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Lack of uptake, release and action of UTP at sympathetic perivascular nerve terminals in rabbit ear artery

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Abstract

A possible role of uridine 5′-triphosphate (UTP) and uridine at sympathetic nerve terminals was studied in the rabbit ear artery after incubation of isolated vessels with [^{3}H]uridine or [^{3}H]noradrenaline. It was found that [^{3}H]uridine was taken up by rabbit ear artery. This uptake was largely suppressed after the removal of endothelium and was inhibited by ethidium bromide and dipyridamole. Chemical denervation of the vessels with 6-hydroxydopamine did not reduce the uptake. Following pre-incubation of the isolated vessels with [^{3}H]uridine, there was a release of radioactivity from the superfused rabbit ear artery. UTP, UDP, UMP and uridine were detected by thin layer chromatography both in the superfusate and inside the vessels. Transmural electric stimulation (30 V, 5 Hz) induced a contraction of the vessels but did not increase the release of uridine nucleotides into the superfusate. [^{3}H]Noradrenaline was released during electric stimulation and the addition of UTP (100 μM) had no effects on this release. To conclude, this study shows that in contrast to endothelial cells, the sympathetic nerve terminals of the rabbit ear artery do not take up uridine and do not release uridine-derived nucleotides. UTP at 100 μM is also unable to modulate the evoked release of noradrenaline. These results mainly confine the role of UTP in endothelium-derived vasodilatation via $P2Y_2$ and/or $P2Y_4$ receptors.

Keywords: UTP; Noradrenaline; Uptake; Release; Endothelium; Sympathetic nerve terminal; Ear artery

1. Introduction

The uptake of purine and pyrimidine nucleotides has been claimed in some tissues but remains disputed (Chaudry et al., 1985). This is due to the fact that nucleotides are generally rapidly broken down by enzymes (ectonucleotidases) located on the surface of the cells, therefore the apparent uptake of nucleotides could in fact be due to an uptake of nucleosides.

Purine and pyrimidine nucleosides (adenosine, uridine, thymidine and guanosine) can be taken up by different cell types such as erythrocytes, epithelial and endothelial cells (Cohen et al., 1979; Jarvis, 1986). Once in the cell cytoplasm, these substances are phosphorylated into nucleotides (Pearson and Gordon, 1979; Gordon et al., 1986, 1989). Nucleotides have been shown to be released by different cell types; ATP can be co-released with noradrenaline from sympathetic nerve terminals in numerous tissues including blood vessels (Sneddon and Burnstock, 1985; Burnstock and Warland, 1987; Lew and White, 1987) while ATP and UTP can be released from endothelial cells (Mahler, 1976; Pearson and Gordon, 1979; Saïag et al., 1995).

In numerous cells, UTP and ATP elicit various actions by acting on either the same or different receptors. UTP can act on vascular smooth muscle and endothelial cells via $P2Y_2$ purinoceptors (O'Connor et al., 1991) and on specific pyrimidinoceptors (Von Kügelgen et al., 1987; Saïag et al., 1987, 1990; Boeynaems and Pearson, 1990) such as the cloned $P2Y_4$ receptor (Communi et al., 1995; Nguyen et al., 1995).

ATP also modifies the release of noradrenaline largely via prejunctional P1 purinoceptors but also by P2

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purinoceptors in some blood vessels (Westfall et al., 1991). The modulation of noradrenaline release by UTP in nonvascular tissues is controversial: UTP has been claimed to decrease noradrenaline release via P1 receptors in mouse vas deferens (Von Kügelgen et al., 1989), however, in contrast, UTP has been reported to trigger the release of noradrenaline via presynaptic pyrimidinoceptors in rat cultured sympathetic neurones (Boehm et al., 1995; Von Kügelgen et al., 1997).

The present study investigates the possible roles of UTP in the sympathetic modulation of the vascular tone and the effects of this nucleotide on the release of noradrenaline in superfused isolated rabbit central ear artery. The uptake and release properties of perivascular nerve terminals for UTP and its derivatives are also examined.

2. Materials and methods

2.1. Uptake experiments

Proximal segments of rabbit ear artery (4 mm long) were incubated for 1 h in Krebs solution at 37°C containing 10^{-7} M of [^{3}H]uridine (45 Ci/mmol; 10 mCi/ml). After washing, the total [^{3}H] radioactivity was measured by dissolving the artery segment in a tissue solubilizer solution overnight (Optisolv, LKB) and adding a scintillation cocktail (Optiphase, LKB).

In some experiments, a fine needle with a cotton thread was passed through the lumen of the artery in order to remove the endothelium. The intima was thus rubbed then flushed away with Krebs solution. The removal of the endothelium was confirmed by the absence of any relaxation to acetylcholine. Some incubations were performed in the presence of dipyridamole or ethidium bromide.

2.2. Spontaneous and evoked [^{3}H]noradrenaline and [^{3}H]UTP release

Proximal rabbit ear artery segments (4 mm) were preincubated with [^{3}H]noradrenaline (57 Ci/mmol) (10^{-7} M) or [^{3}H]uridine (45 Ci/mmol; 10 mCi/ml) (10^{-7} M) for 1 h at 37°C. Preparations were then allowed to equilibrate for 90 min with an initial tension of 0.5–1 g and superfused (1 ml/min) with a modified oxygenated Krebs solution at 37°C (Saïag et al., 1990). The segments were stimulated twice using parallel wire electrodes. The two stimulations (S1 and S2; 30 V, 5 Hz, 90 s) were applied 6 min and 32 min after the start of the experiment, respectively. Contractions were measured as changes in isometric tension. The superfusate was collected for 2 min three times before each stimulation, once during stimulation and twice after stimulation. The radioactivity was measured as described above.

The results were expressed as a percentage fractional rate of the stimulation-evoked release of [^{3}H]noradrenaline or [^{3}H]uridine ratio of the responses to the second stimulation [S2] vs. the responses to the first stimulation [S1] as previously described by Alberts et al. (1981).

In some experiments, the terminals of sympathetic nerves were destroyed with 6-hydroxydopamine for 30 min (Aprigliano et al., 1976). The depletion of noradrenergic vesicles was confirmed by the absence of a contractile response of the vessels during electrical stimulation.

2.3. Thin-layer chromatography

Proximal rabbit ear artery segments were preincubated with [^{3}H]uridine, superfused with modified oxygenated

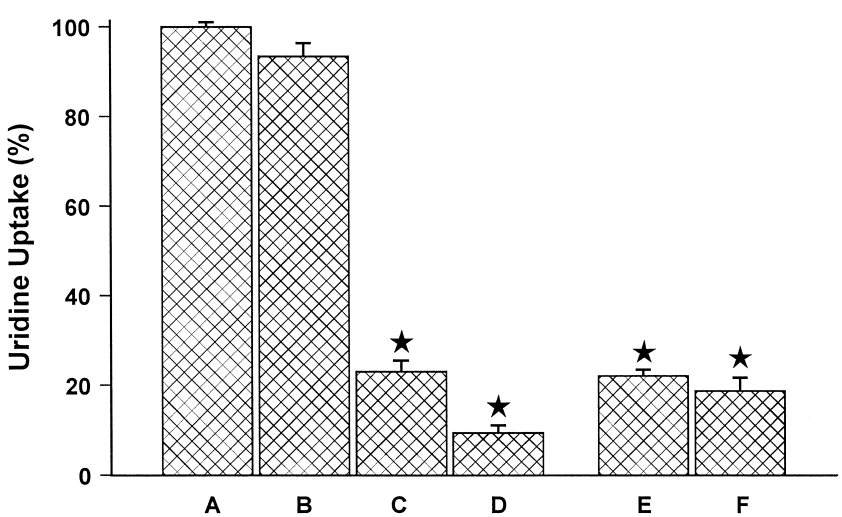

Fig. 1. Uptake of uridine by rabbit ear artery. Radioactivity uptake was measured on rings [A] with endothelium ($n = 4$), [B] treated with 6-hydroxydopamine ($n = 4$), [C] treated with ethidium bromide ($n = 4$), [D] treated with dipyridamole ($n = 4$), [E] without endothelium ($n = 5$), [F] without endothelium and treated with 6-hydroxydopamine ($n = 3$). $*$: $P < 0.001$ compared with [A].

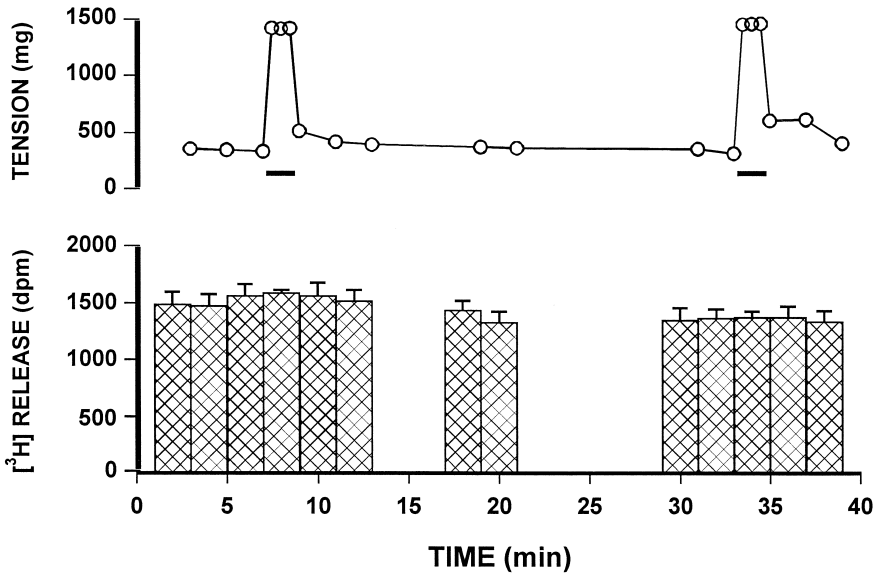

Fig. 2. Release of uridine nucleotides by rabbit ear artery rings. Upper panel, contractile responses of the rings to transmural electrical stimulation (30 V, 5 Hz) for 90 s (—). Lower panel, [^{3}H] radioactivity released in the superfusate as 2-min fractions ($n = 3$).

Krebs solution, then stimulated twice. Samples were analyzed by thin layer chromatography, as previously described (Norman et al., 1974; Saïag et al., 1995).

Briefly, after a 5-h migration, the silica plates (20 × 20 cm) were dried. The standards were visualized in UV light at 254 nm and their R_f values determined. The plates were then divided into 20 equal strips. Each strip was rubbed and the radioactivity in the silica gel powder was measured in a liquid scintillation spectrophotometer. Data which express the total radioactivity refer to the radioactivity present in the 20 strips cut from each plate. In order to quantify the radioactivity specific to each nucleotide, the radioactivity present in the strips located between the migration zones of the different nucleotides (15–20%) was not taken into account. Results were expressed as dpm per 100 μl of sample. The R_f value of each radioactive peak was determined and compared to the non-radioactive standards: UTP, UDP, UMP and uridine.

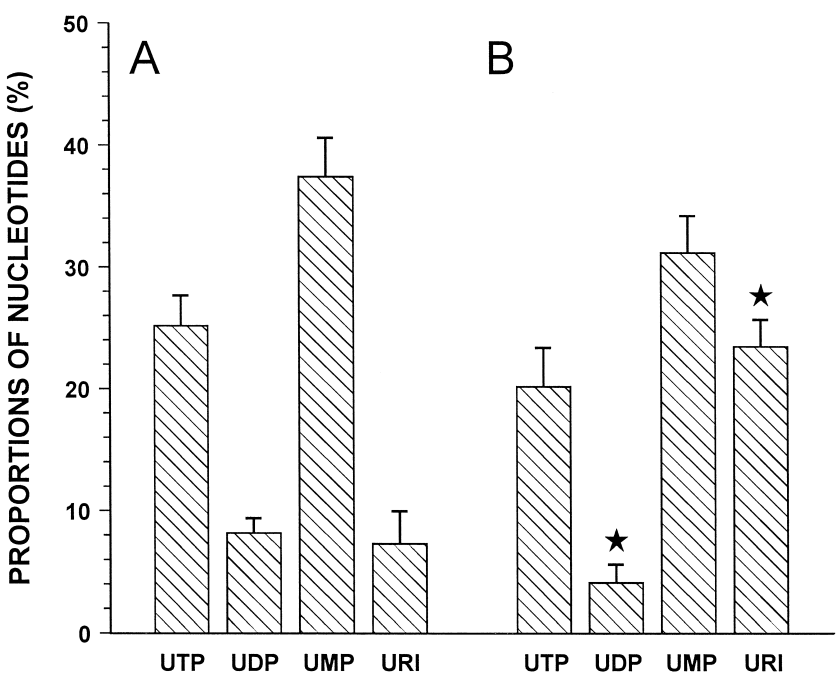

Fig. 3. Thin-layer chromatography of [^{3}H] radioactivity. [A] radioactivity present inside the vascular wall during superfusion. [B] radioactivity released in the superfusate during electrical stimulation (S1). ∗: $P < 0.05$, a significant difference between the amount of radioactivity present in the cells and the amount of radioactivity released during stimulation.

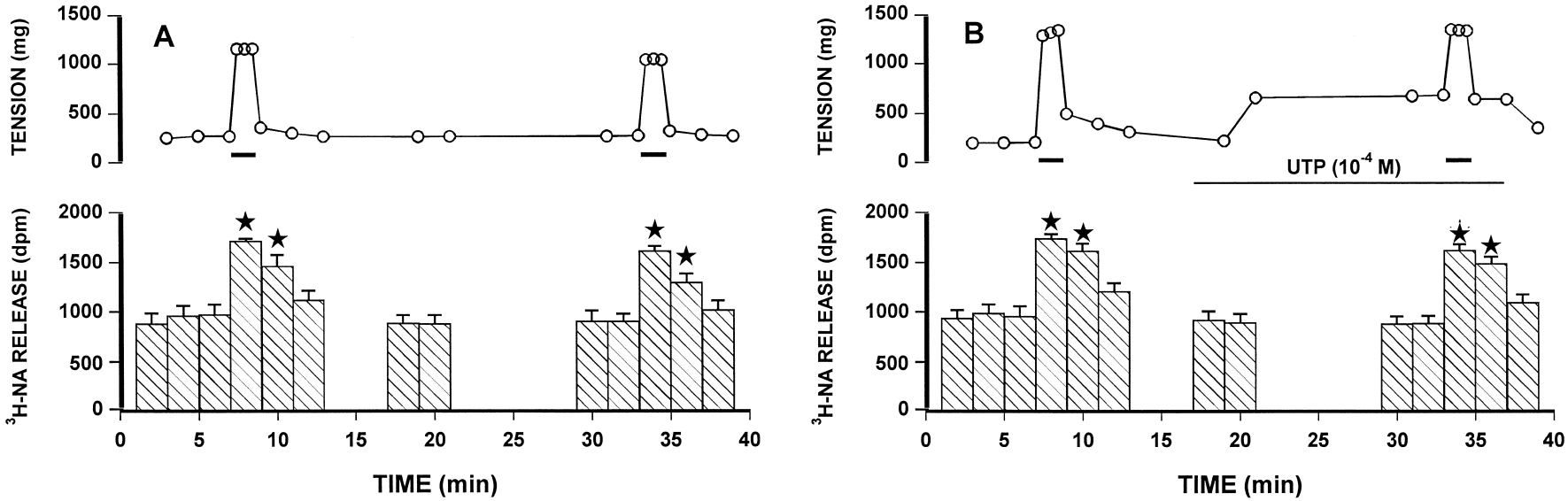

Fig. 4. Effects of UTP on the release of noradrenaline. Upper panels, contractile responses of the rings to transmural electrical stimulation. Lower panels, [^{3}H]noradrenaline (^{3}H-NA) released in the superfusate as 2-min fractions. [A] After incubation, the rings were superfused and stimulated electrically (30 V, 5 Hz) for 90 s (—). [B] UTP (10^{-4} M) was added 18 min after the beginning of the superfusion. Note the post-junctional contractile effect of UTP. $*$: $P < 0.001$ compared with basal release of [^{3}H]noradrenaline.

2.4. Drugs

Ethidium bromide, dipyridamole, uridine, UMP, UDP and UTP were obtained from Sigma, Poole, UK. [^{3}H]Noradrenaline hydrochloride (57 Ci/mmol), 5-6-[^{3}H]uridine ammonium salt (45 Ci/mmol) were obtained from Amersham, UK. Drug solutions were prepared daily.

2.5. Statistical analysis

All data represent arithmetic means ± S.E.M. Comparisons between samples were made using a Mann–Whitney test. Differences were declared significant at a probability of 0.05 or less.

3. Results

3.1. Uptake of uridine

The results on the uptake of uridine are reported in Fig. 1. Segments of rabbit ear artery with intact endothelium took up uridine. Treatment of these segments with 6-hydroxydopamine did not significantly change the uptake ($6.5 \pm 2.4\%$ reduction, $n = 5$). The presence of ethidium bromide at a concentration of 10^{-4} M in the incubation medium significantly reduced the uptake of uridine by $90.6 \pm 2.3\%$ ($n = 4$). Dipyridamole (10^{-4} M) had the same effect as ethidium bromide, reducing the uptake of uridine by $76.9 \pm 1.8\%$ ($n = 4$). The removal of the endothelium significantly reduced the uptake of uridine by $77.8 \pm 1.4\%$ ($n = 5$) and 6-hydroxydopamine did not significantly change this uptake of uridine on the rings without endothelium ($81.2 \pm 2.9\%$ reduction relative to segments with intact endothelium, $n = 3$).

3.2. Release of uridine nucleotides

When rabbit ear artery segments preincubated with [^{3}H]uridine were superfused, there was a spontaneous and stable release of [^{3}H] radioactivity. The transmural stimulation of these vessel rings did induce a contraction under isometric conditions (Fig. 2A) but did not evoke more release of radioactivity (Fig. 2B). Treatment of the rabbit ear artery with 6-hydroxydopamine suppressed the contraction induced by electrical stimulation but did not change the release of radioactivity seen during superfusion (data not shown).

Thin layer chromatography was performed in order to identify the pyrimidines in which the radioactivity was distributed. The radioactivity present in the superfusate during transmural stimulation (S1) of rabbit ear artery segments and that present inside the cells at the end of the experiment was analyzed. The results show that after incubation with [^{3}H]uridine, the radioactivity present in the cells after superfusion (Fig. 3A) was divided between UTP ($25.2 \pm 2.5\%$) and its derivatives UDP ($8.2 \pm 1.2\%$), UMP ($37.4 \pm 3.2\%$) and uridine ($7.3 \pm 2.7\%$). The radioactivity released by the vascular wall during electrical stimulation (Fig. 3B) can be attributed to UTP ($20.2 \pm 3.2\%$), UDP ($4.1 \pm 1.5\%$), UMP ($31.2 \pm 3.0\%$) and uridine ($23.5 \pm 2.2\%$). These proportions were identical for UTP and UMP but were significantly different for UDP ($P \leq 0.05$) and uridine ($P \leq 0.001$).

3.3. Release of noradrenaline

Rabbit ear artery rings were pre-incubated with [^{3}H]noradrenaline for 1 h then washed with modified oxygenated Krebs solution and superfused. Transmural electric stimulations (S1 and S2) resulted in both a contraction of the vessel and a release of [^{3}H]noradrenaline (Fig. 4A). Addition of UTP (10^{-4} M) before the second transmural electric stimulation (S2) did contract the vessel rings but did not change the release of [^{3}H]noradrenaline (Fig. 4B). Treatment of the rings with 6-hydroxydopamine suppressed the release of [^{3}H]noradrenaline (data not shown).

4. Discussion

The aim of this study was to investigate the uptake and release properties of perivascular nerve terminals for UTP derivatives, the possible implication of UTP in the sympathetic modulation of the vascular tone and the effects of this nucleotide on the spontaneous and evoked [^{3}H]noradrenaline release from nerve terminals.

Uridine is taken up by several cell types such as erythrocytes (Plagemann and Wohlhueter, 1984) and intestinal epithelial cells (Schwank et al., 1984). In this study, isolated rabbit ear artery with an intact endothelium demonstrated an uptake of radioactivity in presence of extracellular tritiated uridine. This uptake was inhibited by preincubation of the rings with dipyridamole and ethidium bromide and therefore has common characteristics with the uptake of uridine we have already described in rabbit aortic endothelial cells in culture (Saïag et al., 1995) and the uptake of adenosine shown in pig aortic endothelial cells in culture (Pearson et al., 1978). It thus appears that in terms of mechanism of uptake of uridine and intracellular phosphorylation of this nucleotide, endothelial cells of the ear artery have the same characteristics as the endothelial cells of the aorta.

An uptake of uridine of the order of 20% is still observed on preparations without endothelium. The destruction of sympathetic nerves terminals after treatment with 6-hydroxydopamine does not reduce the uptake of uridine and this excludes the involvement of sympathetic nerve terminals. The cause of this uptake, however, is not known. It has already been shown that despite the fact that

vascular smooth muscle cells lack the high-affinity transport system for adenosine, as displayed by endothelial cells, these cells are able to take up this nucleoside (Pearson et al., 1978). By analogy, the same transport system could exist for uridine in vascular smooth muscle cells but this will need experimental confirmation.

After incubation with tritiated uridine and superfusion of the rabbit ear artery segments, thin layer chromatography showed the presence of uridine, UMP, UDP and UTP both inside the vessels and into the superfusate. The transmural stimulation of the vessel rings did not, by itself, produce any release of uridine nucleotides and the treatment of the arteries with 6-hydroxydopamine did not change the proportions of uridine nucleotides both inside the cells and in the superfusate. These proportions match those we found in rabbit aortic endothelial cells under conditions of increased flow (Saïag et al., 1995). It is therefore likely that the superfusion of the rabbit ear artery segments produced shear stress on the endothelial cells lining the vessels and a consequent release of uridine nucleotides.

Rabbit ear arteries have abundant perivascular sympathetic nerve terminals (Holton, 1959; Edvinsson et al., 1984). In addition, the contractile effect of UTP via specific nucleotide receptors present on vascular smooth muscle cells of this bed has already been described (Von Kügelgen et al., 1987; Von Kügelgen and Starke, 1990). In these vessels, however, we showed that the presence of a high concentration of UTP (100 μM) failed to change the release of noradrenaline observed during transmural electrical stimulation.

UTP-triggered neurotransmitter release via pyrimidinoceptors has recently been demonstrated in the sympathetic nerves of the rat superior ganglia where the addition of UTP has been shown to induce a very significant release of noradrenaline within 30 s (Boehm et al., 1995).

The standard conditions of evoked [^{3}H]noradrenaline release obtained in our experiments were identical to those described previously by Maynard et al. (1991) in a similar model of superfused rabbit ear artery. Our results, however, are different to those obtained by Boehm et al. (1995). It should be emphasised that this last study was performed using isolated neurones from the rat cervical superior ganglia. Our study uses isolated rabbit ear artery and the physiological differences between these two preparations could account for the differences observed. Our results could also mean that the sympathetic nerve terminals of the rabbit ear artery are free of specific presynaptic pyrimidinoceptors like those described in the sympathetic neurones of rat cervical ganglia (Boehm et al., 1995).

To conclude, our results show that endothelial cells from the rabbit ear artery are able to take up uridine and release UTP nucleotides under conditions of flow, as already demonstrated on aortic endothelial cells in culture. In contrast, sympathetic perivascular nerve terminals are unable to take up uridine and unable to release UTP and its derivatives. Furthermore, the present study demonstrates that UTP, at a concentration of 100 μM, does not modulate the release of noradrenaline from sympathetic nerve terminals during electrical stimulation. As uridine nucleotides are able to induce vascular effects when acting on specific receptors (Hardebo et al., 1987; Von Kügelgen et al., 1987; Saïag et al., 1987, 1990; Von Kügelgen and Starke, 1990; Evans and Kennedy, 1994), our results suggest that these substances can only participate in the regulation of the vascular tone when released from endothelial cells.

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