Flow-induced release of adenosine 5′-triphosphate from endothelial cells of the rat mesenteric arterial bed

V. Ralevic, P. Milner, K. A. Kirkpatrick and G. Burnstock

Department of Anatomy and Developmental Biology and Centre for Neuroscience, University College London, Gower Street, London WC1E 6BT (England)
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Abstract. Adenosine 5′-triphosphate (ATP) was released into the perfusate of rat isolated mesenteric arterial beds during each of two consecutive increases in flow. There was no significant difference between the amounts of ATP released on each occasion. Substance P was also released into the perfusate by increased flow, although its release was more variable. Removal of the endothelium of the mesenteric vessels with sodium deoxycholate led to a significant reduction (74%) in the amount of ATP released compared with the release before the endothelium had been removed. This suggests that the ATP released into the mesenteric arterial perfusate during increased flow arises from endothelial cells.
Key words. Endothelium; adenosine 5′-triphosphate; substance P; mesenteric bed.

Systemic blood pressure is largely controlled by changes in the resistance of the peripheral vascular bed, and this is mediated primarily by sympathetic vasoconstrictor fibres. In mesenteric resistance vessels of the rat a contribution to the neural regulation of tone by nonadrenergic, noncholinergic vasodilator nerves is also likely [1]. However, evidence suggests that arterial dilatation evoked by an increase in blood flow velocity is sensed by the luminal side of the vascular wall, and a relationship between flow-dependent dilatation and a factor released from endothelial cells, endothelium-derived relaxing factor (EDRF) [2, 3], has been demonstrated both in large arteries [4–8] and at the microvascular level in the rabbit ear [9] and in rabbit mesenteric arterioles [10].

There is increasing evidence for a dual role for the endothelium as both a target for and a source of vasoactive agents. A number of substances, including serotonin (5-HT), acetylcholine (ACh), substance P (SP) and adenosine 5′-triphosphate (ATP), which elicit vascular relaxation by a direct action on specific endothelial cell surface receptors and subsequent release of EDRF [2, 3, 11, 12], have been shown to be present within endothelial cells [13–15]. Electron microscopic immunocytochemistry was first used to localise choline acetyltransferase (ChAT), the acetylcholine synthesising enzyme, in vascular endothelial cells of the rat brain [16]. Subsequently, 5-HT and SP were localised in endothelial cells of rat femoral and mesenteric arteries [17], SP and ChAT were localised in endothelial cells of rat coronary arteries [13, 18] and rat mesenteric arterial endothelial cells were shown to be a source of vasopressin, 5-HT and angiotensin II [19]. Furthermore, it has been shown that endothelial cells of the rat hindlimb release SP in response to an increase in flow [20]. An endothelial source of these substances was substantiated by the demonstration of release of ATP, ACh and SP from cultures of endothelial cells by shear stress due to increased flow [21, 22].

An attractive hypothesis, therefore, is that vasoactive substances, such as ATP and SP, released from endothelial cells by increased flow, can act on their own and/or neighbouring endothelial receptors to cause release of EDRF with subsequent dilatation of the vessel, and thus may function as mediators of the vascular response to high flow. In this study, the isolated mesenteric arterial bed of the rat was used to determine whether endothelial cells can act as a source of releasable ATP and SP, and by using increased flow as a stimulus to evoke their release, to concomitantly determine a possible physiological role for these substances.

Methods

Adult male Wistar rats were used in the study. Rats were given heparin (1000 units, i.p.) and killed by stunning and exsanguination. Mesenteric beds were prepared for perfusion as described previously [23]. In brief, the superior mesenteric artery was exposed and cannulated and perfusion commenced with Krebs' solution containing (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52, gassed with 95% O_2, 5% CO_2 and maintained at 37 °C, and containing bovine serum albumin (5 g l^{-1}). The vascular bed was placed on gauze in a heated chamber and perfused at 4.8 ml min^{-1}. The preparation was superfused at 1 ml min^{-1} with Krebs solution of the same composition. The preparation was allowed to equilibrate for 30 min prior to experimentation.

In a control group of rats, fractions (20 × 1 ml) were collected before and during each of two consecutive periods of increased flow (about 5 times that of basal flow). A second group of rats were treated as above, but the endothelium was removed after the first period of high flow (see below). All perfusate samples were collected in ice-cold vials and placed on ice until they were assayed for ATP or SP content. Changes in perfusion pressure (mm Hg) were monitored by a pressure transducer (model P23, Gould) on a side arm of the perfusion cannula, and were recorded on a polygraph (model 79D, Grass). Vasodilator responses were investigated in the raised-

tone preparation, with tone raised by the addition of noradrenaline (NA) to the perfusate to a final concentration of 3×10^{-5} M.

Removal of endothelium. The endothelium was removed by perfusing the preparation with 2 ml of a 2 mg ml^{-1} solution of sodium deoxycholate in saline for 30 s [23]. The success of this treatment was assessed using the endothelium-dependent relaxing agent ACh at a range of concentrations (50-µl bolus injections of 10^{-7} to 10^{-3} M). The integrity of the smooth muscle was assessed by the ability of the preparation to constrict to NA.

Adenosine 5'-triphosphate assay. ATP was assayed using the luciferin-luciferase technique as described previously [24]. In these experiments the assay was capable of measuring ATP levels as low as 25 fmol.

Substance P assay. Fractions of perfusate were assayed for SP content by use of an inhibition enzyme-linked immunosorbent assay as previously described [20].

Statistical analysis. Release was expressed as pmol ATP or SP released per min and given as mean ± standard error of the mean. ATP data were analysed using a Student's unpaired two-tailed t-test; SP data were analysed using a Student's paired one-tailed t-test. $p < 0.05$ was taken as significant.

Results

The basal perfusion pressure of the rat isolated mesenteric arterial bed, perfused at a constant flow rate of 4.8 ± 0.15 ml min^{-1} was 17.9 ± 1.30 mm Hg (n = 14). A 5-fold increase in flow to 24.0 ± 0.52 ml min^{-1} was accompanied by an increase in perfusion pressure to 55.5 ± 2.56 mm Hg (n = 14). After treatment of mesenteric beds from the second group of rats with sodium deoxycholate, there was no change in basal perfusion pressure and there was no impairment of the ability of the preparation to contract to NA: with endothelium 60.4 ± 5.34 mm Hg (n = 5), without endothelium 109 ± 16.8 mm Hg (n = 5). In fact, endothelium-denuded preparations showed significantly enhanced vasoconstrictor responses to NA ($p < 0.025$). Relaxant responses to lower doses of ACh were abolished, while those to higher doses were greatly attenuated, confirming that the endothelium had been largely removed.

Release of adenosine 5'-triphosphate. In control animals (n = 5) the first period of increased flow evoked a significant release of ATP above basal release, which was maximal in fraction 6 and then dropped to a lower level which was sustained for subsequent fractions (fig. 1 a). ATP released during the second period of increased flow showed a similar release profile to that during the first period. There was no significant difference in release of ATP between the first and second periods of flow in these animals.

In the second group of rats (n = 5) the first period of increased flow evoked a release of ATP similar to the equivalent period in the control group (fig. 1 b). This also showed a maximal release in fraction 6, and declined through subsequent fractions. After removing the endothelium there was no significant release of ATP in any of the fractions, except for fraction 12 ($p < 0.05$) (fig. 1 b). There was a 74% reduction in release of ATP during the second period of high flow in endothelium-denuded preparations compared with ATP release during the same period in preparations with intact endothelium.

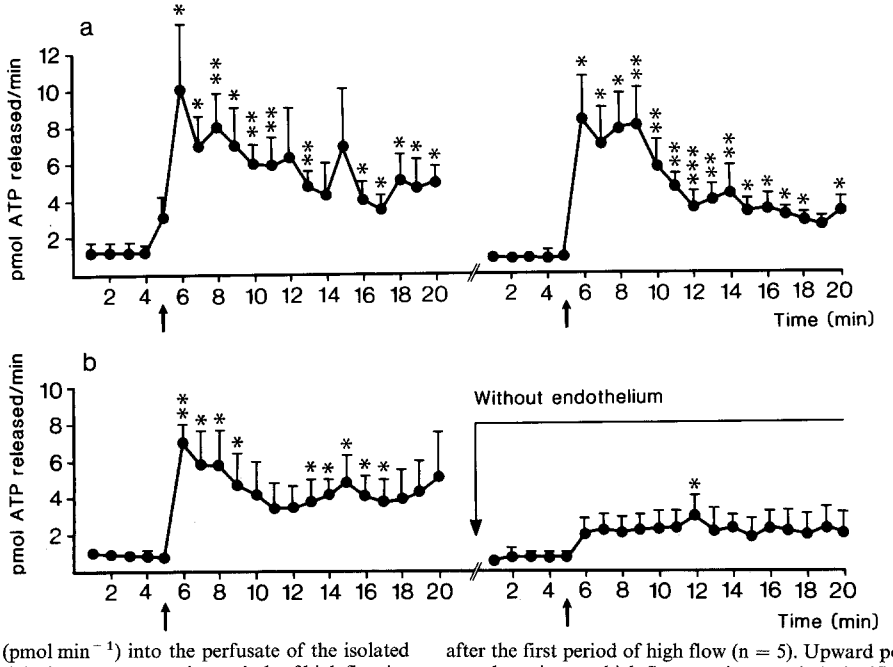

Figure 1. ATP released (pmol min^{-1}) into the perfusate of the isolated rat mesenteric arterial bed during two consecutive periods of high flow in: *a*) control rats (n = 5), and *b*) rats in which the endothelium was removed after the first period of high flow (n = 5). Upward pointing arrows indicate the point at which flow was increased. A significant difference from basal release is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Release of substance P. SP was released into the perfusate of the mesenteric arterial bed preparations during increased flow (fig. 2), although the time course and amounts of SP varied greatly from one vascular bed to another, indeed, from 14 preparations, 4 mesenteric beds showed no release of SP on either period of increased flow. During the first period of increased flow, the level of SP in the perfusate increased from 1.81 ± 0.63 pmol min^{-1} to 6.07 ± 2.56 pmol min^{-1} ($n=10$) and during the second period of increased flow, SP levels increased from 1.43 ± 0.37 pmol min^{-1} to 7.14 ± 2.75 pmol min^{-1} ($n=8$). Paired comparisons between both high and low flow for each animal showed that during both first and second periods of increased flow this release was significant ($p < 0.05$). Two examples of the pattern of SP release are given in figure 2. Since the amount of SP released during the first period of increased flow was not always reproduced in the same preparation during the second period of increased flow, and since some of the preparations showed no detectable release of SP, the effect of endothelium removal on SP release could not be determined.

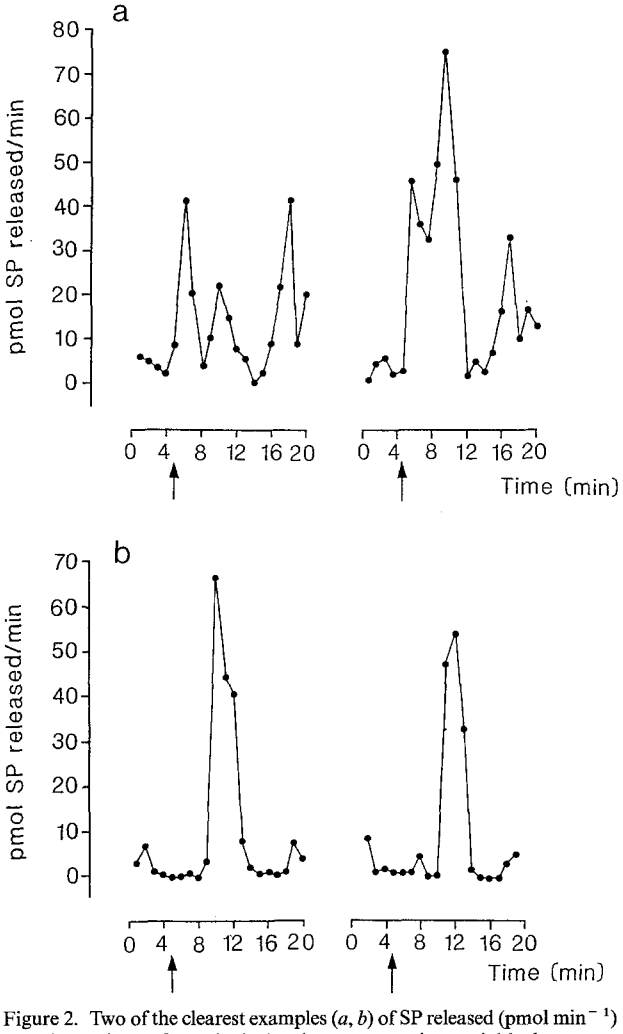

Figure 2. Two of the clearest examples (*a*, *b*) of SP released (pmol min^{-1}) into the perfusate from the isolated rat mesenteric arterial bed preparation during two consecutive periods of high flow. Upward pointing arrows indicate the point at which flow was increased. Large variability of SP release was seen between preparations with some showing no second period of release, some having greater release in the second period of increased flow, and some showing no detectable release on either period of increased flow.

Discussion

This study shows that increased flow through the rat mesenteric arterial bed preparation causes the release of ATP and SP into the perfusate, and that ATP release is abolished following removal of the endothelium. This procedure did not damage the functional integrity of the underlying smooth muscle (see also [23]).

Since ATP is a ubiquitous intracellular constituent, any cell could potentially serve as a source of extracellular ATP. ATP has a role as a transmitter in purinergic nerves [25] and as a co-transmitter in sympathetic nerves [26]; it is also present in the circulating blood as a constituent of blood-borne elements such as platelets [27] and erythrocytes [28], and is present as a component of smooth muscle and endothelial cells [29]. However, for the following reasons, not all of these are likely candidates for the source of the ATP released into the luminal perfusate in this study. The mesenteric arterial bed preparation was perfused with Krebs' solution, therefore, there was no possibility that ATP release was from the blood. Fittingly, flow-induced endothelium-dependent dilatation also occurs in vessels perfused with artificial perfusate [30, 31], which precludes the exclusive participation of blood-borne substances in this mechanism. In view of their position at the adventitial-medial border it is also unlikely that perivascular nerves are the source of ATP, or any other vasoactive substances acting via endothelial receptors, since these substances would have to pass a number of diffusion barriers and would be subject to enzymatic breakdown.

In this study the significant reduction in the release of ATP after removal of the endothelium suggests that the major source of this ATP is the endothelial cells. Our results are supported by a recent study [32] in which it has been shown that while high flow elicits the release of ATP from cultures of endothelial cells, no such release is produced from smooth muscle cells. Even after sodium deoxycholate treatment there was still some release of ATP from the mesenteric bed with increased flow (26% of the amount released before treatment). It is most likely that this comes from the endothelial cells remaining after sodium deoxycholate treatment, which explains the remaining relaxations to high doses of ACh. More drastic treatment to remove the endothelium is possible, but at the expense of the integrity of the underlying smooth muscle.

It was not possible to predict whether SP could be released on a second occasion of increased flow and this, together with the variable pattern of release, did not allow investigation of the effect of endothelium removal on SP release. The variability may have been a conse-

quence of the effects of the trauma of setting up the preparation, although this did not appear to affect ATP release. Immunohistochemical analysis of rat mesenteric resistance vessels has demonstrated the presence of SP-immunoreactive nerve fibres[33], however, it is unlikely that the source of the SP is from perivascular nerves. Indeed, in the perfused rat hindlimb preparation, destruction of SP-containing sensory nerves with capsaicin has no effect on the levels of SP released during increased flow[20].

In rat mesenteric arteries, it is possible that ATP has a more important role than SP as an endothelium-dependent relaxing agent since it has a potent relaxing action via endothelial P_{2Y}-purinoceptors[23], while SP is a poor dilator in this preparation[1]. A physiological role for ATP has also been proposed in the initiation of hypoxic vasodilatation of the coronary vasculature since specific blockade of P_{2Y} receptors attenuated dilatation due to ATP and to hypoxia, an action mediated via the release of EDRF[34].

This study, showing the release of ATP and SP into the perfusate of the rat mesenteric arterial bed, is in accordance with an increasing number of studies which show that endothelial cells are not only effectors, but a source of vasoactive substances. Furthermore, our study confirms that increased flow is an effective stimulus to evoke their release. This, coupled with the fact that an increase in flow causes an endothelium-dependent relaxation in many vessels, and with evidence that ATP and SP initiate endothelium-dependent relaxation in many vessels, makes it possible that substances released from endothelial cells may have a physiological role as mediators of flow-induced dilatation.

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The endothelium-dependent relaxation of human middle cerebral artery: Effects of activated neutrophils

S. E. Akopov, M. R. Grigorian and E. S. Gabrielian

Department of Pharmacology, Yerevan Medical Institute, 2 Kirov street, 375025 Yerevan, Armenia (USSR)
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Abstract. Neutrophils, activated by 4β-phorbol-12β-myristate-13α-acetate, decreased acetylcholine-induced relaxation of strips of human middle cerebral artery precontracted with noradrenaline. This effect was prevented by catalase, but not by superoxide dismutase. Nifedipine, propranolol and, less markedly, captopril reduced the decrease in acetylcholine-induced relaxation. Aspirin and dipyridamole did not reduce it.
Key words. Neutrophils; endothelium; oxygen products; regulation by drugs.