*J. Anat.* (1999) **194**, pp. 335–342 with 2 figures Printed in the United Kingdom


## Review

# Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction

GEOFFREY BURNSTOCK

*Autonomic Neuroscience Institute, Royal Free and University College Medical School, London, UK*

(*Accepted 8 December 1998*)

ABSTRACT

The evidence for release of vasoactive substances from endothelial cells in response to shear stress caused by the viscous drag of passing fluids is reviewed and, in particular, its physiological significance both in short-term regulation of blood vessel tone and in long-term regulation of cell growth, differentiation, proliferation, and cell death in pathophysiological conditions is discussed. A new concept of purinergic mechanosensory transduction, particularly in relation to nociception, is introduced. It is proposed that distension of tubes (including ureter, vagina, salivary and bile ducts, gut) and sacs (including urinary and gall bladders, and lung) leads to release of ATP from the lining epithelium, which then acts on $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey information to the CNS.

*Key words*: Vasculature; $P2X_{2/3}$receptors; ATP.

## VASCULAR ENDOTHELIAL CELLS

### *Short-term regulation of vascular tone*

For many years it was thought that vascular tone was controlled primarily by sympathetic nerves releasing noradrenaline (NA) with antagonistic parasympathetic cholinergic vasodilator control in some vessels. However, there have been some remarkable changes in our understanding of the mechanisms controlling vascular tone more recently, especially since the seminal studies of Furchgott concerning endothelial-mediated vasodilatation (Furchgott & Zawadski, 1980) and dual control of vascular tone by perivascular nerves and endothelial cells (see Burnstock, 1990; Ralevic & Burnstock, 1996).

Perivascular nerves are confined to the adventitial-medial border in most blood vessels and it is now known that they consist of 4 different nerve types, namely: sympathetic nerves, which utilise as principal transmitters NA, ATP and neuropeptide Y (NPY); parasympathetic nerves, which often utilise vasoactive intestinal polypeptide (VIP) together with acetylcholine (ACh); sensory-motor nerves with a chemical coding consisting of calcitonin gene-related peptide (CGRP), substance P (SP) and, in some subpopulations, ATP; and the projections from neurons in intrinsic ganglia in the heart, lung, gut etc., which contain a variety of peptidergic, purinergic, nitrergic and classical transmitters depending on their location and species (see Burnstock & Ralevic, 1996).

Endothelial cells are now known to synthesise, store and release a variety of vasoactive substances in response to shear stress produced by changes in blood flow (see Bodin et al. 1994; Koller & Kaley, 1996; Ralevic & Burnstock, 1996; Loesch & Burnstock, 1998).

*Nitric oxide* (*NO*). Nitric oxide synthase (NOS) has been localised at both light and electron microscope levels in subpopulations of endothelial cells in most vessels and increased flow or shear stress results in an increase in synthesis and release of NO which then acts on smooth muscle to produce vasodilatation and/or trophic actions (Pohl et al. 1986; Rubanyi et al. 1986; Busse et al. 1993; Lincoln et al. 1997). Since NOS inhibitors greatly increase the blood pressure in whole animals (Rees et al. 1989), it is likely that basal release of NO in response to changing blood flow patterns provides vasodilator tone in dynamic balance with

Correspondence to Prof. Geoffrey Burnstock, Autonomic Neuroscience Institute, Royal Free and University College Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK. Tel.: +44 171 830 2948; fax: +44 171 830 2949; e-mail: g.burnstock@ucl.ac.uk

sympathetic vasoconstrictor tone. Shear stress has also been shown to potentiate agonist-stimulated NO release evoked by a number of agonists such as ACh, SP, ATP and 5-hydroxytryptamine (5-HT) (Busse et al. 1994), probably since these substances are also released by shear stress (see below) to occupy receptors that also lead to NO release.

*Endothelin-1*. Endothelial cells synthesise and store constricting agents, notably endothelin (ET) (Yanagisawa et al. 1988). In addition to directly constricting vascular smooth muscle, ET can also act on the endothelium to release NO which produces vasodilatation (Warner et al. 1989). ET is released from aortic endothelial cells isolated from 12-mo-old rabbits when they are exposed to an increased perfusate flow rate (Milner et al. 1990*a*, 1992). Chronic hypoxia changes the ratio of ET to ATP release from rat aortic endothelial cells exposed to high flow (Bodin et al. 1992). It was suggested that, under these conditions of reduced arterial oxygen tension, a dynamic balance between ET (constrictor) and ATP (dilator) release could regulate the responses of vessels to shear stress. In double-labelling experiments using colloidal gold with postembedding electron microscopy, perhaps surprisingly, ET-1 and NOS have been shown by A. Loesch to be colocalised in some endothelial cells (Ralevic & Burnstock, 1995).

*Choline acetyltransferase-acetylcholine*. In the original studies by Furchgott and colleagues ACh, acting via muscarinic receptors on endothelial cells, was shown to release endothelium-derived relaxing factor (EDRF)/NO. However, it is unlikely that ACh released from perivascular nerves in medium to large blood vessels would survive degradation to reach the endothelial receptor sites. Therefore it was of interest when Parnavelas et al. (1985) demonstrated localisation of choline acetyltransferase, the synthetic enzyme for ACh, in vascular endothelial cells of vessels in the rat brain and they suggested that ACh was synthesised in the cells and released to cause NO release from adjacent endothelial cells. Later ACh was shown to be released during shear stress (Milner et al. 1989, 1990*b*; Kawashima et al. 1990).

*Substance P*. SP was shown subsequently also to be stored in subpopulations of endothelial cells in various arteries (Loesch & Burnstock, 1988; Linnik & Moskowitz, 1989; Milner et al. 1989) and released by shear stress (Milner et al. 1989, 1990*b*, 1995). In the perfused rat hindlimb, increase in flow caused release of SP into the effluent (Ralevic et al. 1990). After removal of the endothelium by perfusion with air bubbles, increased flow no longer evoked release of SP, showing that it was not originating from perivascular sensory nerves. Furthermore, neonatal capsaicin treatment, which destroys SP containing sensory nerves, had no effect on flow-induced release of SP.

*Other vasoactive agents*. There is now evidence that subpopulations of endothelial cells that vary in different vascular beds store a number of other vasoactive agents, including 5-HT, arginine-vasopressin, angiotensin II, histamine, atrial natriuretic peptide (ANP) and under some rare circumstances, CGRP, VIP and NPY (see Loesch & Burnstock, 1998). There is documented evidence for the release of some of these substances in response to increase in perfusion rate (e.g. Domer et al. 1992, 1993; Bodin et al., 1994, 1995).

*ATP*. ATP has been shown to be rapidly released from freshly dissociated or cultured endothelial cells (see Bodin et al. 1991,1992; Bodin & Burnstock, 1995) and from different vascular beds in response to increased perfusion flow rate (Milner et al. 1989, 1990*b*; Bodin et al. 1991, 1992; Vials & Burnstock, 1996). It is a feature that with successive increases in flow there is progressive reduction in ATP release from endothelial cells, which is in marked contrast to the release of peptides such as endothelin which shows increased release with successive flow stimuli (Fig. 1; Bodin et al. 1991). The diminished release of ATP may be due to the limiting source of adenosine in the perfusion medium for reuptake into endothelial cells and conversion to ATP (Pearson & Gordon, 1979), while shear stress is known to increase synthesis of endothelin (Yoshizumi et al. 1989) which could account for the larger release of ET during the second flow-induced stimulation.

Flow-induced release of uridine nucleotides as well as purine nucleotides has been demonstrated from cultured endothelial cells of rabbit aorta (Saïag et al. 1995).

### *Long-term trophic actions of substances released from endothelial cells*

While the early interest in substances released from endothelial cells was focused on their influence on vascular tone and platelet aggregation, it became increasingly clear that some of the substances released could also have long-term influences. For example, ET, SP, ATP and angiotensin II have all been shown to produce growth and/or proliferation of smooth muscle cells (see Schachter, 1990; Erlinge, 1998; Saita et al. 1998). This is of particular interest for pathological conditions such as hypertension, atherosclerosis, restenosis following angioplasty, diabetes

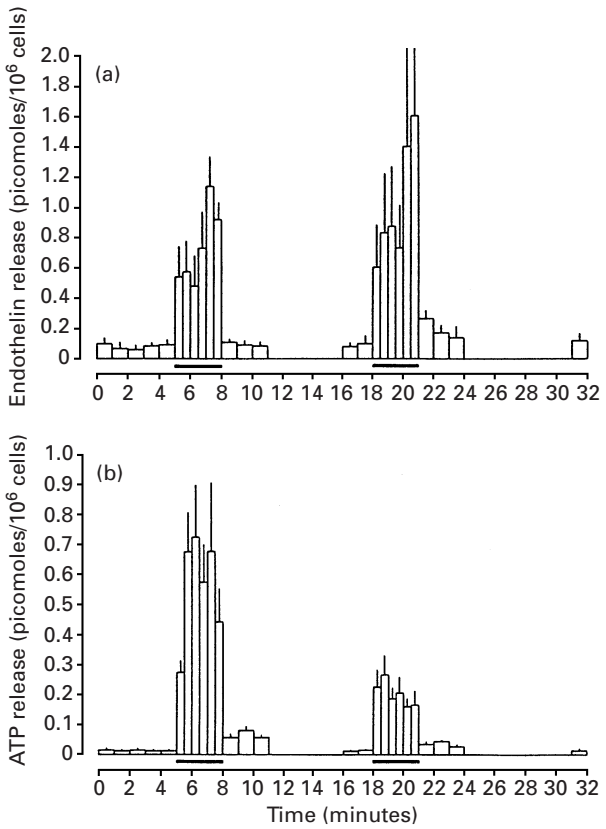


Fig. 1. Release of (*a*) endothelin and (*b*) ATP into Krebs' buffer perfusing freshly isolated rabbit aortic endothelial cells at low flow rate (0.5 ml/min) and a higher flow rate (3.0 ml/min, black bar on horizontal axis). Note that release of endothelin and ATP was significantly enhanced during high flow ($P < 0.001$, $n = 5$) and that a second period of increase in flow led to a further increase in endothelin release, in contrast to ATP release which was reduced with the second stimulus (see text) (from Milner et al. 1990*a*)

and malignancies (see Schachter, 1990; Kohler et al. 1991; Milner & Burnstock, 1994; Abbracchio, 1996). NO can inhibit proliferation of endothelial cells; this may occur in pathological states where production of NO in plaques and diseased vessels impedes re-endothelialisation, thereby contributing to adverse thrombotic or vasospastic activities (Sarker et al. 1995). In contrast, SP has growth-promoting actions on endothelial cells (Villablanca et al. 1994), whereas adenosine, a breakdown product of ATP, stimulates retinal microvascular endothelial cell migration and tube formation (Lutty et al. 1998)

Human umbilical vessels are unusual in that not all the substances released during shear stress act on receptors on adjacent endothelial cells to influence local vascular tone. Histamine, 5-HT, ATP and ET, when released from umbilical endothelial cells in response to shear stress, produce local vasoconstriction (Sexton et al. 1996). However, endothelial cells in human umbilical veins that contain and release NPY and ANP, as well as NO, are inactive locally and it has been suggested that they may have trophic effects on fetal development (Cai et al. 1993*a*, *b*; Salas et al. 1995; Sexton et al. 1995).

Long-term sympathectomy suppresses flow-induced release of ATP and endothelin from endothelial cells isolated from the adult rat aorta (Milner et al. 1996).

Apart from its role as a vasoconstrictor, endothelin is a mitogenic agent to smooth muscle (Komuro et al. 1988; Hirata et al. 1989). ET is greatly increased in blood vessels in cancer where it appears to contribute to tumour enlargement (Shichiri et al. 1991; Shankar et al. 1998), and perhaps in atherosclerosis and restenosis (Dashwood et al. 1998).

## MECHANISMS OF ENDOTHELIAL MECHANOTRANSDUCTION

By virtue of their position at the luminal surface of blood vessels, endothelial cells are ideally situated to act as sensors of, and as modulators of, changes in blood flow. The sensitivity of the endothelium to flow is supported by the morphological and cytoskeletal changes that occur in cultured endothelial cells subjected to shear stress (Flaharty et al. 1972). In some vessels, endothelial cells are subject to pulsatile laminar blood flow; in other regions, particularly arterial branch sites, blood flow is turbulent (Davies, 1988), and it has been suggested that this is more important in the predisposition of vessels for the development of atherosclerotic lesions.

The cellular and molecular pathways mediating mechanotransduction in endothelial cells have not been resolved. There is debate about whether shear-stressed NO release is $Ca^{2+}$-dependent or not (Korenaga et al. 1994; Kuchan & Frangos, 1994). It has been suggested that shear stress is sensed by integrins on the cell surface which interact with cytoskeletal proteins to recruit signalling proteins into focal adhesion complexes (Davies, 1995). Support for the involvement of the cytoskeleton in flow-mediated responses comes from studies of rabbit abdominal aorta endothelial cells (Hutcheson & Griffith, 1996). Cytochalasin B, an F-actin depolymerising agent, phalloidin, an F-actin stabilising agent and colchicine, a tubulin dimerisation inhibitor were employed to assess the contribution of the actin microfilament and microtubule lattice. The results were interpreted to suggest that the endothelial F-actin microfilament and microtubule networks are involved in the mechano-transduction pathway for flow-evoked EDRF release in rabbit aorta, athough, interestingly, these cyto-skeletal elements appear to play no role in acetyl-

choline-induced EDRF release. Shear stress also activates G protein, $Gi_{2\alpha}$, which may be linked to the activation of $K^+$ channels (Olesen et al. 1988) and increase in $[Ca^{2+}]_i$ (Rubanyi et al. 1986; Lewis & Smith, 1991). Three temporal signal responses to shear stress have been postulated: the immediate production of NO; a rapid response involving activation of extracellularly regulated kinases including MAPK and ERK1/2; and a sustained response involving tyrosine phosphorylation of focal adhesion kinase (FAK) (Takahashi et al. 1997). The 'immediate' response is concerned with short-term physiological control of vascular tone, while the 'rapid' and 'sustained' responses appear to be more related to long-term trophic events involved in vessel wall remodelling and adaptation (see also Davies, 1997). It has also been suggested that stretch-activated ion channels in vascular endothelial cells may act as mechanotransducers (Lansman et al. 1987).

It is interesting to note that mechanical stretch induces ET-1 mRNA and stimulates release of ET-1 from cardiomyocytes (Yamazaki et al. 1996). These authors also showed that stretching activates MAPK after 8 min, followed by an increase in protein synthesis in cardiomyocytes after 24 h. These effects were inhibited by an angiotensin II antagonist and, since the local renin-angiotensin system is considered to play a key role in the mechanism of stretch-induced hypertrophy of cardiomyocytes (Sadoshima et al. 1993), ET-1 and angiotensin II may cooperate in the induction of cardiomyocyte hypertrophy.

## PURINERGIC MECHANOSENSORY TRANSDUCTION

Recent findings suggest that stretch deformation of epithelial cells leads to release of ATP via a selective ATP transport process to act on subepithelial sensory nerves initiating messages to be relayed to the CNS. Two such systems will now be explored.

### *Nociception*

There were early hints about the actions of ATP on nociceptive sensory nerves (Bleehan & Keele, 1977; Bleehan, 1978; Burnstock, 1981; Coutts et al. 1981; Krishtal et al. 1988; Bouvier et al. 1991). However, more recently, cloning and characterisation of an extracellular receptor for ATP has provided direct evidence for $P2X_3$ receptor homomultimers and $P2X_{2/3}$ receptor heteromultimers on nociceptive sensory neurons (Chen et al. 1995; Lewis et al. 1995; Burnstock & Wood, 1996). These receptors have been localised on subpopulations of nerve cell bodies in dorsal root, trigeminal and nodose ganglia and on their central and peripheral extensions with in situ hybridisation and immunohistochemical methods (Vulchanova et al. 1996, 1997; Bradbury et al. 1998; Llewellyn-Smith & Burnstock, 1998). Evidence in support of this hypothesis is beginning to appear from application of ATP and purinoceptor antagonists to in vivo pain models (e.g. Bland-Ward & Humphrey, 1997; Trezise & Humphrey, 1997; Dowd et al. 1998).

The possible sources of the ATP acting on $P2X_{2/3}$ receptors was discussed by Burnstock (1996*a*) in relation to pain associated with causalgia, reflex sympathetic dystrophy, cancer and vascular pain such as migraine, angina and ischaemia, where it was suggested that endothelial cells in the microcirculation might provide the source of the ATP acting on perivascular sensory nerve terminals.

It is now proposed (see Fig. 2) that in tubes such as ureter, vagina, gut, salivary and bile ducts, and sacs such as bladder and lung, the pain caused by distention works through a purinergic mechanosensory transduction mechanism, i.e. that the epithelial cells lining these organs release ATP to act on $P2X_{2/3}$ nociceptive receptors on subepithelial sensory nerve terminals which relay impulses to the CNS to be registered as pain. There is already supportive evidence for this concept in the bladder (see Ferguson et al. 1997; Morrison et al. 1998). This hypothesis is currently being tested further in our laboratory in distended sensory innervated preparations of ureter (Cervero & Sann, 1989) and vagina (Friese et al. 1997).

### *Hearing*

There is extensive evidence for purinergic signalling in the inner ear, the outer hair cells showing particularly high sensitivity to ATP (see Housley, 1997; Chen & Bobbin, 1998). Studies with quinacrine, a fluorescent dye that has been shown to selectively label high levels of ATP bound to peptides in large granular vesicles (Irvin & Irvin, 1954; da Prada et al. 1978; Ekelund et al. 1980) have revealed that marginal cells of the stria vascularis in the inner ear are positively stained and these cells have been suggested as the source of ATP release involved in mechanosensory transduction in this system (White et al. 1995). ATP release has also been demonstrated from the organ of Corti (Wangermann, 1996). Levels of ATP measured in the endolymph (13 nM) and in perilymph (10 nM) even after some ectoenzymatic breakdown (Munoz et al.

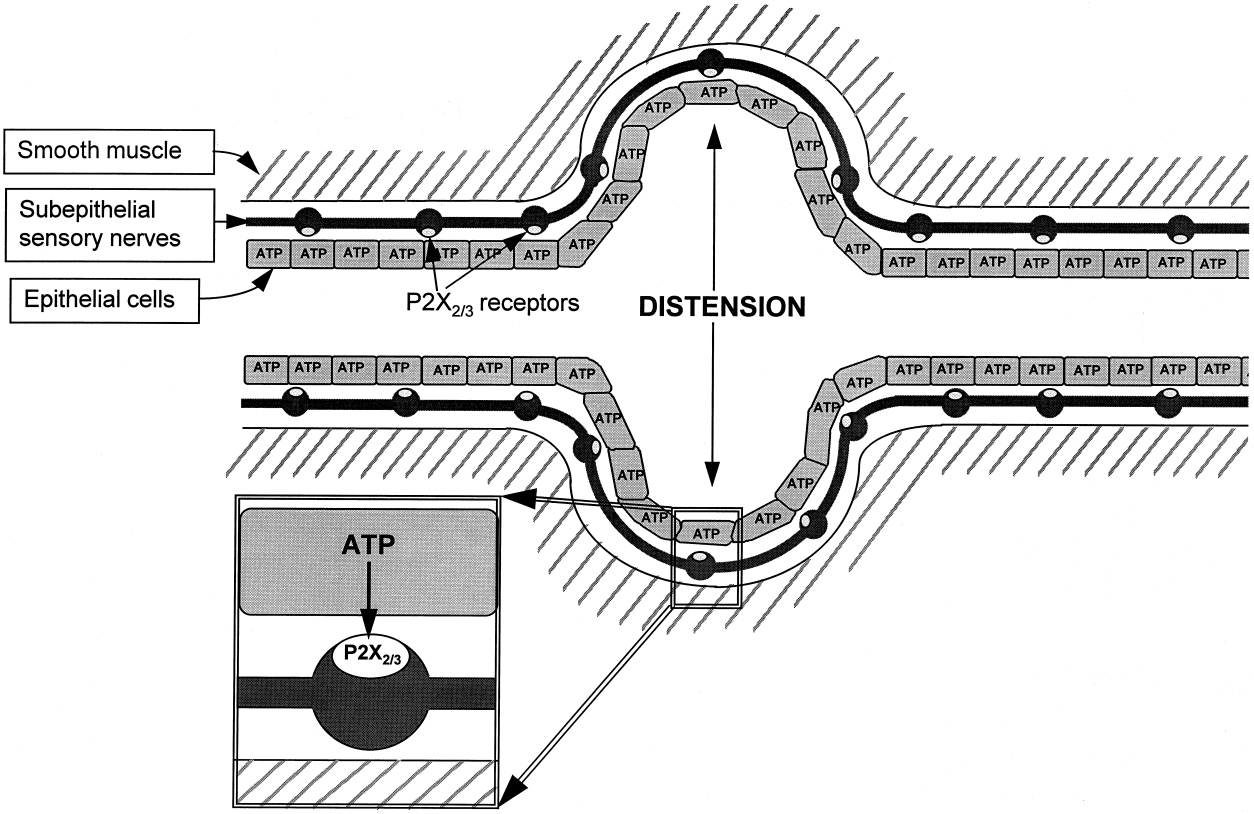


Fig. 2. Schematic representation of hypothesis for purinergic mechanosensory transduction in tubes (e.g. ureter, vagina, salivary and bile ducts, gut) and sacs (e.g. urinary and gall bladders, and lung). It is proposed that distention leads to release of ATP from epithelium lining the tube or sac, which then acts on $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey sensory/nociceptive information to the CNS.

1995) approach the threshold reported for functional responses in isolated guinea pig cochlea outer hair cells (Housley et al. 1992).

## SUMMARY AND FUTURE DIRECTIONS

This article has focused on shear stress release of vasoactive substances from endothelial cells, but it has also been proposed that release of ATP from epithelial cells lining tubes and sacs in response to stretch is part of a mechanosensory transduction mechanism involved in pain. Another possible mechanosensory mechanism in the inner ear has been described and there are hints that mechanically released ATP may also be involved in purinergic signalling in embryonic development (see Burnstock, 1996*b*) and bone remodelling (Bowler et al. 1998), in cystic fibrosis (Watt et al. 1998). However, these proposals will need substantial further experimental work to establish them as biological processes of physiological significance. Discovering precisely how ATP is released from a variety of cell types, including endothelial and epithelial cells, odontoblasts and osteoblasts in response to mechanical stimuli is an exciting challenge. It appears likely that this release involves a special ATP transport mechanism as distinct from exocytotic release from nerves. There is considerable current interest in the possibility that mechanically stimulated ATP transport involves ATP-binding cassette (ABC) proteins, sulphonylurea receptors and/or cystic fibrosis transmembrane conductance regulator (CFTR) channel proteins. It is interesting in this respect that glibenclamide was reported to block flow-induced release of ATP from endothelial cells of the rat pulmonary vascular bed (Hasséssian et al. 1993). Clearly, discovery of ATP transporters and of agents that can enhance or inhibit release of ATP to mechanical stimulation would have significant therapeutic potential.

## ACKNOWLEDGEMENTS

The editorial assistance of Mr R. Jordan in the preparation of the manuscript and figures is greatly appreciated.

## REFERENCES

ABBRACCHIO MP (1996) P1 and P2 receptors in cell growth and differentiation. *Drug Development Research* **39**, 393–406.
BLAND-WARD PA, HUMPHREY PPA (1997) Acute nociception

mediated by hindpaw $P_{2X}$ receptor activation in the rat. *British Journal of Pharmacology* **122**, 365–371.

BLEEHEN T (1978) The effects of adenine nucleotides on cutaneous afferent nerve activity. *British Journal of Pharmacology* **62**, 573–577.

BLEEHEN T, KEELE CA (1977) Observations on the algogenic actions of adenosine compounds on human blister base preparation. *Pain* **3**, 367–377.

BODIN P, BAILEY DJ, BURNSTOCK G (1991) Increased flow-induced ATP release from isolated vascular endothelial but not smooth muscle cells. *British Journal of Pharmacology* **103**, 1203–1205.

BODIN P, MILNER P, WINTER R, BURNSTOCK G (1992) Chronic hypoxia changes the ratio of endothelin to ATP release from rat aortic endothelial cells exposed to high flow. *Proceedings of the Royal Society of London*, *Series B* **247**, 131–135.

BODIN P, LOESCH A, MILNER P, BURNSTOCK G (1994) Effect of increased flow on release of vasoactive substances from vascular endothelial cells. In *The Society of Experimental Biology Seminar Series* 54: *Biomechanics and Cells*. Part I. *Soft Tissue* (ed. Lyall F, El Haj AJ), pp. 37–60. Cambridge: Cambridge University Press.

BODIN P, BURNSTOCK G (1995) Synergistic effect of acute hypoxia on flow-induced release of ATP from cultured endothelial cells. *Experientia* **51**, 256–259.

BODIN P, MILNER P, MARSHALL J, BURNSTOCK G (1995) Cytokines suppress the shear-stress stimulated release of vasoactive peptides from human endothelial cells. *Peptides* **16**, 1433–1438.

BOUVIER MM, EVANS ML, BENHAM CD (1991) Calcium influx induced by stimulation of ATP receptors on neurons cultured from rat dorsal root ganglia. *European Journal of Neuroscience* **3**, 285–291.

BOWLER WB, TATTERSALL JA, HUSSEIN R, DIXON CJ, COBBOLD PH, GALLAGHER JA (1998) Release of ATP by osteoblasts; modulation by fluid shear forces. *Bone* **22**, 3S

BRADBURY E, McMAHON SB, BURNSTOCK G (1998) The expression of $P2X_3$ purinoceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. *Molecular and Cellular Neuroscience* **12**, 256–258.

BURNSTOCK G (1981) Pathophysiology of migraine: a new hypothesis. *Lancet* **i**, 1397–1399.

BURNSTOCK G (1990) Dual control of local blood flow by purines. In *Biological Actions of Extracellular ATP*. *Annals of the New York Academy of Sciences* **603** (ed. Dubyak GR, Fedan JA), pp. 31–45. New York: New York Academy of Sciences.

BURNSTOCK G (1996*a*) A unifying purinergic hypothesis for the initiation of pain. *Lancet* **347**, 1604–1605.

BURNSTOCK G (1996*b*) Purinoceptors: ontogeny and phylogeny. *Drug Development Research* **39**, 204–242.

BURNSTOCK G, RALEVIC V (1996) Cotransmission. In *The Pharmacology of Vascular Smooth Muscle* (ed. Garland CJ, Angus JA), pp. 210–232. Oxford: Oxford University Press.

BURNSTOCK G, WOOD JN (1996) Purinergic receptors: their role in nociception and primary afferent neurotransmission. *Current Opinion in Neurobiology* **6**, 526–532.

BUSSE R, FLEMING I, HECKER M (1993) Signal transduction in endothelium-dependent vasodilatation. *European Heart Journal* **14** (Suppl I), 2–9.

BUSSE R, HECKER M, FLEMING I (1994) Control of nitric oxide and prostacyclin synthesis in endothelial cells. *Arzneimittel-Forschung* **44**, 392–396.

CAI WQ, BODIN P, LOESCH A, SEXTON A, BURNSTOCK G (1993*a*) Endothelium of human umbilical blood vessels: ultrastructural immunolocalization of neuropeptides. *Journal of Vascular Research* **30**, 348–355.

CAI WQ, TERENGHI G, BODIN P, BURNSTOCK G, POLAK JM (1993*b*) In situ hybridization of atrial natriuretic peptide mRNA in the endothelial cells of human umbilical vessels. *Histochemistry* **100**, 277–283.

CERVERO F, SANN H (1989) Mechanically evoked responses of afferent fibres innervating the guinea-pig's ureter: an in vitro study. *Journal of Physiology* (*London*) **412**, 245–266.

CHEN C, BOBBIN RP (1998) P2X receptors in cochlear Deiters' cells. *British Journal of Pharmacology* **124**, 337–344.

CHEN C-C, AKOPIAN AN, SIVILOTTI L, COLQUHOUN D, BURNSTOCK G, WOOD JN (1995) A P2X purinoceptor expressed by a subset of sensory neurons. *Nature* **377**, 428–431.

COUTTS AA, JORIZZO JL, EADY RAJ, GREAVES MW, BURNSTOCK G (1981) Adenosine triphosphate-evoked vascular changes in human skin: mechanism of action. *European Journal of Pharmacology* **76**, 391–401.

DA PRADA M, RICHARDS JG, LOREZ HP (1978) Blood platelets and biogenic monoamines: biochemical, pharmacological, and morphological studies. In *Platelets: A Multidisciplinary Approach* (ed. De Gaetano G, Garattini S), pp. 331–353. New York: Raven Press.

DASHWOOD MR, ANGLINI GD, MEHTA D, JEREMY JY, MUENTER K, KIRCHENGAST M (1998) Effect of angioplasty and grafting on porcine vascular nerves: a potential neurotropic role for endothelin-1. *Journal of Anatomy* **192**, 435–437.

DAVIES PF (1988) Endothelial cells, hemodynamic forces, and the localization of atherosclerosis. In *Endothelial Cells*, vol. II (ed. Ryan US), pp. 123–138. Boca Raton, FL: CRC Press.

DAVIES PF (1995) Flow-mediated endothelial mechanotransduction. *Physiological Reviews* **75**, 519–560.

DAVIES PF (1997) Overview: temporal and spatial relationships in shear stress-mediated signalling. *Journal of Vascular Research* **34**, 208–211.

DOMER F, ALEXANDER B, MILNER P, BODIN P, BURNSTOCK G (1992) Cerebrovascular perfusion of the rabbit brain: a method that permits evaluation of compounds in effluent samples. *Journal of Physiology* **452**, 323P(Abstract).

DOMER F, ALEXANDER B, MILNER P, BODIN P, BURNSTOCK G (1993) Effect of changes in rate of vascular perfusion on release of substances into the effluent from the brain of the rabbit. *Brain Research* **630**, 88–94.

DOWD E, MCQUEEN DS, CHESSELL IP, HUMPHREY PPA (1998) P2X receptor-mediated excitation of nociceptive afferents in the normal and arthritic rat knee joint. *British Journal of Pharmacology* **125**, 341–346.

EKELUND M, AHREN B, HÅKANSON R, LUNDQUIST I, SUNDLER F (1980) Quinacrine accumulates in certain peptide hormone-producing cells. *Histochemistry* **66**, 1–9.

ERLINGE D (1998) Extracellular ATP: a growth factor for vascular smooth muscle cells. *General Pharmacology* **31**, 1–8.

FERGUSON DR, KENNEDY I, BURTON TJ (1997) ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes – a possible sensory mechanism? *Journal of Physiology* (*London*) **505**, 503–511.

FLAHERTY JT, PIERCE JE, FERRANS VJ, PATEL DJ, TUCKER WK, FRY DL (1972) Endothelial nuclear patterns in the canine arterial tree with particular reference to hemodynamic events. *Circulation Research* **30**, 23–33.

FRIESE N, DIOP L, LAMBERT C, RIVIERE PJ, DAHL SG (1997) Antinociceptive effects of morphine and U-50,488H on vaginal distension in the anesthetized rat. *Life Sciences* **61**, 1559–1570.

FURCHGOTT RF, ZAWADZKI JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**, 373–376.

HASSÉSSIAN H, BODIN P, BURNSTOCK G (1993) Blockade by glibenclamide of the flow-induced endothelial release of ATP that contributes to vasodilatation in the pulmonary vascular bed of the rat. *British Journal of Pharmacology* **109**, 466–472.

HIRATA Y, TAKAGI Y, FUKUDA Y, MARUMO F (1989) Endothelin is a potent mitogen for rat vascular smooth muscle cells. *Atherosclerosis* **78**, 225–228.

HOUSLEY GD (1997) Extracellular nucleotide signaling in the inner ear. *Molecular Neurobiology* **16**, 21–48.

HOUSLEY GD, GREENWOOD D, ASHMORE JF (1992) Localization of cholinergic and purinergic receptors on outer hair cells isolated from the guinea-pig cochlea. *Proceedings of the Royal Society of London*, *Series B* **249**, 265–273.

HUTCHESON IR, GRIFFITH TM (1996) Mechanotransduction through the endothelial cytoskeleton: mediation of flow- but not agonist-induced EDRF release. *British Journal of Pharmacology* **249**, 720–726.

IRVIN JL, IRVIN EM (1954) The interaction of quinacrine with adenine nucleotides. *Journal of Biological Chemistry* **210**, 45–56.

KAWASHIMA K, WATANABE N, OOHATA H, FUJIMOTO K, SUZUKI T, ISHIZAKI Y et al. (1990) Synthesis and release of acetylcholine by cultured bovine arterial endothelial cells. *Neuroscience Letters* **119**, 156–158.

KOHLER TR, KIRKMAN TR, KRAISS LW, ZIERLER BK, CLOWES AW (1991) Increased blood flow inhibits neointimal hyperplasia in endothelialized vascular grafts. *Circulation Research* **69**, 1557–1565.

KOLLER A, KALEY G (1996) Shear stress dependent regulation of vascular resistance in health and disease: role of endothelium. *Endothelium* **4**, 247–272.

KOMURO I, KURIHARA H, SUGIYAMA T, YOSHIZUMI M, TAKAKU F, YAZAKI Y (1988) Endothelin stimulates *c-fos* and *c-myc* expression and proliferation of vascular smooth muscle cells. *FEBS Letters* **238**, 249–252.

KORENAGA R, ANDO J, TSUBOI H, YANG W, SAKUMA I, TOYO OKA T et al. (1994) Laminar flow stimulates ATP- and shear stress-dependent nitric oxide production in cultured bovine endothelial cells. *Biochemical and Biophysical Research Communications* **198**, 213–219.

KRISHTAL OA, MARCHENKO SM, OBUKHOV AG (1988) Receptors for ATP in rat sensory neurones: the structure-function relationship for ligands. *British Journal of Pharmacology* **95**, 1057–1062.

KUCHAN MJ, FRANGOS JA (1994) Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. *American Journal of Physiology* **266**, C628–636.

LANSMAN JB, HALLAM TJ, RINK TJ (1987) Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? *Nature* **325**, 811–813.

LEWIS MJ, SMITH JA (1991) Factors regulating the release of endothelium-derived relaxing factor. In *Endothelial Regulation of Vascular Tone* (ed. Ryan US, Rubanyi GM), pp. 139–154. New York: Marcel Dekker.

LEWIS C, NEIDHART S, HOLY C, NORTH RA, SURPRENANT A (1995) Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. *Nature* **377**, 432–435.

LINCOLN J, HOYLE CHV, BURNSTOCK G (1997) *Nitric Oxide in Health and Disease*, pp. 355. Cambridge: Cambridge University Press.

LINNIK MD, MOSKOWITZ MA (1989) Identification of immunoreactive substance P in human and other mammalian endothelial cells. *Peptides* **10**, 957–962.

LLEWELLYN-SMITH IJ, BURNSTOCK G (1998) Ultrastructural localization of $P2X_3$ receptors in rat sensory neurons. *NeuroReport* **9**, 2245–2250.

LOESCH A, BURNSTOCK G (1988) Ultrastructural localisation of serotonin and substance P in vascular endothelial cells of rat femoral and mesenteric arteries. *Anatomy and Embryology* **178**, 137–142.

LOESCH A, BURNSTOCK G (1998) The endothelium: electron immunocytochemistry of vasoactive agents. In *Modern Visualisation of the Endothelium* (ed. Polak JM), pp. 3–44. Amsterdam: Harwood Acedemic Publishers.

LUTTY GA, MATHEWS MK, MERGES C, McLEOD DS (1998) Adenosine stimulates canine retinal microvascular endothelial cell migration and tube formation. *Current Eye Research* **17**, 594–607.

MILNER P, RALEVIC V, HOPWOOD AM, FEHÉR E, LINCOLN J, KIRKPATRICK KA et al. (1989) Ultrastructural localisation of substance P and choline acetyltransferase in endothelial cells of rat coronary artery and release of substance P and acetylcholine during hypoxia. *Experientia* **45**, 121–125.

MILNER P, BODIN P, LOESCH A, BURNSTOCK G (1990*a*) Rapid release of endothelin and ATP from isolated aortic endothelial cells exposed to increased flow. *Biochemical and Biophysical Research Communications* **170**, 649–656.

MILNER P, KIRKPATRICK KA, RALEVIC V, TOOTHILL V, PEARSON JD, BURNSTOCK G (1990*b*) Endothelial cells cultured from human umbilical vein release ATP, substance P and acetylcholine in response to increased flow. *Proceedings of the Royal Society*, *Series B* **241**, 245–248.

MILNER P, BODIN P, LOESCH A, BURNSTOCK G (1992) Increased shear stress leads to differential release of endothelin and ATP from isolated endothelial cells from 4- and 12-month-old male rabbit aorta. *Journal of Vascular Research* **29**, 420–425.

MILNER P, BURNSTOCK G (1994) Trophic factors and the control of smooth muscle development and innervation. In *Airways Smooth Muscle: Development and Regulation of Contractility* (ed. Raeburn D, Giembycz MA), vol. 1, pp. 1–39. Switzerland: Birkhauser.

MILNER P, BODIN P, LOESCH A, BURNSTOCK G (1995) Interactions between sensory perivascular nerves and the endothelium in brain microvessels. *International Journal of Microcirculaton* **15**, 1–9.

MILNER P, BODIN P, BURNSTOCK G (1996) Long-term guanethidine sympathectomy suppresses flow-induced release of ATP and endothelin from endothelial cells isolated from adult rat aorta. *Journal of Vascular Research* **33**, 139–145.

MORRISON, JFB, NAMASIVAYAM, S, EARDLEY I (1998) ATP may be a natural modulator of the sensitivity of bladder mechanoreceptors during slow distention, In *Proceedings of 1st International Consultation on Incontinence*, *Monaco*, June 28–July 1, 1998, p. 84 (Abstract).

MUNOZ DJ, THORNE PR, HOUSLEY GD, BILLETT TE (1995) Adenosine 5′-triphosphate (ATP) concentrations in the endolymph and perilymph of the guinea-pig cochlea. *Hearing Research* **90**, 119–125.

OLESEN SP, CLAPHAM DE, DAVIES PF (1988) Haemodynamic shear stress activates a $K^+$ current in vascular endothelial cells. *Nature* **331**, 168–170.

PARNAVELAS JG, KELLY W, BURNSTOCK G (1985) Ultrastructural localization of choline acetyltransferase in vascular endothelial cells in rat brain. *Nature* **316**, 724–725.

PEARSON JD, GORDON JL (1979) Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotide. *Nature* **281**, 384–386.

POHL U, HOLTZ J, BUSSE R, BASSENGE E (1986) Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension* **8**, 37–44.

RALEVIC V, BURNSTOCK G (1995) Neuropeptides in blood pressure control. In *Hypertension: Pathophysiology*, *Diagnosis and Management*, 2nd edn (ed. Laragh JH, Brenner BM), pp. 801–831. New York: Raven Press.

RALEVIC V, BURNSTOCK G (1996) Interactions between perivascular nerves and endothelial cells in control of local vascular tone. In *The Autonomic Nervous System*, vol. 8. *Nervous Control of Blood Vessels* (ed. Bennett T, Gardiner S), pp. 135–175. Switzerland: Harwood Academic.

RALEVIC V, MILNER P, HUDLICKA O, KRISTEK F, BURNSTOCK G (1990) Substance P is released from the endothelium of normal and capsaicin-treated rat hindlimb vasculature, *in vivo*, by increased flow. *Circulation Research* **66**, 1178–1183.

REES DD, PALMER RMJ, MONCADA S (1989) Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proceedings of the National Academy of Sciences of the USA* **86**, 3375–3378.

RUBANYI GM, ROMERO JC, VANHOUTTE PM (1986) Flow-induced release of endothelium-derived relaxing factor. *American Journal of Physiology* **250**, H1145–1149.

SADOSHIMA J, XU Y, SLAYTER HS, IZUMO S (1993) Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* **75**, 977–984.

SAÏAG B, BODIN P, SHACOORI V, CATHELINE M, RAULT B, BURNSTOCK G (1995) Uptake and flow-induced release of uridine nucleotides from isolated vascular endothelial cells. *Endothelium* **2**, 279–285.

SAITA Y, YAZAWA H, KOIZUMI T, MORITA T, TAMURA T, TAKENAKA T et al. (1998) Mitogenic activity of endothelin on human cultured prostatic smooth muscle cells. *European Journal of Pharmacology* **349**, 123–128.

SALAS SP, ALTERMATT F, CAMPOS M, GIACAMAN A, ROSSO P (1995) Effects of long-term nitric oxide synthesis inhibition on plasma volume expansion and fetal growth in the pregnant rat. *Hypertension* **26**, 1019–1023.

SARKER R, WEBB RC, STANLEY JC (1995) Nitric oxide inhibition of endothelial cell mitogenesis and proliferation. *Surgery* **118**, 274–279.

SCHACHTER M (1990) Endothelium and smooth muscle: trophic interactions and potential for therapeutic intervention. *Journal of Human Hypertension* **4**, 17–21.

SEXTON AJ, LOESCH A, TURMAINE M, MIAH S, BURNSTOCK G (1995) Nitric oxide and human umbilical vessels: pharmacological and immunohistochemical studies. *Placenta* **16**, 277–288.

SEXTON AJ, LOESCH A, TURMAINE M, MIAH S, BURNSTOCK G (1996) Electron-microscopic immunolabelling of vasoactive substances in human umbilical endothelial cells and their actions in early and late pregnancy. *Cell and Tissue Research* **284**, 167–175.

SHANKAR A, LOIZIDOU M, ALIEV G, FREDERICKS S, BOULOS PB, HOLT D et al. (1998) Raised endothelin-1 levels in patients with colorectal liver metastases. *British Journal of Surgery* **85**, 502–506.

SHICHIRI M, HIRATA Y, NAKAJIMA T, ANDO K, IMAI T, YANAGISAWA M et al. (1991) Endothelin-1 is an autocrine/paracrine growth factor for human cancer cell lines. *Journal of Clinical Investigation* **87**, 1867–1871.

TAKAHASHI M, ISHIDA T, TRAUB O, CORSON MA, BERK BC (1997) Mechanotransduction in endothelial cells: temporal signaling events in response to shear stress. *Journal of Vascular Research* **34**, 212–219.

TREZISE DJ, HUMPHREY PPA (1997) Activation of cutaneous afferent neurones by ATP. In *Experimental Headache Models*, *Frontiers in Headache Research*, vol. 6 (ed. Olesen SP, Moscowitz MA), pp. 111–116. New York: Raven Press.

VIALS A, BURNSTOCK G (1996) ATP release from the isolated perfused guinea pig heart in response to increased flow. *Journal of Vascular Research* **33**, 1–4.

VILLABLANCA AC, MURPHEY CJ, REID TW (1994) Growth-promoting effects of substance P on endothelial cells in vitro. Synergism with calcitonin gene-related peptide, insulin and plasma factors. *Circulation Research* **75**, 1113–1120.

VULCHANOVA L, ARVIDSSON U, RIEDL M, WANG J, BUELL G, SURPRENANT A et al. (1996) Differential distribution of two ATP-gated channels (P2X receptors) determined by imunocytochemistry. *Proceedings of the National Academy of Sciences of the USA* **93**, 8063–8067.

VULCHANOVA L, ARVIDSSON U, RIEDL M, WANG J, BUELL G, SURPRENANT A et al. (1997) Imunocytochemical study of the $P2X_2$ and $P2X_3$ receptor subunits in rat and monkey sensory neurons and their central terminals. *Neuropharmacology* **36**, 1229–1242.

WANGEMANN P (1996) $Ca^{2+}$-dependent release of ATP from the organ of Corti measured with a luciferin-luciferase bioluminescence assay. *Auditory Neuroscience* **2**, 187–192.

WARNER TD, MITCHELL JA, NUCCI G, VANE JR (1989) Endothelin-1 and endothelin-3 release EDRF from isolated perfused arterial vessels of the rat and rabbit. *Journal of Cardiovascular Pharmacology* **13**, 585–588.

WATT WC, LAZAROWSKI ER, BOUCHER RC (1998) Cystic fibrosis transmembrane regulator-independent release of ATP. Its implications for the regulation of $P2Y_2$ receptors in airway epithelia. *Journal of Biological Chemistry* **273**, 14053–14058.

WHITE PN, THORNE PR, HOUSLEY GD, MOCKETT BE, BILLETT TE, BURNSTOCK G (1995) Quinacrine staining of marginal cells in the stria vasculares of the guinea-pig cochlea: a possible source of extracellular ATP? *Hearing Research* **90**, 97–105.

YAMAZAKI T, KOMURO I, KUDOH S, ZOU Y, SHIOJIMA I, HIROI Y et al. (1996) Endothelin-1 is involved in mechanical stress-induced cardiomyocyte hypertrophy. *Journal of Biological Chemistry* **271**, 3221–3228.

YANAGISAWA M, KURIHARA H, KIMURA S, TOMOBE Y, KOBAYASHI M, MITSUI Y et al. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332**, 411–415.

YOSHIZUMI M, KURIHARA H, SUGIYAMA T, TAKAKU F, YANAGISAWA M, MASAKI T et al. (1989) Hemodynamic shear stress stimulated endothelin production by cultured endothelial cells. *Biochemical and Biophysical Research Communications* **161**, 859–864.