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Adenosine modulation of neurotransmission in human uterine arteries

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The relaxing effects of adenosine, N-[(R)-1-methyl-2 phenylethyl]-adenosine (R-PIA) and 5-N-ethylcarboxamide adenosine (NECA) were investigated in human uterine arteries precontracted by phenylephrine *in vitro*. Adenosine, R-PIA and NECA relaxed isolated uterine arteries with intact endothelium, the potency order was NECA > R-PIA > adenosine. When tested on vessels devoid of their endothelium, the relaxing effect of adenosine was the same. These results suggest the vasodilatation effect on human uterine arteries is endothelium-independent, and might be via the A_2 receptor (by pharmacological classification). By administering adenosine to human uterine arterial cell culture, single cell intracellular calcium change was also determined by laser cytometry. Decreased intracellular calcium was observed after administration of adenosine 10^{-6} M and 2×10^{-5} M. We concluded from the results that adenosine acts on human uterine artery cell by A2 receptor, independently of the endothelium, and decreases the intracellular calcium concentration, thus causing uterine artery relaxation.

Key words: adenosine/human uterine artery/intracellular calcium concentration

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Introduction

The uterine arteries are composed of the most important blood vessels supplying the human uterus, and are richly supplied by noradrenergic nerves (Owman and Stjernquist, 1988). Noradrenaline contracts the human uterine artery in a dose-dependent manner (Stjernquist and Owman, 1985). Acetylcholine induces vasodilatation in endothelium intact vessel, and this relaxation effect has been proposed to be mediated via relaxing substances derived from the endothelium. Three such substances have been identified, i.e. the endothelium-derived relaxing factor (Furchgott, 1983), now characterized as nitric oxide (Palmer *et al.*, 1987); prostacyclin (Moncada *et al.*, 1976); and the endothelium-derived hyperpolarization factor (Feletou and Vanhoutte, 1986). The endothelium-derived relaxing substances should be released when acetylcholine acts on muscarinic receptors on the endothelial cells. Other neurotransmitters which act by means of endothelium to induce uterine artery dilatation have been recently identified as substance P (SP), and vasoactive intestinal polypeptide (VIP). On the other hand, calcitonin gene-related peptide (CGRP) and atrial natriuretic peptide (ANP) act on the uterine vessel independently of the endothelium (Bodelsson and Stjernquist, 1992).

Adenosine is a purine nucleoside of fundamental importance, serving as a building block in nucleic acid and as a regulator of biological function. Adenosine has recently been demonstrated to modulate the non-adrenergic non-cholinergic (NANC) neurotransmitter (Chiang *et al.*, 1994). It has a potent effect of vasodilatation, thereby increasing uterine artery blood flow in sheep (Resnik *et al.*, 1976). However, to our knowledge, the adenosine receptor or the relationship with endothelium in human uterine artery has seldom been investigated.

Therefore, the aim of the present study was to assess the pharmacological characterization of adenosine receptors in the human uterine artery *in vitro*. Additionally, endothelium and endothelium-denuded preparations were investigated to clarify whether the endothelium-dependent vasodilatation effect of adenosine is involved in the human uterine artery. For further study, the modulation of intracellular calcium by adenosine in a single uterine artery cell was determined with laser cytometry.

Materials and methods

Human material

Specimens of the ascending branch of the uterine artery were obtained from 40 women (mean age 48.2 years, range 40–66) undergoing abdominal hysterectomy for benign gynaecological diseases (endometriosis, fibromyoma, adenomyosis, menometrorrhagia). The patients received epidural anaesthesia with 2% xylocaine. Premedication was administered by use of atropine and demerol. Immediately following removal of the uterus, the helically formed ascending branches of the uterine arteries were identified on the lateral sides of the uterus. Segments of the vessel were excised and placed in cold (+4°C) Krebs–Ringer solution. We had provided the patients with detailed information regarding the purpose of this investigation and had obtained their consents for the excision of the specimens.

In-vitro pharmacology

Following excision, the vessels were transported to the laboratory immediately, where they were dissected free from adjacent veins and

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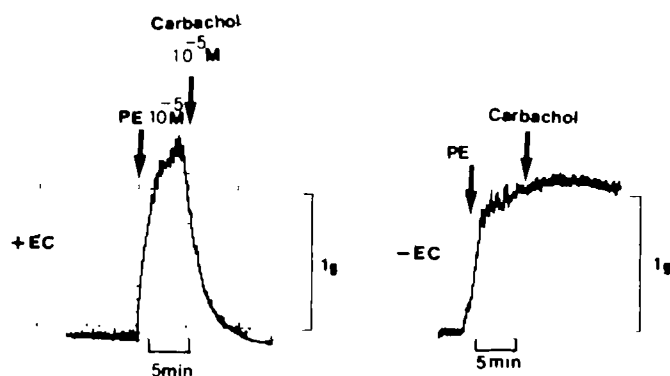

Figure 1. Bioassay for the removal of endothelial cells (EC) from human uterine arteries. In the intact vessels (+EC), the maximal contraction induced by phenylephrine (phenylephrine) 10^{-5} M (usually after 5 min) was abolished by carbachol 10^{-5} M, whereas the denuded vessels (−EC) did not respond to carbachol

connective tissue They were cut into cylindrical segments with a length of 3–5 mm The circular specimens were placed in an organ chamber (volume 20 ml) between two platinum hooks, one of which was attached to a Grass FT03C force-displacement transducer and one of which could be moved to adjust the smooth muscle tension. The isometric tension was recorded on a Grass model 7D polygraph. The organ bath contained a modified Krebs–Ringer solution of the composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$-$2H_2O$ 2.0, $MgSO_4$ $7H_2O$ 1 2, $NaHCO_3$ 24.8, KH_2PO_4 1 2 and glucose 5 6 The specimens were mounted at 2 g resting tension in organ chambers containing Krebs–Ringer solution at 37°C, gassed with 95% O_2 and 5% CO_2; pH was maintained at 7 4–7.6. The drug was removed by several washes with Krebs–Ringer solution, and the tension was allowed to return to the baseline. Specimens were allowed to re-equilibrate for 30 min after the drug response to ensure maximum wash-out of the drug as well as to minimize the possibility of receptor desensitization.

After an equilibration period of 1 h, the specimens were contracted by phenylephrine 10^{-5} M. We removed the endothelium from 10 of the vessel preparations by two gentle injections of O_2/CO_2 gas mixture through the vessel lumen during 5 min at an interval of 10 min (Mikkelsen *et al.*, 1988; Bodelsson *et al*, 1989). Another 10 untreated preparations acted as controls. Before a maximal vasoconstriction was elicited with phenylephrine (10^{-5} M), we added carbamylcholine chloride (carbachol, 10^{-5} M) for 5 min to ensure that the endothelium had been completely removed (Figure 1) After washing, the preparations were contracted by an addition of 10^{-5} M phenylephrine When a stable contraction was established (after 5–10 min), adenosine (10^{-5} to 10^{-3} M) was added to the organ chamber in a cumulative manner on both the intact and endothelium-denuded vessels to obtain a concentration–response curve. The higher dose was added when a stable muscle tension had been established, usually after 5 min Next, the potency of adenosine and adenosine agonist was investigated by adding adenosine 10^{-5} to 10^{-3} M, N-[(R)-1-methyl-2 phenylethyl]-adenosine (R-PIA) 10^{-8} to 10^{-5} M, 5-N-ethylcarboxamide adenosine (NECA) 10^{-8} to 10^{-5} M to the organ chamber in a cumulative manner on intact vessels.

Culture of cells

Vascular smooth muscle cells (VSMC) were isolated from uterine arteries by collagenase/elastase digestion (Davies *et al.*, 1991). The cells were grown on glass cover slips in medium 199 supplemented with 10% fetal calf serum, penicillin 100 IU/ml, and fungizone 2.5 µg/ml Positive identification of VSMC (>90%) was made possible by use of an *a*-smooth muscle actin immuno-fluorescent technique. The medium was changed every 2–3 days. Cells were passaged with trypsin and EDTA (0.5 g/l and 0.2 g/l respectively) after confluence. Cells were subcultured into two or four chamber slides 1–2 days before the start of the experiment The cells selected for study of intracellular free calcium ($[Ca^{2+}]i$) were firmly attached to the chamber slides and placed in areas with little cell debris in healthy cultures. We analysed data on single, well-separated cells that showed a normal response to phenylephrine.

Measurements of $[Ca^{2+}]i$

We incubated cells with 5 µM of the calcium indicator Fluo-3 acetoxy-methyl ester (Fluo-3/AM) for 45 min at 37°C, and washed the cells three times with Hank's solution Then the cells were incubated for 30 min in Hank's solution to complete the de-esterification of Fluo-3/AM into Fluo-3. An ACAS 570 interactive laser cytometer (Meridian Instruments Inc., Okemos, MI, USA) was used to perform calcium measurement. We were able to identify individual arterial myocytes by their characteristic morphology using phase optics and image-scanning for fluorescence to determine the fluorescence intensity of Fluo-3 within the cell. We measured the intensity of Fluo-3 by image scanning at 30 s intervals with an argon laser at 488 nm A computer was used to convert the fluorescent image into a pseudogrey level image, coded according to fluorescence intensity. Analysis consisted of 500 s to establish a baseline. After addition of phenylephrine 10^{-6} M, adenosine 10^{-6} M and 2×10^{-5} M, changes in relative $[Ca^{2+}]i$ concentration were monitored for 360 s.

Drugs

Phenylephrine and carbachol were obtained from Sigma (St Louis, MO, USA), adenosine, R-PIA, NECA were obtained from Research Biochemicals Incorporated (Natick, MA, USA) Fluo-3/AM was purchased from Molecular Probes Inc (Eugene, OR, USA).

Calculations and statistics

The percentage of relaxation refers to the decrease in phenylephrine induced tone. Data were expressed as means ± SEM. We estimated the concentration of the drug producing 50% of the relaxation (ED_{50} value) by use of a statistical package for the personal computer. Student's *t*-test was used for one or two samples to evaluate the possible significance, each experiment was performed only once on material from the same patient. The number of experiments was equal to the number of patients (*n*).

Results

Relaxing effect of adenosine in uterine artery with intact and denuded endothelium

Segments of uterine artery, with and without the presence of endothelium, were contracted with phenylephrine (10^{-5} M). Adenosine (10^{-5} to 10^{-3} M), added cumulatively, relaxed both types of uterine artery in a dose dependent manner. Figure 2 reveals the dose–response curves for the action of adenosine in both preparations. Adenosine induced a relaxation of the isolated uterine arteries which was similar in intact and endothelium-denuded preparations (Table I).

Effect of adenosine analogues in uterine artery

As shown in Figure 3, adenosine (10^{-5} to 10^{-3} M), R-PIA (10^{-8} to 10^{-5} M) and NECA (10^{-8} to 10^{-5} M) induced relaxation

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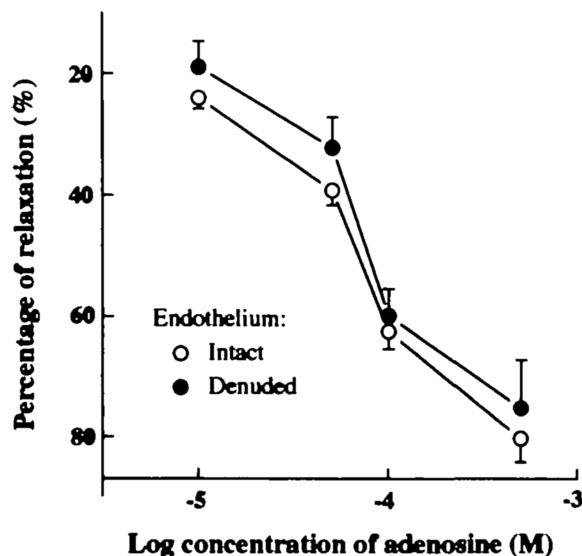

Figure 2. Concentration–response curves showing the relaxing effect of adenosine (10^{-5} to 10^{-3} M) on endothelium-intact (n = 10) and on endothelium-denuded (n = 10) human uterine arteries. The relaxing effect is expressed as a percentage of the contractile force developed by phenylephrine. Each point is the mean, and vertical bars indicate the SEM. Adenosine induced a concentration-dependent relaxation in both intact and denuded vascular preparations.

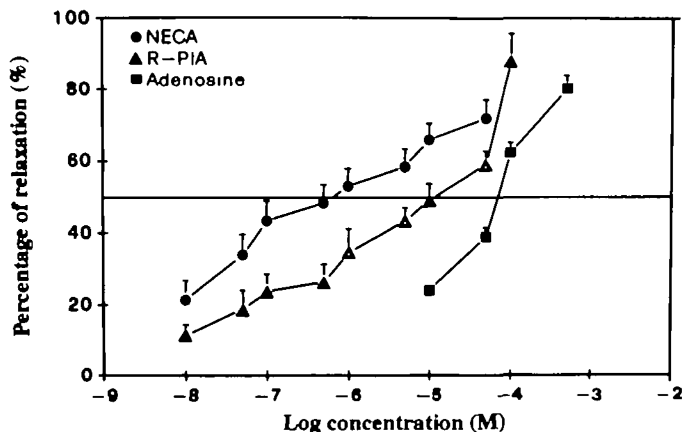

Figure 3. The effect of 5-N-ethylcarboxamide adenosine (NECA) (n = 10), N-[(R)-1-methyl-2 phenylethyl]-adenosine (R-PIA) (n = 10) and adenosine (n = 10) on the endothelium-intact human uterine arteries. The increase in contractility induced by phenylephrine stimulation before addition of each agonist was taken as 100% response. Each point is the mean, and vertical bars indicate the SEM. The respective ED_{50} values are summarized in Table II.

in the uterine artery in a dose-dependent manner. NECA were the most potent of the adenosine analogues tested.

High doses of adenosine (5×10^{-4} to 5×10^{-3} M) elicited greater response than lower doses (10^{-5} to 5×10^{-4} M). The ED_{50} values of NECA, R-PIA and adenosine on the relaxation of contractions were 7.8 ± 1.3×10^{-7} M (n = 10), 4.8×10^{-6} ± 4.5×10^{-7} M (n = 10) and 6.34×10^{-5} ± 8.9×10^{-6} M (n = 10) respectively. The ratios of the ED_{50} values of the other agonists relative to NECA were 6.2 for R-PIA and 81.3 for adenosine. Adenosine and its analogues caused inhibited contractile responses which were induced by

Table I. Percentage relaxation response to adenosine in isolated human uterine artery precontracted with phenylephrine 10^{-5} M. Comparison between vascular preparations either intact or denuded of endothelium

Concentration of adenosine (M)	Relaxation (%)		Significance of difference[a]
	Intact	Denuded	
10^{-5}	24.00 ± 1.78	20.33 ± 6.80	NS
5×10^{-5}	38.96 ± 2.50	31.97 ± 5.01	NS
10^{-4}	62.34 ± 2.97	59.77 ± 4.49	NS
5×10^{-3}	80.25 ± 3.93	75.12 ± 8.07	NS

Data are means ± SEM, n = 10 in each group.
[a]Student's t-test for two samples.
NS = not significant.

Table II. Sensitivity of human uterine artery to adenosine receptor agonists

Agonist	ED_{50} ($\times10^{-7}$ M)	Ratio relative to NECA	n
NECA	7.8 (± 1.3)	1.0	10
R-PIA	48.0 (± 4.5)	6.2	10
Adenosine	634.5 (± 89.1)	81.3	10

NECA = 5-N-ethylcarboxamide adenosine; R-PIA = N-[(R)-1-methyl-2 phenylethyl]-adenosine; ED_{50} = concentration ± SEM necessary to produce a response which was 50% of the response to maximal stimulation by phenylephrine.
n = number of experiments.

phenylephrine with the potency order: NECA > R-PIA > adenosine (Table II).

Relative intracellular calcium changes induced by adenosine

VSMC intracellular calcium concentrations increased 1.6-fold after exposure to 10^{-6} M phenylephrine comparing to mean base line. Thereafter, adenosine 10^{-6} M decreased $[Ca^{2+}]i$, by 1.3-fold compared to the base line. Cumulative administration of adenosine 2×10^{-5} M brought $[Ca^{2+}]i$ to the base level (Figure 4).

Discussion

Although the vessel activity of human uterine arteries has been assumed to be controlled by noradrenaline and acetylcholine, other neurotransmitters, e.g. CGRP, VIP, SP, ANP, nitric oxide have been found to be involved in vessel relaxation. Also, CGRP, VIP, SP, nitric oxide have been proposed to act as NANC neurotransmitters (Ottesen *et al.*, 1984; Kim *et al.*, 1991; Rajfer *et al.*, 1992; Said, 1992). As a local hormone, neurotransmitter, or neuromodulator, adenosine is an ubiquitous biological compound, being present in every cell of the human body. It is quite well established as an efficient local chemical regulatory signal that facilitates communication between cells and exerts a wide spectrum of effects on various tissues and organs under physiological and pathophysiological conditions (Wihlund *et al.*, 1991). In sheep uterine arteries, intra-arterial injection of adenosine (24 μM) produces blood flows equal to those initiated by 1 μg of oestradiol-17β (0.24 μM) (Resnik

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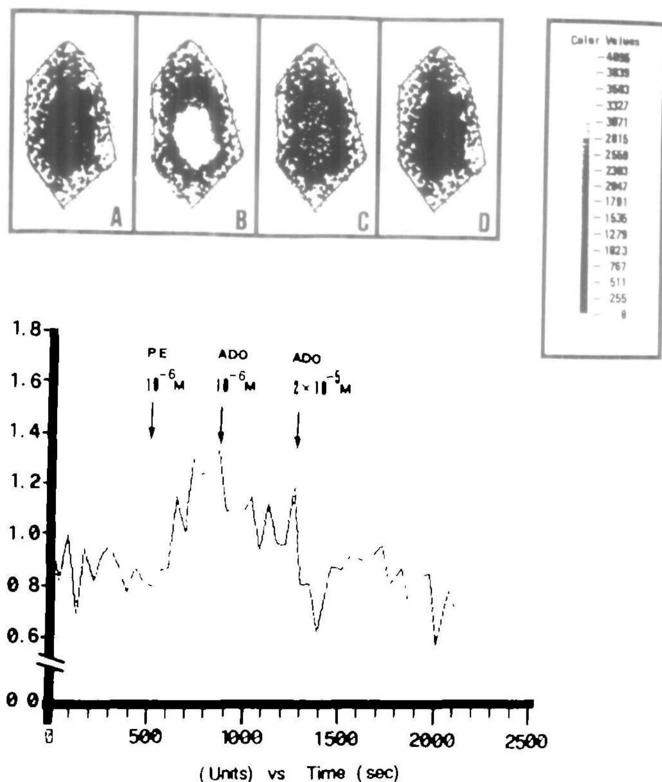

Figure 4. Effect of phenylephrine and adenosine on human uterine arterial myocyte intracellular free calcium $[Ca^{2+}]i$. Myocytes were loaded with the calcium indicators, Fluo-3/AM (for image scanning, A to D). Single individual myocytes were identified (**A**) shows the baseline scans before addition of drug; (**B**) shows the result of treatment with phenylephrine 10^{-6} M, (**C**) shows result of treatment with adenosine (ADO) 10^{-6} M; and (**D**) shows the result of treatment with adenosine 2×10^{-5} M. The fluorescence intensity of each image was indicated according to the grey level scan shown in the upper right quadrant. The *y* axis shows the ratio of fluorescence represented as the relative intracellular calcium concentration. Exposure to 10^{-6} M phenylephrine caused a rapid increase in the intracellular calcium concentration. After sequential addition of 10^{-6} M and 2×10^{-5} M adenosine, the intracellular calcium concentration decreased. The results shown are representative of repeated experiments using 10 individual myocytes

et al., 1976). However, little information is available regarding the effects of adenosine on human uterine arteries.

In our study, all adenosine and agonists exerted a vasodilatation effect on human uterine vessels and the potency for this relaxing effect was NECA > R-PIA > adenosine. Subclasses of adenosine receptors may be distinguished by the relative agonist potencies of certain adenosine analogues (Daly *et al.*, 1985). At the adenosine A2 receptor, NECA is more potent than R-PIA; whereas, R-PIA is equally or more potent than NECA at the A1 receptor (Collis, 1983; Gustafsson *et al.*, 1985; Wihlund *et al.*, 1986). The agonist potency in human uterine arteries may be A_2 receptor dominant by pharmacological classification in our study. In adenosine A_2 receptors, NECA is at least 10-fold more potent than adenosine in rat brain (Daly, 1982). In the present experiments, NECA revealed an 81.3-fold stronger potency than adenosine according to ED_{50}. The slope of the dose–response curve at high adenosine doses (5×10^{-4} to 5×10^{-3} M) is steeper than NECA and R-PIA. Though the reason for this is unclear, it may be that adenosine has different affinity to A_2 receptors at different concentrations. Low dose (10^{-5} to 5×10^{-5} M) adenosine showed almost parallel affinity to NECA and R-PIA. But, high-dose (5×10^{-5} to 5×10^{-3} M) adenosine elicited a greater response than did low-dose adenosine. The slope became even steeper than for NECA and R-PIA.

As shown in this study, adenosine induced dilatation independently of the endothelium, i.e. the same effect that has been reported for ANP and CGRP (Bodelsson and Stjernquist, 1992). These regulatory substances seem to exert their effects on this vascular smooth muscle preparations without involving EDRF in the signal chain. Several lines of evidence have shown that adenosine does not require the presence of endothelial cells to elicit the relaxation of isolated arteries (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981). By contrast, endothelium-denuded preparations of pig aorta reduced the degree of relaxation produced by adenosine (Gordon and Martin, 1983). Therefore it remains unclear whether the endothelium was related to the relaxing effect of adenosine or not, and it seems to be species and specimen specific. The present experimental results reveal that the relaxing effect of adenosine may be endothelium-independent (implying EDRF-independent) and may have a direct effect on the A_2 receptors of human uterine artery smooth muscle. Data have been obtained with a number of tissues indicating that adenosine A_1 receptor is associated with an inhibition of adenylate cyclase and a decrease in intracellular cyclic AMP (Linden, 1991). A second subtype, the A_2 receptor, is associated with the stimulation of adenylate cyclase and an increase in cyclic AMP (van Calker *et al.*, 1979; Londos *et al.*, 1980). An increase in cAMP levels has long been associated with smooth muscle relaxation. Though the full mechanisms by which the cyclic nucleotides induce smooth muscle relaxation are still obscure, it is thought to lead to a reduction in intracellular calcium concentration, causing myosin dephosphorylation and relaxation (Torphy *et al.*, 1986; Lincoln *et al.*, 1990; De Lanerolle and Paul, 1991).

In our results, adenosine produced $[Ca^{2+}]i$ decrease in a single uterine artery cell. Theoretically, the following possibilities might exist for the effect of adenosine on calcium: it may be indirect by (i) activation of cyclic nucleotide-dependent protein kinase, resulting in the lowering of $[Ca^{2+}]i$ concentration; (ii) decreasing the apparent affinity for calcium of an intracellular calcium receptor or site responsible for the release of transmitter; (iii) increasing the intracellular organelles' calcium sequestration; or (iv) by promoting calcium efflux. Otherwise, the effect may be direct by inhibiting the voltage-dependent calcium channel. Since the uterine artery is very important in human reproduction systems, mediation via multiple and complex pathways offers a major advantage: e.g. failure of the relaxant response is less likely than if only one mechanism existed, and the potential for augmentation of the relaxation is greatly enhanced. In the present study of adenosine acting on human uterine arteries, we have demonstrated that adenosine relaxed human uterine arteries by the A2 receptor, which is endothelium-independent. Incidentally, we have also presented the phenomenon that adenosine decreases $[Ca^{2+}]i$ in a single human uterine artery cell. Further investigations

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are needed to reveal other mechanisms involving human uterine artery and adenosine.

References

Bodelsson, G. and Stjernquist, M. (1992) Smooth muscle dilatation in the human uterine artery induced by substance P, vasoactive intestinal polypeptide, calcitonin gene-related peptide and atrial natriuretic peptide: relation to endothelium-derived relaxing substance. *Hum. Reprod.*, **7**, 1185–1188.

Bodelsson, M., Arneklo-Nobin, B. and Tornebrandt, K. (1989) Cooling augments contractile response to 5-hydroxytryptamine via an endothelium-dependent mechanism. *Blood Vessels*, **26**, 347–359.

Chiang, P.H., Wu, S.N., Tsai, E.M., Wu, C.C., Shen, M.R. *et al.* (1994) Adenosine modulation of neurotransmission in penile erection. *Br. J. Clin. Pharmacol.*, **38**, 357–362.

Collis, M.G. (1983) Evidence for an A_1 adenosine receptor in the guinea-pig atrium. *Br. J. Pharmacol.*, **78**, 207–212.

Daly, J.W. (1982) Adenosine receptors: targets for future drugs. *J. Med. Chem.*, **25**, 197–207.

Daly, J.W., Padget, W., Shamin, M.T., Butts-Lamb, P. and Waters, J. (1985) 1,3-Dialkyl-8-(p-sulfophenyl) xanthines: potent water-soluble antagonists for A1 and A2-adenosine receptors. *J. Med. Chem.*, **28**, 487–492.

Davies, E., Westphal, S., Gillespie, J.I. and Dunlop,W. (1991) Isolation and characterization of single vascular smooth muscle cell derived from human uterine arteries. *J. Physiol.*, **435**, 108.

De Lanerolle, P. and Paul, R.J. (1991) Myosin phosphorylation/ dephosphorylation and regulation of airway smooth muscle contractility. *Am. J. Physiol.*, **261**, L1–L14.

De Mey, J.G. and Vanhoutte, P.M. (1981) Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol. (Lond.)*, **316**, 347–355.

Feletou, M. and Vanhoutte, P.M. (1986) Endothelium-derived factor(s) and membrane potential of vascular smooth muscle. *Blood Vessels*, **23**, 68.

Furchgott, R.F. and Zawadzki, J.V. (1980) ATP relaxes rabbit aortic smooth muscle by both an indirect action via endothelial cells and a direct action (Abstr.) *Pharmacologist*, **22**, 271.

Furchgott, R.F. (1983) Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **35**, 557–573.

Gordon, J.L. and Martin, W. (1983) Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of Rb efflux from isolated endothelial cells. *Br. J. Pharmacol.*, **79**, 531–541.

Gustafsson, L.E., Wiklund, N.P., Lundin, J. and Hedqvist, P. (1985) Characterization of pre- and post-junctional adenosine receptors in guinea-pig ileum. *Acta Physiol. Scand.*, **123**, 195–203.

Kim, N., Azadzoi, K.M., Goldstein, I. and Saenz de Tejada, I. (1991) A nitric oxide-like factor mediates nonadrenergic-noncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle. *J. Clin. Invest.*, **88**, 112–118.

Lincoln, T.M., Cornwell, T.L. and Taylor, A.E. (1990) cGMP-dependent protein kinase mediates the reduction of Ca^{2+} by cAMP in vascular smooth muscle cells. *Am. J. Physiol.*, **258**, C399–C407.

Linden, J. (1991) Structure and function of A_1 adenosine receptors. *FASEB J.*, **5**, 2668–2676.

Londos, C., Cooper, D.M.F. and Wolff, J. (1980) Subclasses of external adenosine receptors. *Proc. Natl. Acad. Sci. USA*, **77**, 2551–2554.

Mikkelsen, E.M., Costa, G. and Nyborg, N.C.B. (1988) Influence of endothelium on the response to calcium agonists, calcium, potassium and noradrenaline in rat aorta. *Pharmacol. Toxicol.*, **62**, 22–28.

Moncada, S., Gryglewski, R., Bunting, S. and Vane, J.R. (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*, **263**, 663–665.

Ottesen, B., Wagner, G., Virag, R. and Fahrenkrug, J. (1984) Penile erection: possible role for vasoactive intestinal polypeptide as a neurotransmitter. *Br. Med. J.*, **288**, 9–11.

Owman, Ch. and Stjernquist,M. (1988) Origin, distribution, and functional aspects of aminergic and peptidergic nerves in the male and female reproductive tracts. In Bjorklund, A., Hokfelt, T. and Owman, Ch. (eds), *Handbook of Chemical Neuroanatomy, Vol. 6: The Peripheral Nervous System.* Elsevier, Amsterdam, pp. 445–544.

Palmer, R.M.J., Ferrige, A.G. and Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium derived relaxing factor. *Nature*, **327**, 524–526.

Rajfer, J., Aronson, W.J., Buch, P.A., Dorey, F.J. and Ignarro, L.J. (1992) Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *N. Engl. J. Med.*, **326**, 90–94.

Resnik, R., Killan, A.P., Barton, M.D., Battaglia, F.C., Makowski, E.L. and Meschia, G. (1976) The effect of various vasoactive compounds upon the uterine vascular bed. *Am. J. Obstet. Gynecol.*, **125**, 201–206.

Said, S.I. (1992) Nitric oxide and vasoactive intestinal peptide: cotransmitters of smooth muscle relaxation. *News Physiol. Sci.*, **7**, 181–183.

Stjernquist, M. and Owman, Ch.(1985) Cholinergic and adrenergic neuronal control of smooth muscle function in the non-pregnant rat uterine cervix. *Acta Physiol. Scand.*, **124**, 429–436.

Torphy, T.J., Fine, C.F., Burman, M., Barnette, M.S. and Ormsbee, H.S. (1986) Lower esophageal sphincter relaxation is associated with increased cyclic nucleotide content. *Am. J. Physiol.*, **251**, G786–G793.

Van Calker, D., Muller, M. and Hamprecht, B. (1979) Adenosine regulates, via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem.*, **33**, 99–105.

Wihlund, N.P., Samuelson, U.E. and Brundin, J. (1986) Adenosine modulation of adrenergic neurotransmission in the human Fallopian tube. *Eur. J. Pharmacol.*, **123**, 11–18.

Wihlund, N.P., Samuelson, U.E. and Hammarstrom, M. (1991) Adenosine modulation of neuroeffector transmission in guinea-pig uterine smooth muscle. *Acta Physiol. Scand.*, **143**, 33–43.

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