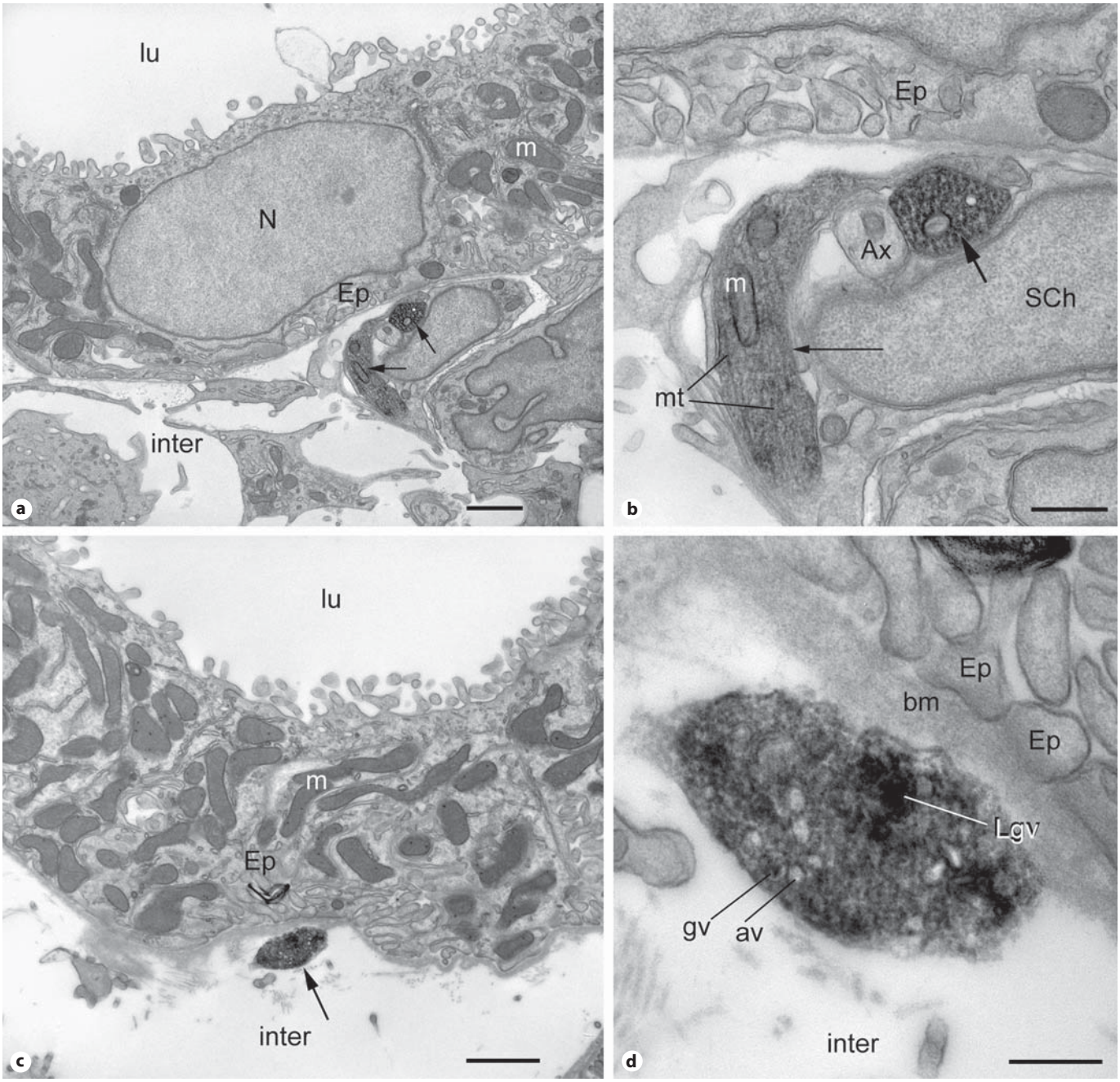

Fig. 2. Electron microscopy of CDs of renal cortex region immunolabelled for TH. **a** A general view of a fragment of CD with surrounding interstitium (inter) shows location of TH-positive nerve fibres (arrows). CD's principal epithelial cell (Ep), nucleus (N) and duct's lumen (lu) can be seen. m = Mitochondria. 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 the short arrow; perhaps 1 or 2 vesicle-like structures are present. Also note a TH-negative axon profile (Ax) and Schwann cell (SCh). Bar: 0.5 μm. **c** A low magnification of a fragment of CD 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.

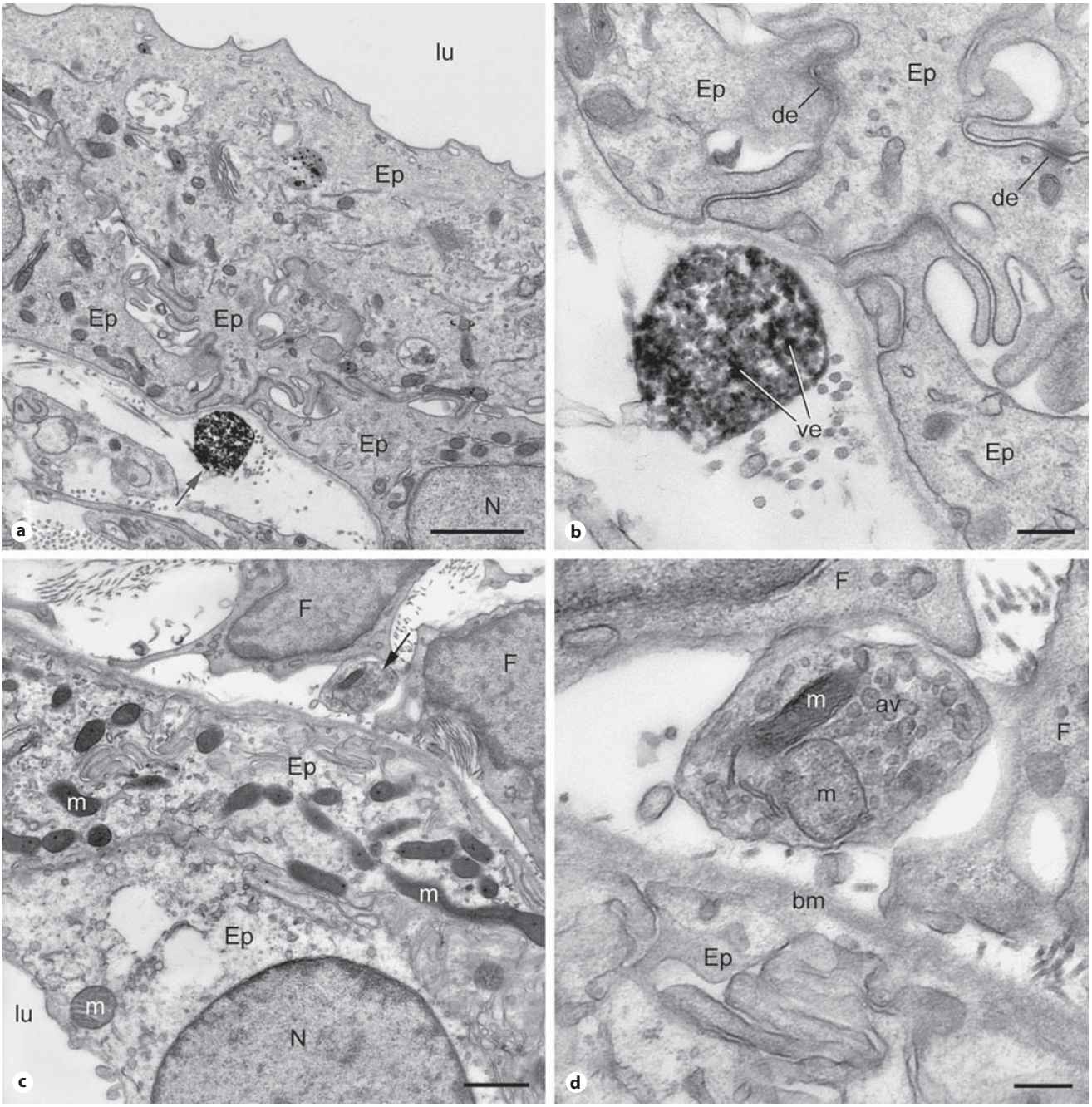

Fig. 3. Electron microscopy of CDs of renal cortex region immunolabelled for TH (**a**, **b**) and unlabelled control preparation (**c**, **d**). **a** At low magnification, note the TH-positive axon varicosity (arrow) located at the base of CD displaying intercalated epithelial cells (Ep); note that 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 CD with surrounding interstitium including fibroblasts (F) at low magnification; note the presence of an axon varicosity (arrow) close to the CD's epithelium containing numerous mitochondria (m). Bar: 0.5 μm. **d** At high magnification, note the 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.

glomerular granular cells have been elegantly presented by DiBona et al. [37–39]. It has been assumed that coordination between sympathetic nerves and intrarenal effectors is essential for overall renal function [40]. A recent example of such an interaction (which may be neural or paracrine) between NA and ATP (costimulation) has been clearly demonstrated for vasoconstriction of the glomerular afferent arteriole [41]. For healthy renal function, functioning of both sequential and non-sequential junctions made by sympathetic nerves on various effectors is required; projections from renal perivascular sympathetic innervation make sequential junctions, while non-perivascular central neurons, with specific and selective afferent reflex input, are associated with non-sequential junctions [28, 36, 39]. It should be mentioned, however, that non-sympathetic nerves can also influence renal physiology. Here, TH-negative nerve fibres appeared together with TH-positive ones. It is likely they represented sensory afferent fibres containing substance P and calcitonin gene-related peptide [42], and perhaps also ATP involved in 'axon reflex' activity via antidrome impulses in sensory collaterals (see [43]), or they may be nerve fibres of intrarenal ganglia containing nitric oxide synthase that are known to modulate the activities of renal sympathetic nerves [34].

We have shown in the present study at the electron microscopic level that sympathetic nerve varicosities form close relationships with the basolateral surfaces of kidney CD epithelial cells. Similar neuroeffector cell relationships have been considered for cells in salivary [44], lacrimal [45], sweat [46], adrenal [47] and endocrine glands [48–53]. Close apposition of sympathetic nerve varicosities with mast cells, vascular endothelial cells, ciliary epithelium and secretory epithelial cells in the lung, gut, liver and bone have also been reported (see [21, 35]).

In summary, on the basis of our findings, we speculate that basolateral P2X and P2Y receptors on collecting duct epithelial cells can be activated by ATP released as a co-transmitter with NA from sympathetic nerves, which may alter sodium and water transport. Moreover, the close proximity of these sympathetic nerve terminals to intercalated cells (as well as principal cells), suggests potential for a P2-mediated effect on collecting duct acid-base transport; but as yet, there are no functional data to support this. In addition, ATP released from renal tubular epithelial cells into the lumen by paracrine and autocrine mechanisms can also act on P2X and P2Y receptors to inhibit sodium and water transport [7, 9, 12]. Thus, there is obvious potential for sophisticated interplay between these 2 ATP-dependent systems; we suggest that while the paracrine/autocrine ATP may dominate under normal physiological circumstances, in pathophysiological states associated with sympathetic activation, these nerves may come into play to modulate collecting duct sodium and water transport.

Acknowledgements

We would like to thank Dr. J. Marks for help with the perfusion-fixation of rat kidney and Dr. G. E. Knight for editorial assistance. This work was supported by a Leverhulme Emeritus Fellowship (www.leverhulme.ac.uk) to G.B.

References

1 Wang Y, Roman R, Lidofsky SD, Fitz JG: Autocrine signaling through ATP release represents a novel mechanism for cell volume regulation. Proc Natl Acad Sci USA 1996;93:12020–12025.

2 Schwiebert EM: ATP release mechanisms, ATP receptors and purinergic signalling along the nephron. Clin Exp Pharmacol Physiol 2001;28:340–350.

3 Ma HP, Saxena S, Warnock DG: Anionic phospholipids regulate native and expressed epithelial sodium channel (ENaC). J Biol Chem 2002;277:7641–7644.

4 Vekaria RM, Unwin RJ, Shirley DG: Intraluminal ATP concentrations in rat renal tubules. J Am Soc Nephrol 2006;17:1841–1847.

5 Hovater MB, Olteanu D, Hanson EL, Cheng NL, Siroky B, Fintha A, Komlosi P, Liu W, Satlin LM, Bell PD, Yoder BK, Schwiebert EM: Loss of apical monocilia on collecting duct principal cells impairs ATP secretion across the apical cell surface and ATP-dependent and flow-induced calcium signals. Purinergic Signal 2008;4:155–170.

6 Vallon V: P2 receptors in the regulation of renal transport mechanisms. Am J Physiol Renal Physiol 2008;294:F10–F27.

7 Wildman SS, Marks J, Turner CM, Yew-Booth L, Peppiatt-Wildman CM, King BF, Shirley DG, Wang W, Unwin RJ: Sodium-dependent regulation of renal amiloride-sensitive currents by apical P2 receptors. J Am Soc Nephrol 2008;19:731–742.

8 Kunzelmann K, Schreiber R, Boucherot A: Mechanisms of the inhibition of epithelial Na^+ channels by CFTR and purinergic stimulation. Kidney Int 2001;60:455–461.

9 Lehrmann H, Thomas J, Kim SJ, Jacobi C, Leipziger J: Luminal $P2Y_2$ receptor-mediated inhibition of Na^+ absorption in isolated perfused mouse CCD. J Am Soc Nephrol 2002;13:10–18.

10 Leipziger J: Control of epithelial transport via luminal P2 receptors. Am J Physiol Renal Physiol 2003;284:F419–F432.

11 Shirley DG, Bailey MA, Unwin RJ: In vivo stimulation of apical P2 receptors in collecting ducts: evidence for inhibition of sodium reabsorption. Am J Physiol Renal Physiol 2005;288:F1243–F1248.

12 Wildman SS, Boone M, Peppiatt-Wildman CM, Contreras-Sanz A, King BF, Shirley DG, Deen PM, Unwin RJ: Nucleotides downregulate aquaporin 2 via activation of apical P2 receptors. J Am Soc Nephrol 2009;20:1480–1490.
13 Kishore BK, Chou CL, Knepper MA: Extracellular nucleotide receptor inhibits AVP-stimulated water permeability in inner medullary collecting duct. Am J Physiol 1995; 269:F863–F869.
14 Zhang Y, Sanchez D, Gorelik J, Klenerman D, Lab M, Edwards C, Korchev Y: Basolateral $P2X_4$-like receptors regulate the extracellular ATP-stimulated epithelial Na^+ channel activity in renal epithelia. Am J Physiol Renal Physiol 2007;292:F1734–F1740.
15 DiBona GF: Neural control of renal function: cardiovascular implications. Hypertension 1989;13:539–548.
16 McLachlan EM, Luff SE: Sympathetic innervation of renal and extra-renal arterial vessels. Kidney Int Suppl 1992;37:S56–S60.
17 Barajas L, Powers K: Monoaminergic innervation of the rat kidney: a quantitative study. Am J Physiol 1990;259:F503–F511.
18 Burnstock G: Co-transmission. The fifth Heymans memorial lecture – Ghent, February 17, 1990. Arch Int Pharmacodyn Therap 1990;304:7–33.
19 Burnstock G: Noradrenaline and ATP as co-transmitters in sympathetic nerves. Neurochem Int 1990;17:357–368.
20 Burnstock G: Cotransmission. Curr Opin Pharmacol 2004;4:47–52.
21 Burnstock G: Physiology and pathophysiology of purinergic neurotransmission. Physiol Rev 2007;87:659–797.
22 Ramme D, Regenold JT, Starke K, Busse R, Illes P: Identification of the neuroeffector transmitter in jejunal branches of the rabbit mesenteric artery. Naunyn-Schmiedebergs Arch Pharmacol 1987;336:267–273.
23 Evans RJ, Surprenant A: Vasoconstriction of guinea-pig submucosal arterioles following sympathetic nerve stimulation is mediated by the release of ATP. Br J Pharmacol 1992; 106:242–249.
24 Burnstock G: Structural and chemical organisation of the autonomic neuroeffector system; in Bolis CL, Licinio J, Govoni S, (eds): Handbook of the Autonomic Nervous System in Health and Disease. Neurological Disease and Therapy. New York, Marcel Dekker, Inc., 2002, vol 55, pp 1–54.
25 Burnstock G: The Autonomic Neuroeffector Junction; in Robertson D, Low P, Burnstock G, Biaggioni I, (eds): Primer on the Autonomic Nervous System, ed 2. Amsterdam, Elsevier, 2004, pp 29–33.
26 Verlander JW, Madsen KM, Tisher CC: Effect of acute respiratory acidosis on two populations of intercalated cells in rat cortical collecting duct. Am J Physiol 1987;253: F1142–F1156.
27 Verlander JW, Madsen KM, Stone DK, Tisher CC: Ultrastructural localization of H^+ATPase in rabbit cortical collecting duct. J Am Soc Nephrol 1994;4:1546–1557.
28 Müller J, Barajas L: Electron microscopic and histochemical evidence for a tubular innervation in the renal cortex of the monkey. J Ultrastruct Res 1972;41:533–549.
29 Barajas L, Powers K, Wang P: Innervation of the renal cortical tubules: a quantitative study. Am J Physiol 1984;247:F50–F60.
30 Barajas L, Liu L, Powers K: Anatomy of the renal innervation: intrarenal aspects and ganglia of origin. Can J Physiol Pharmacol 1992;70:735–749.
31 Tsuneki K, Kobayashi H, Gallardo R, Pang PK: Electron microscopic study of the innervation of the renal tubules and urinary bladder epithelium in *Rana catesbeiana* and *Necturus maculosus*. J Morphol 1984;181: 143–153.
32 Ferguson M, Ryan GB, Bell C: The innervation of the renal cortex in the dog. An ultrastructural study. Cell Tissue Res 1988;253: 539–546.
33 Luff SE, Hengstberger SG, McLachlan EM, Anderson WP: Two types of sympathetic axon innervating the juxtaglomerular arterioles of the rabbit and rat kidney differ structurally from those supplying other arteries. J Neurocytol 1991;20:781–795.
34 Liu L, Liu GL, Barajas L: Distribution of nitric oxide synthase-containing ganglionic neuronal somata and postganglionic fibers in the rat kidney. J Comp Neurol 1996;369: 16–30.
35 Burnstock G: Non-synaptic transmission at autonomic neuroeffector junctions. Neurochem Int 2008;52:14–25.
36 Luff SE, Hengstberger SG, McLachlan EM, Anderson WP: Distribution of sympathetic neuroeffector junctions in the juxtaglomerular region of the rabbit kidney. J Auton Nerv Syst 1992;40:239–253.
37 DiBona GF, Sawin LL, Jones SY: Differentiated sympathetic neural control of the kidney. Am J Physiol 1996;271:R84–R90.
38 DiBona GF, Kopp UC: Neural control of renal function. Physiol Rev 1997;77:75–197.
39 DiBona GF: Neural control of the kidney: functionally specific renal sympathetic nerve fibers. Am J Physiol Regul Integr Comp Physiol 2000;279:R1517–R1524.
40 DiBona GF: Physiology in perspective: the wisdom of the body. Neural control of the kidney. Am J Physiol Regul Integr Comp Physiol 2005;289:R633–R641.
41 Hultström M, Lai EY, Ma Z, Källskog Ö, Patzak A, Persson AE: Adenosine triphosphate increases the reactivity of the afferent arteriole to low concentrations of norepinephrine. Am J Physiol Regul Integr Comp Physiol 2007;293:R2225–R2231.
42 Ferguson M, Bell C: Ultrastructural localization and characterization of sensory nerves in the rat kidney. J Comp Neurol 1988;274:9–16.
43 Burnstock G: Introduction: Changing face of autonomic and sensory nerves in the circulation; in Edvinsson L, Uddman R, (eds): Vascular Innervation and Receptor mechanisms: New Perspectives. San Diego, Academic Press Inc., USA, 1993, pp 1–22.
44 Takemura H, Horio Y: Spatial microenvironment defines Ca^{2+} entry and Ca^{2+} release in salivary gland cells. Biochem Biophys Res Commun 2005;336:223–231.
45 Gromada J, Jorgensen TD, Dissing S: Role of protein kinase C in the regulation of inositol phosphate production and Ca^{2+} mobilization evoked by ATP and acetylcholine in rat lacrimal acini. Pflügers Arch – Eur J Physiol 1995;429:578–586.
46 Ko WH, O'Dowd JJM, Pediani JD, Bovell DL, Elder HY, Jenkinson DM, Wilson SM: Extracellular ATP can activate autonomic signal transduction pathways in cultured equine sweat gland epithelial cells. J Exp Biol 1994; 190:239–252.
47 Jurányi Z, Orsó E, Jánossy A, Szalay KS, Sperlágh B, Windisch K, Vinson GP, Vizi ES: ATP and [^{3}H]noradrenaline release and the presence of ecto-Ca^{2+}-ATPases in the capsule-glomerulosa fraction of the rat adrenal gland. J Endocrinol 1997;153:105–114.
48 Miller RE: Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the Islets of Langerhans. Endocr Rev 1981;2:471–494.
49 Green ST: Intrathyroidal autonomic nerves can directly influence hormone release from rat thyroid follicles: a study in vitro employing electrical field stimulation and intracellular microelectrodes. Clin Sci (Lond) 1987; 72:233–238.
50 Grunditz T, Hakanson R, Sundler F, Uddman R: Neuronal pathways to the rat thyroid revealed by retrograde tracing and immunocytochemistry. Neuroscience 1988;24:321–335.
51 Prince FP: Ultrastructural evidence of indirect and direct autonomic innervation of human Leydig cells: comparison of neonatal, childhood and pubertal ages. Cell Tissue Res 1992;269:383–390.
52 Troadec JD, Thirion S: Multifaceted purinergic regulation of stimulus-secretion coupling in the neurohypophysis. Neuroendocrinol Lett 2002;23:273–280.
53 Kim CH, Kim SS, Choi JY, Shin JH, Kim JY, Namkung W, Lee JG, Lee MG, Yoon JH: Membrane-specific expression of functional purinergic receptors in normal human nasal epithelial cells. Am J Physiol Lung Cell Mol Physiol 2004;287:L835–L842.