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Impaired cyclic nucleotide-mediated vasorelaxation may contribute to closure of the human umbilical artery after birth

S. Renowden, *D.H. Edwards & [1]T.M. Griffith

Departments of Diagnostic Radiology and *Cardiology, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN

1 The mechanical and biochemical effects of agents that relax vascular smooth muscle either through elevation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) or adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels were compared in isolated ring preparations of human umbilical artery and rat aorta. Tone was established by preconstriction with 5-hydroxytryptamine.

2 The endothelium-dependent vasodilator calcium ionophore (A23187) (which stimulates endothelium-derived relaxing factor [EDRF] release and thus acts through soluble guanylyl cyclase), sodium nitroprusside (which stimulates soluble guanylyl cyclase directly), and atrial natriuretic peptide (which stimulates particulate guanylyl cyclase) relaxed rat aorta but not human umbilical artery.

3 Sodium nitroprusside, 10 μM, increased cyclic GMP levels from 10 to 390 pmol mg^{-1} protein at 2 min in rat aorta, as compared with a slower, relatively attenuated rise from 5 to 116 pmol mg^{-1} protein after 15 min in human umbilical artery. The rise in cyclic GMP in the umbilical artery was not significantly augmented by the cyclic GMP phosphodiesterase inhibitor, MB22948. Atrial natriuretic peptide increased cyclic GMP levels in rat aorta but not in human umbilical artery.

4 Forskolin, 10 μM, which stimulates both soluble and particulate adenylyl cyclase, maximally relaxed rat aorta and increased cyclic AMP levels from 15 to 379 pmol mg^{-1}protein at 15 min, but did not significantly relax or increase cyclic AMP levels in human umbilical artery. After preincubation with the cyclic nucleotide phosphodiesterase inhibitor, IBMX, 10 μM forskolin increased cyclic AMP levels to 1365 pmol mg^{-1} protein at 30 min in human umbilical arteries, but these high levels were not accompanied by mechanical relaxation.

5 8-Bromo-cyclic GMP and 8-bromo-cyclic AMP which are lipophilic analogues of cyclic GMP and cyclic AMP, both maximally relaxed the rat aorta at a concentration of 10 μM, but did not significantly relax the human umbilical artery.

6 The findings indicate that elevated cyclic nucleotide levels are not associated with mechanical relaxation of the post-partum human umbilical artery, as in other vessels such as rat aorta. This impaired response to cyclic nucleotides may contribute to closure of the umbilical artery after birth.

Keywords: Human umbilical artery; rat aorta; vasorelaxation; cyclic GMP; cyclic AMP

Introduction

The umbilical artery constricts rapidly after birth, thus preventing foetal blood loss (Eltherington *et al.*, 1968; Bjoro & Stray-Pedersen, 1986). The mechanisms underlying the spontaneous closure of this vessel, however, remain obscure. Neural effects are unlikely to play a role as umbilical vessels are not innervated (Spivak, 1943; Reilly & Russell, 1977). One possible stimulus to contraction may be the decrease in cord temperature from body to room temperature which occurs at delivery. Boura and coworkers (1979) attributed spasm of the umbilical vein on cooling to prostaglandin synthesis as the effect was inhibited by indomethacin. The tone of umbilical arteries is also dependent on the ambient oxygen tension. Thus, Eltherington and coworkers (1968) demonstrated that elevation of the Po_2 of the umbilical artery from the normal *in vivo* value of 15 mmHg (Bartels *et al.*, 1962) to that of air (120 mmHg) induced vasoconstriction, which was non-specifically potentiated by bradykinin, 5-hydroxytryptamine (5-HT) and adrenaline. Thromboxane A_2, prostaglandins PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$, bradykinin and 5-HT have all been detected in human umbilical cord blood (Karim, 1967, Tuvemo *et al.*, 1976; Melmon *et al.*, 1968; Altura *et al.*, 1972, Bjoro & Stray-Pedersen, 1986). Whether these agents actually contribute to closure of the umbilical artery post partum is, however, unknown.

In many artery types elevated cyclic nucleotide levels (guanosine 3':5'-cyclic monophosphate [cyclic GMP] and adenosine 3':5'-cyclic monophosphate [cyclic AMP]) are associated with relaxation of vascular smooth muscle (Bhalla *et al.*, 1978; Ignarro *et al.*, 1981; Lincoln, 1983; Lincoln & Fisher-Simpson, 1983; Sano *et al.*, 1983; Reid *et al.*, 1988). We have accordingly investigated whether impairment of relaxation, rather than active constriction, might be involved in the closure of the umbilical artery. Responses in the human umbilical artery were compared with those of the rat aorta.

Methods

Human umbilical cords were obtained from normal, spontaneous, full-term transvaginal deliveries, ligated at the placental and foetal ends, and within 15 min of delivery, placed in Holmans buffer at room temperature (composition, mM:NaCl 120, KCl 5, $CaCl_2$ 2.5, $NaHCO_3$ 25, NaH_2PO_4 1.3, glucose 11, sucrose 10, pH 7.2–7.4) (Griffith *et al.*, 1985; Collins *et al.*, 1986). The umbilical arteries were carefully dissected away from the cord which was bathed continuously in Holman's buffer, and were prepared either for mechanical studies or measurement of cyclic nucleotide levels.

For mechanical studies, 3 mm rings of umbilical artery were mounted in an organ bath containing Holmans' buffer

[1] Author for correspondence.

at 37°C and changes in isometric tension measured with a force transducer. The preparations were continuously oxygenated with a mixture of 95% O_2/5% CO_2 as umbilical arteries are known to be more reactive at high oxygen concentrations than at their normal physiological Po_2 of 15 mmHg (Eltherington *et al.*, 1968; Silva de Sa, 1981). All arteries were placed under a resting tension of 1.5 g and equilibrated for 1–2 h, with frequent adjustment of baseline tension during stress relaxation. To construct concentration-response curves to vasodilators the vessels were preconstricted (by a further 1.0–4.0 g) with 5-HT at a concentration that induced maximal constriction (10 μM). In separate experiments, relaxation was also studied after constriction with submaximal concentrations of 5-HT (0.01, 0.1 and 1 μM). All experiments were performed within 4 h of delivery.

Male Wistar rats (250–300 g) were killed by a blow to the head and the aorta quickly removed and placed in Holmans' buffer. Adherent tissue was dissected away, and 3 mm rings were suspended in an organ bath as described above under a resting tension of 1.0 g. After equilibration for 1–2 h, they were preconstricted (by a further 0.5–1.0 g) with a maximal concentration of 5-HT (also 10 μM) in order to study relaxant responses.

No attempt was made to remove the endothelium from the arterial segments under study. The presence of an intact endothelium in both the umbilical artery and the rat aortic rings was confirmed by binocular microscopy after staining the luminal surface of the rings with 1% silver nitrate for 2 min, followed by a solution containing 3% cobalt bromide and 3% ammonium bromide for a further 2 min.

To determine cyclic nucleotide levels, 5 mm rings of endothelium-intact human umbilical artery or rat aorta were incubated in oxygenated (95% O_2/5% CO_2) Holmans' solution at 37°C for 2 h in the absence of 5-HT, before addition of stimulants of guanylyl or adenylyl cyclase. Additional experiments were performed with the umbilical artery to study the effects of preincubation with 10 μM MB22948 or 1 mM IBMX. The rings were subsequently immersed in liquid nitrogen and stored at − 80°C for later determination of cyclic GMP and cyclic AMP levels. The frozen arterial segments were homogenized in ice cold 6% (v/v) trichloroacetic acid, the homogenate centrifuged and the supernatant then treated with 0.5 M tri-n-octylamine dissolved in 1,1,2 trichloro-trifluoroethane, to remove the trichloroacetic acid. The samples were then centrifuged and the cyclic GMP and cyclic AMP content of the neutralised aqueous phase determined by radioimmunoassay (New England Nuclear). The residue remaining after centrifugation was treated with 1 mM NaOH and its protein content determined by the method of Bradford (1976).

Statistics

Results are expressed as mean ± s.e.mean. Comparison of data sets was performed with the unpaired Student's *t* test, $P<0.05$ being taken as significant.

Drugs

Sodium nitroprusside, calcium ionophore (A23187), 5-hydroxytryptamine, forskolin, 8-bromo-cyclic GMP, 8-bromo-cyclic AMP, 3-isobutyl-1-methylxanthine (IBMX) and atrial natriuretic peptide (ANP) were obtained from Sigma Chemical Co., Poole, Dorset. 2-0-propoxyphenyl-8-azapurin-6-one (MB22948) was a gift from Rhone-Poulenc.

Results

Preconstriction by 5-hydroxytryptamine

5-HT induced concentration-dependent constriction of the human umbilical artery ($n = 15$) and rat aorta ($n = 6$) with half-maximal concentrations (EC_{50}) of 0.007 ± 0.001 μM and 1.12 ± 0.23 μM respectively. These values were significantly different from each other ($P<0.001$), so that 5-HT was ca. 150 fold more potent in the umbilical artery than the rat aorta (Figures 1, 2). Vasoconstriction by 5-HT was completely reversible in both artery types on washout. In approximately 50% of the umbilical artery preparations, there was a slight decline in tone (pharmacological 'fade') as a function of time (up to 10% by ca. 30 min), which was independent of the presence of a vasodilator (Figure 2). Such 'fade' can account for the minor degree of relaxation of the umbilical artery preparations observed following challenge with potential relaxants and described in the following sections.

Mechanical responses

Agents acting through cyclic GMP

Endothelium-dependent relaxation in human umbilical arteries Preparations with intact endothelium and constricted by 10 μM 5-HT did not relax in response to the calcium ionophore A23187 (0.001 to 1 μM) ($n = 6$, data not shown).

Sodium nitroprusside Sodium nitroprusside (to 10 μM) did not relax human umbilical arteries constricted by 10 μM 5-HT to any significant extent (3.0 ± 1.5%, $n = 6$), and the response to 10 μM sodium nitroprusside was not affected by preincubation with 10 μM MB22948 for 20 min (10.0 ± 6.3%, $n = 6$) (Figures 2, 3). In contrast, sodium nitroprusside induced concentration-dependent relaxation of rat aortic preparations constricted by 10 μM 5-HT, which was 95.0 ± 2.1% of the established tone at the highest concentration employed (10 μM) ($n = 6$, Figure 3). This relaxation began within 30 s and was maximal within 4 min with an EC_{50} value of 0.33 ± 0.20 μM.

Atrial natriuretic peptide (ANP) Human umbilical arteries preconstricted by 10 μM 5-HT did not relax significantly in response to ANP, there being only a 9.0 ± 5.6% ($n = 6$) fall in tension after 15–20 min incubation with the highest concentration studied (1 μM) (Figure 3). In contrast, the preconstricted rat aorta relaxed by 103.8 ± 10.4% of established tone with 1 μM ANP ($n = 6$, Figure 3). This relaxation began within 1 min and was maximal by 10–15 min with an EC_{50} value of 1.8 ± 0.5 nM.

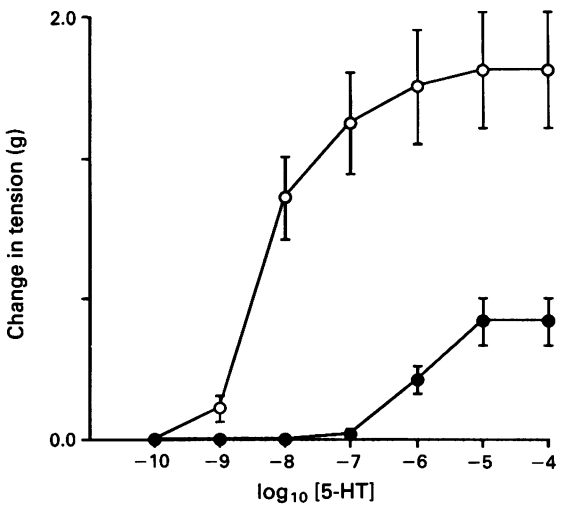
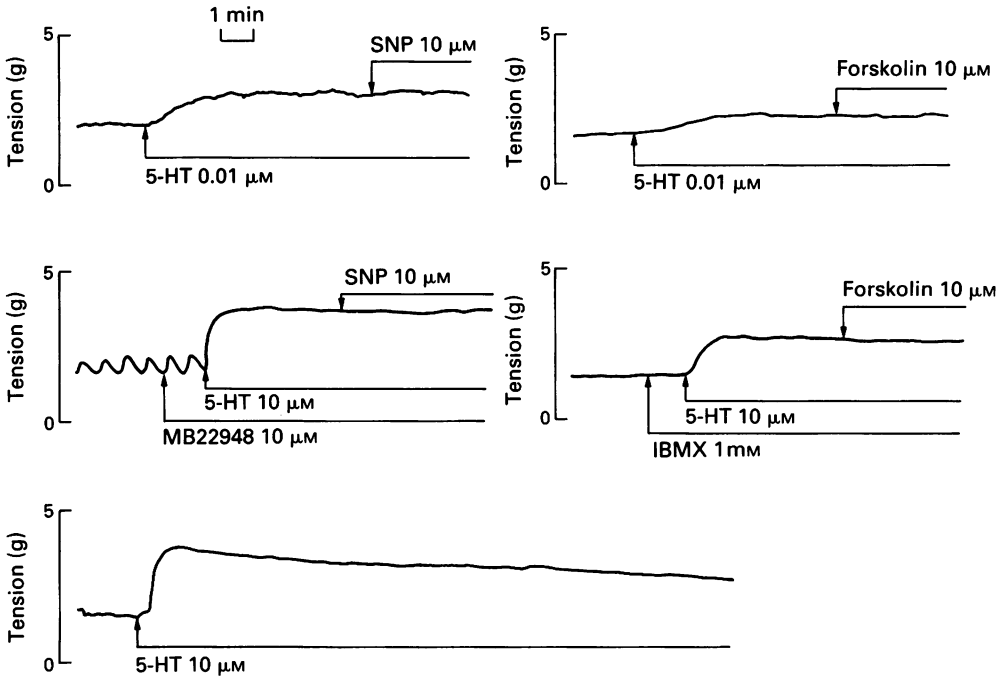


Figure 1 Concentration-response curves to 5-hydroxytryptamine (5-HT) plotting increases in tension (g) from baseline in human umbilical artery (○) and rat aorta (●). EC_{50} concentrations were 0.007 ± 0.001 and 1.12 ± 0.23 μM respectively, and were significantly different from each other ($P<0.001$). Maximal constriction to 5-HT was obtained at a concentration of 10 μM in both artery types.



Figue 2 Representative human umbilical artery traces illustrating lack of relaxation to 10 μM sodium nitroprusside (SNP) and 10 μM forskolin, in both the absence (upper) and the presence (middle) of phosphodiesterase inhibitors (10 μM MB22948 and 1 mM isobutylmethylxanthine (IMBX), respectively). This impaired response cannot be attributed to functional antagonism between 5-hydroxytryptamine (5-HT) and cyclic nucleotides as relaxation could not be elicited even when tone was established with low (i.e. submaximal) concentrations of 5-HT (0.01 μM illustrated here). In approximately 50% of umbilical artery preparations there was a slow decline in tension which was independent of the presence of a vasodilator (lower). Note that spontaneous oscillations in tone were observed in some preparations.

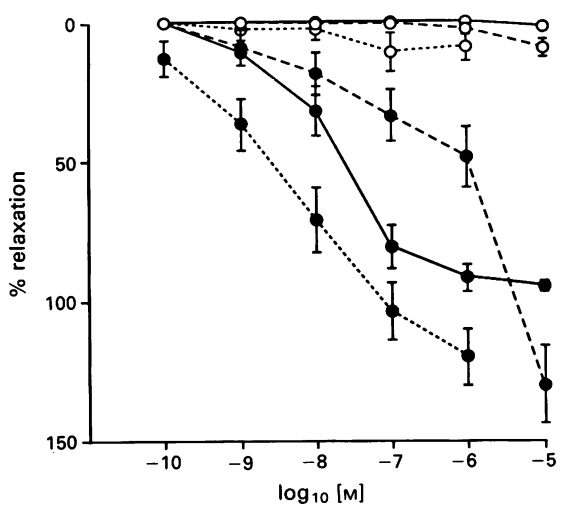


Figure 3 Effects of (a) sodium nitroprusside (continuous lines), (b) atrial natriuretic peptide (dotted lines) and (c) 8-bromo-cyclic GMP (dashed lines) in human umbilical artery (○) and rat aorta (●).

8-Bromo-cyclic GMP Human umbilical arteries preconstricted by 10 μM 5-HT were unresponsive to 8-bromo-cyclic GMP (to 10 μM), the fall in tension after 15–20 min incubation being 9.7 ± 3.2% ($n = 6$, Figure 3). In contrast, the preconstricted rat aorta relaxed by 130.3 ± 13.7% of established tone in response to 10 μM 8-bromo-cyclic GMP ($n = 6$, Figure 3). This relaxation began within 1 min and was maximal within 15–20 min with an EC_{50} value of 1.17 ± 0.25 μM.

Agents acting through cyclic AMP

Forskolin Forskolin (to 10 μM) caused no significant relaxation of human umbilical arteries preconstricted by 10 μM 5-HT (Figure 4), and the small decline in tension at 15–20 min (10.6 ± 2.5%, $n = 8$) was not significantly enhanced by preincubation with 1 mM IBMX (13.7 ± 7.4%, $n = 6$). In contrast, forskolin induced concentration-dependent relaxation of the preconstricted rat aorta which was 117.6 ± 9.7% ($n = 6$) of established tone at the highest concentration studied (10 μM) (Figure 4). This relaxation began within 1 min and was maximal within 15–20 min with an EC_{50} value of 0.29 ± 0.12 μM.

8-Bromo-cyclic AMP Human umbilical arteries preconstricted by 10 μM 5-HT were unresponsive to 8-bromo-cyclic

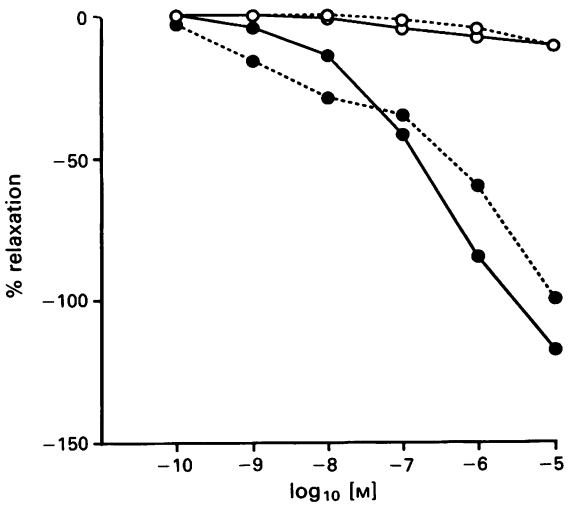


Figure 4 Effects of forskolin (continuous lines) and 8-bromo-cyclic AMP (dotted lines) in human umbilical artery (○) and rat aorta (●).

AMP (to 10 μM), the fall in tension after 15–20 min incubation being 11.2 ± 2.5% ($n = 6$) (Figure 4). In contrast, the preconstricted rat aorta relaxed by 100.0 ± 2.0% with 10 μM 8-bromo-cyclic AMP ($n = 6$, Figure 4). This relaxation began within 1 min and was maximal at 15–20 min with an EC_{50} value of 0.85 ± 0.47 μM.

Concentration of 5-HT used to establish tone

In the above experiments, 10 μM 5-HT was used to preconstrict both the umbilical artery and rat aortic preparations. This concentration induced the maximum attainable constrictor response to 5-HT in both artery types (Figure 1). In further experiments with the umbilical artery, tone was established with lower concentrations of 5-HT (0.01, 0.10 and 1 μM) to determine whether the lack of cyclic nucleotide-mediated relaxation was attributable to functional antagonism by high concentrations of 5-HT. As in the case of 10 μM 5-HT, however, sodium nitroprusside (to 10 μM) and forskolin (to 10 μM) failed to elicit mechanical relaxation at these lower concentrations of 5-HT, either in the absence or the presence of 10 μM MB22948 or 1 mM IBMX as appropriate ($n = 5$ in each case, Figure 2).

Cyclic nucleotide levels

The effects of vasodilator agents on tissue cyclic GMP and AMP levels were assessed in the absence of a constrictor agonist.

Effects of sodium nitroprusside, atrial natriuretic peptide and forskolin Basal levels of cyclic GMP were statistically similar in the umbilical artery (4.6 ± 1.4 pmol mg^{-1} protein, $n = 6$) and rat aorta (9.7 ± 2.3 pmol mg^{-1} protein, $n = 6$). In the umbilical artery, 10 μM sodium nitroprusside slowly increased cyclic GMP levels to 115.6 ± 46.4 pmol mg^{-1} protein by 15 min, after which they declined but still remained above control levels for up to 60 min (Figure 5). After preincubation with 10 μM MB22948, 10 μM sodium nitroprusside increased cyclic GMP levels to a peak of 158.8 ± 69.9 pmol mg^{-1} protein after 20 min. This was not significantly greater than the maximum induced with 10 μM sodium nitroprusside alone, but cyclic GMP levels remained elevated at 89.2 ± 25.5 pmol mg^{-1} protein after 60 min, a value ca. four-fold greater than that 60 min after administration of 10 μM sodium nitroprusside alone (Figure 5). In rat aorta, by contrast, 10 μM sodium nitroprusside stimulated a substantially greater, and considerably more rapid increase in cyclic GMP levels, with a peak of 388.3 ± 45.6 pmol mg^{-1} protein at 2 min, followed by a rapid subsequent decline (Figure 6).

ANP 1 μM did not elevate cyclic GMP levels in human umbilical artery (Figure 5). In contrast, in rat aortic cyclic GMP levels peaked after 5 min at 157.7 ± 84.7 pmol mg^{-1} protein and quickly declined thereafter (Figure 6).

Forskolin, 10 μM, had no significant effect on cylic GMP levels in either umbilical artery or rat aorta (Figures, 5, 6).

Effects of forskolin (10 μM) and sodium nitroprusside (10 μM) on cyclic AMP levels Basal cyclic AMP levels did not differ significantly between umbilical artery (9.93 ± 3.87 pmol mg^{-1} protein, $n = 6$) and rat aorta (15.1 ± 4.9 pmol mg^{-1} protein, $n = 6$). In umbilical artery, forskolin alone did not alter cyclic AMP levels over a period of 0–50 min (Figure 7), but after preincubation with 1 mM IBMX it increased cyclic AMP levels at 30 min to 1365.0 ± 427.8 pmol mg^{-1} protein after which it declined (Figure 7).

In rat aorta, forskolin increased cyclic AMP levels to a peak of 379.0 ± 62.6 pmol mg^{-1} protein at 15 min which then declined (Figure 8).

Sodium nitroprusside did not signficantly alter cyclic AMP levels in either umbilical artery or rat aorta (Figures 7 and 8).

Discussion

We have investigated the hypothesis that impaired mechanisms of vasodilatation contribute to closure of the human umbilical artery after birth, by comparing the mechanical and biochemical responses of the human umbilical artery and rat aorta to agents which normally mediate vascular smooth muscle relaxation through elevation of cyclic nucleotide levels. To study relaxation, tone was established with 5-hydroxytryptamine (5-HT) which induced reversible, concentration-dependent constriction in both vessel types. The umbilical artery preparations were found to be ca. 150 fold more sensitive to 5-HT than the rat aorta.

To elevate cyclic GMP levels, we used the calcium ionophore A23187, which has previously been shown to be a receptor-independent stimulant of EDRF release from the human umbilical artery (Van de Voorde *et al.*, 1987; Chaudhuri *et al.*, 1991); sodium nitroprusside, a direct stimulant of soluble guanylyl cyclase (Ignarro *et al.*, 1981); and atrial

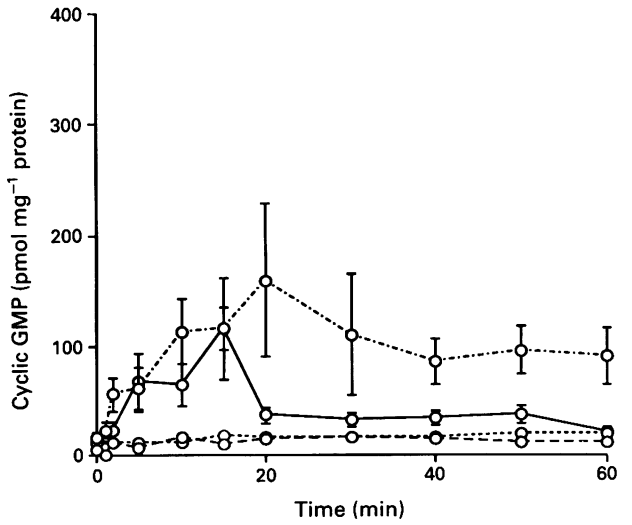


Figure 5 Time course of cyclic GMP levels in human umbilical artery following administration of 10 μM sodium nitroprusside (—), 10 μM atrial natriuretic peptide (– – –), 10 μM forskolin (- - -) and 10 μM sodium nitroprusside in the presence of 10 μM MB22948 (–·–·–).

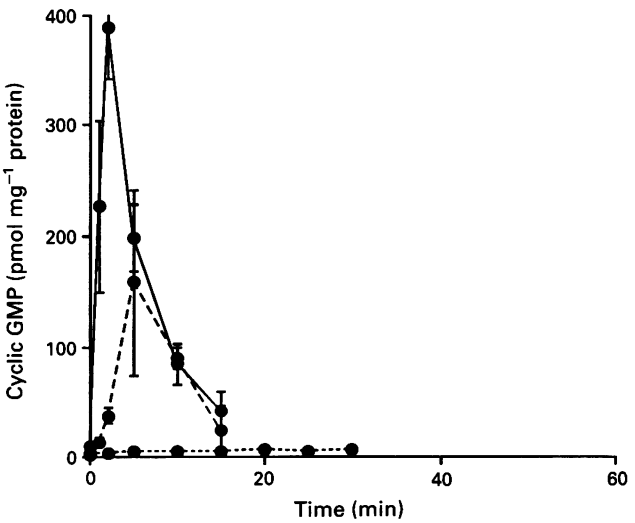


Figure 6 Time course of cyclic GMP levels in rat aorta following administration of 10 μM sodium nitroprusside (continuous lines), 10 μM forskolin (dotted lines) and 10 μM atrial natriuretic peptide (dashed lines).

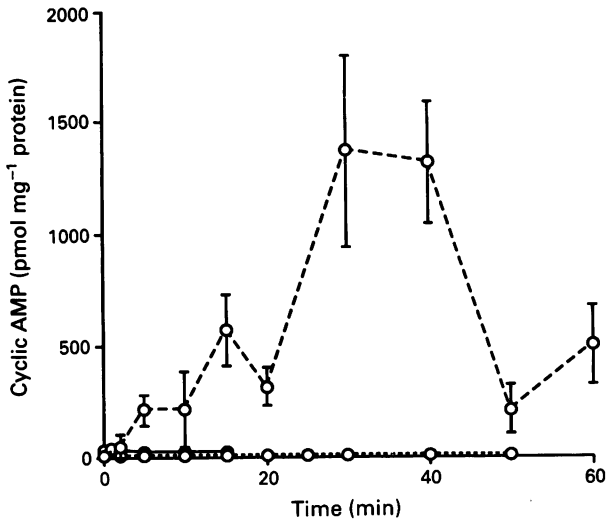


Figure 7 Time course of cyclic AMP levels in human umbilical artery following administration of 10 μM forskolin (dotted lines), 10 μM sodium nitroprusside (continuous lines) and 10 μM forskolin in the presence of 1 mM isobutyl methylxanthine (dashed lines).

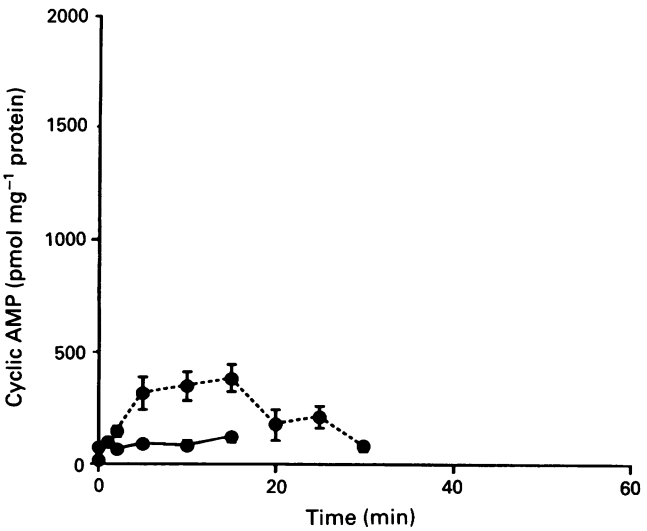


Figure 8 Time course of cyclic AMP levels in rat aorta following administration of 10 μM forskolin (dotted lines) and 10 μM sodium nitroprusside (continuous lines).

natriuretic peptide, a stimulant of particulate guanylyl (Winquist *et al.*, 1984). To elevate cyclic AMP levels, we used forskolin, a reversible stimulator of the catalytic subunit of both soluble and particulate adenylyl cyclase (Lincoln & Fisher-Simpson, 1983). In marked contrast to the rat aorta and many other blood vessels (Sano *et al.*, 1983; Lincoln & Fisher-Simpson, 1983; Reid *et al.*, 1988), the human umbilical artery did not relax to any significant extent with either of these classes of vasodilators. The findings are consistent with a previous report which demonstrated that histamine fails to induce mechanical relaxation of preconstricted endothelium-intact human umbilical artery preparations, but is nevertheless able to stimulate EDRF release from them in cascade bioassay (Van de Voorde, 1987). More recently, the ability of EDRF to relax endothelium-denuded human umbilical arteries has been shown to be markedly attenuated when compared to the response of chorionic plate and pulmonary arteries (Chaudhuri *et al.*, 1991).

The apparent lack of normal relaxation could be a consequence of impaired cyclic nucleotide formation, more rapid hydrolysis of cyclic GMP and cyclic AMP by their respective phosphodiesterases, or decreased sensitivity of the intracellular mechanisms which bring about relaxation to cyclic nucleotides. Experiments were therefore performed to measure cyclic nucleotide levels both in the absence and in the presence of a relatively specific inhibitor of cyclic GMP phosphodiesterase (MB22948) and a non-specific inhibitor of cyclic nucleotide phosphodiesterase (IBMX) (Weishaar, 1987). The mechanical effects of permeable lipophilic analogues of cyclic GMP and cyclic AMP were also examined. It has previously been shown that the sensitivity of the rat aorta to acetylcholine, a vessel in which it is an endothelium-dependent vasodilator (in contrast to the situation in the human umbilical artery; Van de Voorde *et al.*, 1987), is inversely related to the concentration of agonist used to establish tone (Dainty *et al.*, 1990). Functional antagonism between high concentrations of 5-HT and cyclic nucleotide-mediated mechanisms of vasorelaxation was excluded, however, by the demonstration that both classes of vasodilator also failed to elicit relaxation when the umbilical arteries were preconstricted by submaximal concentrations of 5-HT (0.01, 0.1 and 1 μM).

Although sodium nitroprusside did not relax the human umbilical artery, it did elevate cyclic GMP levels, albeit more slowly and to a smaller extent than in rat aorta and other artery types (Lincoln & Fisher-Simpson, 1983; Tanaka *et al.*, 1989). Previous workers have shown that nitrovasodilators (including EDRF) can elevate cyclic GMP levels in the human umbilical artery, but did not study the time course of this response (Chaudhuri *et al.*, 1991). ANP caused no rise in cyclic GMP levels in human umbilical artery. These observations suggest an attenuated soluble guanylyl cyclase response and an absence of particulate guanylyl cyclase response in the human umbilical artery. The latter finding could be explained by an absence of either the particulate enzyme or of specific ANP receptors. ANP has been reported not to act as a vasodilator in other vessels, including small arteries in the rabbit and some veins (Faison *et al.*, 1985).

MB22948, a relatively specific inhibitor of cyclic GMP phosphodiesterase (Weishaar, 1987), did not increase peak levels of cyclic GMP attained in response to sodium nitroprusside in the human umbilical artery, but significantly prolonged their elevation. This indicates that cyclic GMP phosphodiesterase is active in the human umbilical artery, and suggests that the impaired mechanical response to nitrovasodilators probably does not involve enhanced hydrolysis of cyclic GMP. Consistently, 8-bromo-cyclic GMP, a lipophilic analogue of cyclic GMP which mediates relaxation in the micromolar range in rat tail artery (Cheung & MacKay, 1985), rat aorta (Lincoln, 1983) and rabbit aorta (Schultz *et al.*, 1979; Napoli *et al.*, 1980), also failed to elicit significant relaxation of the human umbilical artery. These findings indicate that the responsiveness of the post-partum human umbilical artery to elevated levels of intracellular cyclic GMP is impaired.

Forskolin activates both soluble and particulate adenylyl cyclase (Sano *et al.*, 1983), but elevated cyclic AMP levels in the human umbilical artery only in the presence of the cyclic nucleotide phosphodiesterase inhibitor, IBMX. This suggests that there may be abnormally rapid hydrolysis of cyclic AMP in this vessel. Other mechanisms are also likely to be involved in the failure of relaxation, however, as even these high levels of cyclic AMP failed to affect tone, and there was a corresponding lack of response to the lipophilic cyclic AMP analogue, 8-bromo-cyclic AMP, which relaxes other artery types in the micromolar range (Ousterhout & Sperelakis, 1987). The findings suggest that there is generalised impairment of the intracellular response to elevated cyclic nucleotide levels in the post-partum human umbilical artery. In conclusion, we suggest that impaired mechanisms of relaxation may contribute to the closure of the human umbilical artery after birth and that this is associated both with par-

tially attenuated cyclic nucleotide responses to conventional stimulants and with impaired relaxation in response to elevated cyclic nucleotide levels. It remains to be established at what cellular level failure of relaxation occurs in view of the fact that cyclic GMP and cyclic AMP exert multiple effects on Ca^{2+} homeostasis and protein phosphorylation (Scheid & Pay, 1984; Suematsu *et al.*, 1984; Popescu *et al.*, 1985; Collins *et al.*, 1986; Rapoport, 1986; Twort & Van Breeman, 1988; Cornwall & Lincoln, 1989; Hirata *et al.*, 1990).

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