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Endothelium-dependent and endothelium-independent vasodilatation of the hepatic artery of the rabbit

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1 The isolated hepatic artery of the rabbit contracted to exogenously applied noradrenaline (NA). There was no significant difference in the maximal contraction or the EC_{50} value in vessels where the endothelium was present and in endothelium-denuded preparations.

2 Acetylcholine (ACh) induced a vasodilatation of vessels preconstricted with NA which was entirely dependent on the endothelium.

3 Adenosine 5'-triphosphate (ATP), 2-methylthio ATP, adenosine and sodium nitroprusside induced concentration-dependent, sustained relaxations of vessels in which tone had been induced with NA. The relaxation responses were not reduced after removal of the endothelium. 8-Phenyltheophylline antagonized the relaxation response produced by adenosine, but not that due to ATP at lower concentrations. The maximum response to ATP was reduced in the presence of 8-phenyltheophylline.

4 α,β-Methylene ATP produced further contraction of vessels preconstricted with NA in both endothelium-denuded preparations and in vessels where the endothelium remained intact.

5 Immunohistochemical analysis was used to show the presence of nerve fibres containing substance P (SP), calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP) in the hepatic artery. Application of SP induced a concentration-dependent relaxation which was entirely dependent on the presence of an intact endothelium. CGRP and VIP, however, elicited concentration-dependent relaxations which were independent of the endothelium.

7 It is concluded that in the rabbit hepatic artery, responses to ACh are dependent on the presence of intact endothelium. P_1-, P_{2x}- and P_{2y}-purinoceptors, mediating relaxation to adenosine, vasoconstriction to ATP and vasodilatation to ATP respectively, are located on vascular smooth muscle. Furthermore, CGRP and VIP mediate a direct vasodilatation of smooth muscle both in the absence and the presence of the endothelium, whereas SP produces a relaxation via receptors located on the endothelium.

Keywords: Hepatic artery; endothelium; vasodilatation

Introduction

The involvement of sympathetic noradrenaline (NA)-containing nerves in the control of vascular smooth muscle is well known (Bevan *et al.*, 1980). During recent years, the results from several studies suggest that perivascular nerves of man and other mammals also contain regulatory substances other than NA; these include purines and peptides (Lundberg & Hökfelt, 1983; Ganten *et al.*, 1984; Burnstock, 1988). Purine nucleosides and nucleotides have been shown to have widespread vascular actions (Drury & Szent-Györgyi, 1929; Burnstock & Brown, 1981). In 1978, Burnstock proposed that the receptors mediating the responses to purines should be categorized as P_1- and P_2-purinoceptors, with selectivity for adenosine and adenosine 5'-triphosphate (ATP) respectively. ATP can be released as a cotransmitter with NA from sympathetic nerves and acts on a P_{2x}-purinoceptor subtype on vascular smooth muscle to produce a contraction (Burnstock & Kennedy, 1985); this has been demonstrated in both the hepatic and the saphenous artery of the rabbit (Burnstock & Warland, 1987; Brizzolara & Burnstock, 1990). In addition, since the discovery that relaxation to acetylcholine (ACh) in a number of vessels is dependent on the presence of an intact endothelium (Furchgott & Zawadzki, 1980; Furchgott *et al.*, 1981; De Mey & Vanhoutte, 1982; Vanhoutte & Rimele 1983; Peach *et al.*, 1985), it has been shown that, in all vessels, except the rabbit portal vein (Kennedy & Burnstock, 1985a) and the rabbit mesenteric artery (Mathieson & Burnstock, 1985), ATP induces vasodilatation wholly or partly via P_{2y}-purinoceptors located on the endothelium (De Mey & Vanhoutte, 1980; 1982; Cocks & Angus, 1983; Furchgott, 1983; Kennedy *et al.*, 1985; Burnstock & Kennedy, 1985; Liu *et al.*, 1989). In contrast, adenosine has been shown to mediate a vasodilatation via P_1-purinoceptors that is dependent on the presence of an intact endothelium in only a few cases (Gordon & Martin, 1983; Kennedy & Burnstock, 1985b).

Endothelium-independent vasodilatation is produced by several peptides including calcitonin gene-related peptide (CGRP) (Brain *et al.*, 1985; Hanko *et al.*, 1985; Uddman *et al.*, 1986; Kawasaki *et al.*, 1988; Marshall & Craig, 1988), and vasoactive intestinal polypeptide (VIP) (Hand *et al.*, 1984; Lee *et al.*, 1984; Schoeffter & Stoclet, 1985; Varga *et al.*, 1986; Fazekas *et al.*, 1987). Other neuropeptides, including substance P (SP), vasopressin and angiotensin II, have been shown to be stored in and released from endothelial cells (Lincoln *et al.*, 1990). Furthermore, SP has been shown to produce a relaxation of the vasculature that is dependent on the presence of an intact endothelium (Mione *et al.*, 1990).

These experiments were designed to examine the role of the endothelium in the local control of the vascular tone of the rabbit isolated hepatic artery. The vascular location of P_1- and P_2-purinoceptors was also investigated by use of ATP, 2-methylthio ATP, α,β-methylene ATP and adenosine in the presence and absence of endothelium. The responses to ACh and sodium nitroprusside (which acts directly on the smooth muscle) (Murad *et al.*, 1979) were also investigated in the presence and absence of the endothelium and acted as controls of endothelial and smooth muscle cell integrity. Immunocytochemical staining was used to examine the presence of nerve fibres containing SP, CGRP and VIP (Costa *et al.*, 1980), while the relaxant action of these peptides was investigated by pharmacological methods.

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Methods

Tissue preparation

Male New Zealand White rabbits (2.3–3.3 kg) were killed by an overdose of pentobarbitone sodium (Sagatal), which was injected via the ear vein, and exsanguinated. The proper hepatic artery, which runs from the gastroduodenal artery to the porta hepatis, was cleaned of excess connective tissue and fat. Two 4 mm ring segments were cut and the endothelium of one of the rings was removed by pulling a braided silk suture through the lumen of the vessel. Each ring was then mounted horizontally under isometric conditions in a 5 ml organ bath by inserting a tungsten wire through the lumen of the vessel ring, which was anchored to a stationary support. Another wire, similarly inserted, was connected to a Grass FT03C force-displacement transducer. The responses were recorded on a Grass ink-writing polygraph. The preparations were placed under a resting tension of 0.75–1.0 g and allowed to equilibrate for 1.5–2 h in Bülbring-modified Krebs solution of the following composition (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, pH 7.2 (Bülbring, 1953). The solution was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Bovine serum albumin (0.005%) and bacitracin (30 mg l^{-1}) were added to the Krebs solution in order to prevent peptide degradation and adhesion to the surfaces of the glassware.

Pharmacology

A cumulative concentration-response curve for NA was established for each segment in order to find the maximum contractile response. The absence of endothelium was assessed by lack of relaxation to ACh (Furchgott & Zawadzki, 1980). Before tissues were exposed to any test drug, they were contracted to approximately 75% of maximal tension with NA. After contractions reached a plateau, ACh was administered. If tissues did not relax to ACh, the endothelium was considered removed. In some cases, rubbed and unrubbed preparations were opened longitudinally and stained with silver nitrate as described by Caplan *et al.* (1974). Briefly, the preparations were immersed successively in the dark at room temperature in: (1) HEPES (20 mM) buffered (pH 7.4) solution containing 4.6% glucose for 150 s; (2) 0.4% $AgNO_3$ in 4.2% glucose solution for 60 s, and (3) 4.6% glucose solution for 60 s. The arteries were then fixed at room temperature in 0.1 M sodium cacodylate containing 7.5% sucrose and examined under the light microscope.

Application of purines and sodium nitroprusside

In the presence and absence of endothelium, vessels were preconstricted to 75% of maximal tension with NA. Cumulative concentration-response curves to ATP, 2-methylthio ATP, α,β-methylene ATP, adenosine and sodium nitroprusside were constructed.

Construction of cumulative concentration-response curves to ATP and adenosine was repeated for the preparations preincubated with 8-phenyltheophylline (10 μM) for 20 min.

Application of peptides

In the presence and absence of endothelium, the preparations were constricted to 75% of their maximal tension with NA. SP, CGRP, and VIP were added to the bath as single additions, each at 30 min intervals. The peptides were washed out of the Krebs solution either once a maximum relaxant response had been reached or after the drug had been in contact with the vessel for approximately 2 min.

Immunocytochemistry

Vessels were cleaned of excess connective tissue and fat. Vessel segments were slit open longitudinally and stretched out, adventitial side uppermost, onto strips of Sylgard silicone rubber. The segments were then processed for immunofluorescent localization of SP, CGRP, and VIP according to the method of Costa *et al.* (1980).

The preparations were immersion-fixed in 4% paraformaldehyde for 1–1.5 h. They were then washed three times in phosphate buffered saline (PBS) for 10 min and then placed for 30 min in 80%, 90% and 100% alcohol to dehydrate the tissues. Following a 20 min period of immersion in Histoclear, the tissues were rehydrated by placing them for 30 min in 100%, 90% and 80% alcohol. Following three washes in PBS/Triton X solution at 10 min intervals, the primary antibody to SP, CGRP or VIP (1:200 in each case) was placed on a segment and incubated for 12–18 h at room temperature in a moist atmosphere. After three 10 min washes in PBS/Triton X, the tissue was incubated with the second goat anti-rabbit fluorescein-isothiocyanate (FITC)-conjugated antibody (1:50) for 1 h. The tissue was then washed for 10 min in PBS, followed by PBS containing 0.05% pontamine sky blue and 1% dimethylsulphoxide for 15 min. After washing in PBS twice for a further 20 min, the tissues were stretched out on slides and left to dry before being mounted in Citifluor and viewed under a Zeiss microscope.

Drugs and chemicals

(−)-Noradrenaline bitartrate (NA), α,β-methylene adenosine 5′-triphosphate (α,β-methylene ATP) (lithium salt), adenosine (hemisulphate salt), adenosine 5′-triphosphate (ATP) (disodium salt), acetylcholine bromide (ACh), sodium nitroprusside (sodium nitroferricyanide), 8-phenyltheophylline, bacitracin and bovine serum albumin were obtained from Sigma Chemical Co. Ltd; Sagatal was supplied by May and Baker; 2-methylthio adenosine 5′-triphosphate (2-methylthio ATP) (Research Biochemicals Inc., U.S.A.), substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), antibody for SP, CGRP and VIP were obtained from Cambridge Research Biochemicals; goat anti-rabbit IgG conjugated to FITC was obtained from Nordic and Citifluor was obtained from Citifluor Ltd, London.

NA was dissolved and diluted in 100 μM ascorbic acid. 8-Phenyltheophylline was dissolved in a solution of 80% methanol and 0.1% sodium hydroxide. All other drugs were dissolved in distilled water.

Statistical analysis

Data are given as a mean ± s.e.mean. Results were analysed by Student's t test (paired or unpaired data as appropriate) and a probability of less than or equal to 0.05 was considered significant.

Results

Both in the absence and presence of the endothelium, NA caused a concentration-dependent contraction of the rabbit hepatic artery. The maximum response to NA and the EC_{50} in those preparations where the endothelium was intact (2.06 ± 0.13 g and 5.39 ± 3.75 μM respectively, $n = 8$) were comparable with those in preparations denuded of endothelium (1.96 ± 0.17 g and 6.0 ± 0.91 μM respectively, $n = 8$). This demonstrates that the tissue had not been damaged by the mechanical removal of the endothelium. Addition of NA (10 μM) produced a sustained contractile response of the vessel that was approximately 75% of the maximum NA contraction.

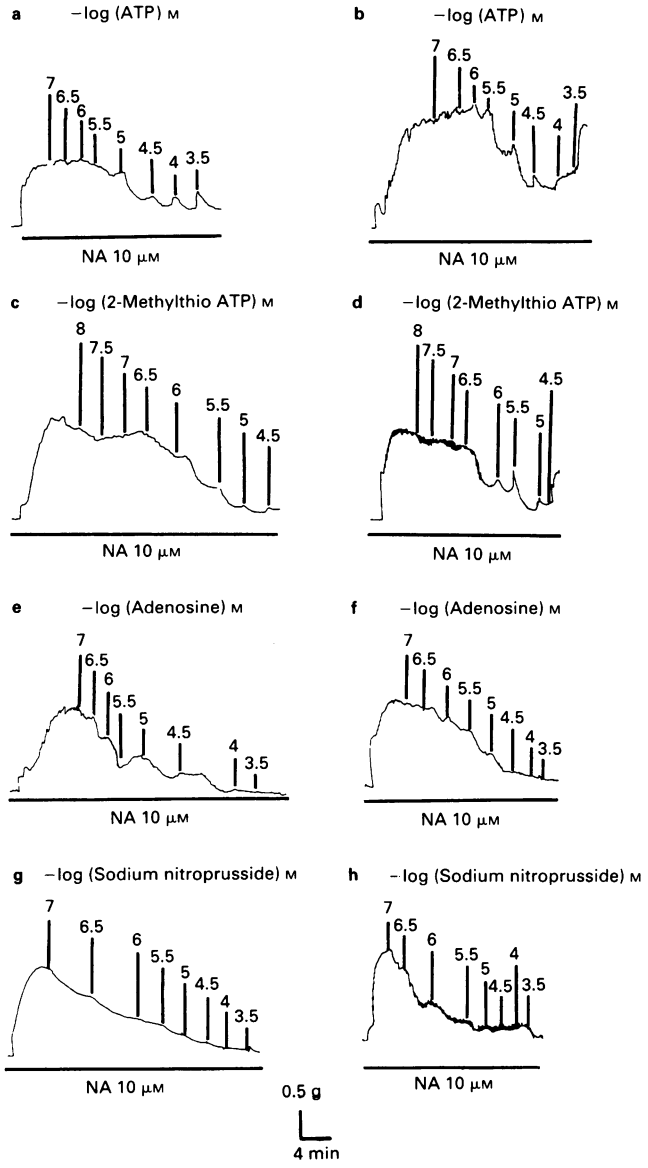

Figure 1 Isolated ring-preparations of the rabbit hepatic artery preconstricted with noradrenaline (NA) to 75% of the maximal constriction. Response to ATP: (a) endothelium intact; (b) endothelium removed, 2-methylthio ATP; (c) endothelium intact; (d) endothelium removed, adenosine; (e) endothelium intact; (f) endothelium removed, sodium nitroprusside; (g) endothelium intact; (h) endothelium removed.

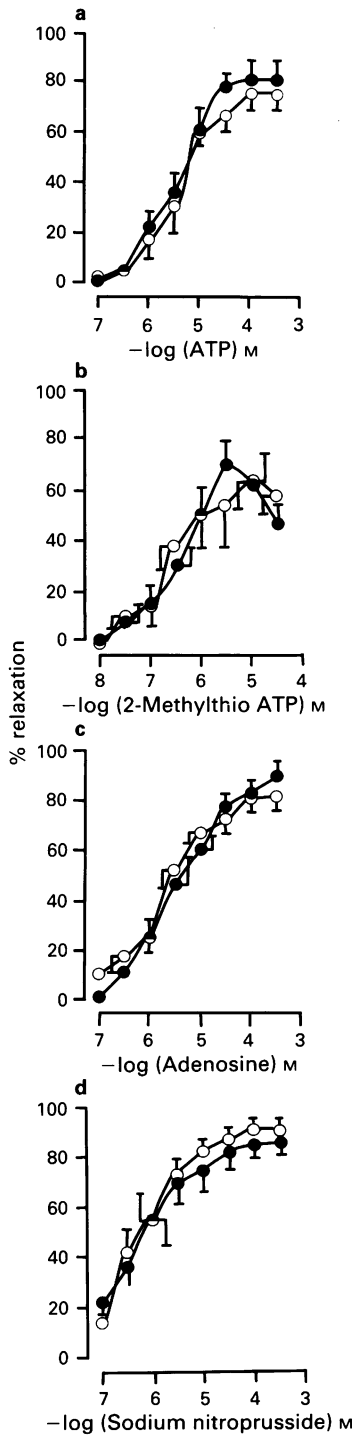

Figure 2 Isolated ring-preparations of the rabbit hepatic artery preconstricted with noradrenaline (NA) (10 μM). (a) Concentration-response curve to ATP in the presence (●) ($n = 6$) and in the absence (○) ($n = 6$) of endothelium. (b) Concentration-response curve to 2-methylthio ATP in the presence (●) ($n = 5$) and in the absence (○) ($n = 6$) of endothelium. (c) Concentration-response curve to adenosine in the presence (●) ($n = 10$) and absence (○) ($n = 6$) of endothelium. (d) Concentration-repsonse curve to sodium nitroprusside in the presence (●) ($n = 6$) and absence (○) ($n = 5$) of endothelium. Each point represents the mean percentage relaxation of the NA-induced contraction and vertical bars denote the s.e.mean.

In the presence of endothelium, addition of ACh to preconstricted vessels produced concentration-dependent, sustained relaxant responses. When the endothelium was removed, the relaxant response to ACh was abolished.

Responses to ATP, 2-methylthio ATP, adenosine, α,β-methylene ATP and sodium nitroprusside

In the presence of endothelium, ATP, 2-methylthio ATP, adenosine and sodium nitroprusside produced concentration-dependent, sustained relaxant responses (Figures 1 and 2). Occasionally, a small contraction preceded the relaxant response to ATP and 2-methylthio ATP. Removal of the endothelium did not significantly affect the response of the vessel to these agents. In preconstricted vessels, α,β-methylene ATP elicited concentration-dependent contractions in vessels where the endothelium remained intact and in those preparations where the endothelium had been removed by mechanical rubbing (Figure 3). Occasionally, a relaxation was observed at higher concentrations of α,β-methylene ATP.

Preincubation of the tissues with 8-phenyltheophylline (10 μM), a potent antagonist at P_1-purinoceptors (Smellie *et al.*,

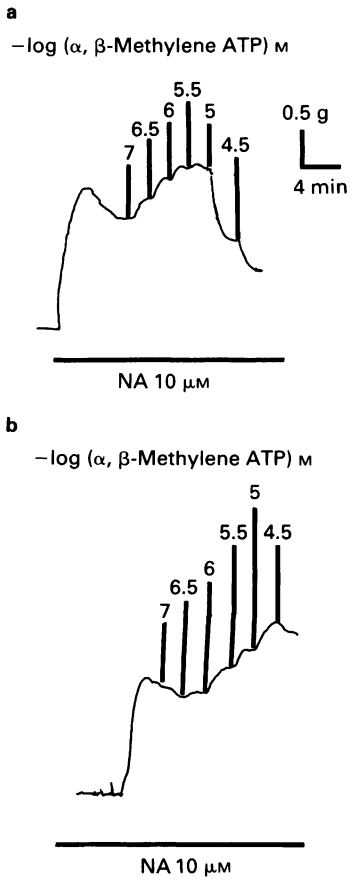

Figure 3 Rabbit isolated hepatic artery preconstricted with noradrenaline (NA) to 75% of the maximal constriction. Effect of α,β-methylene ATP in (a) the presence and (b) the absence of endothelium.

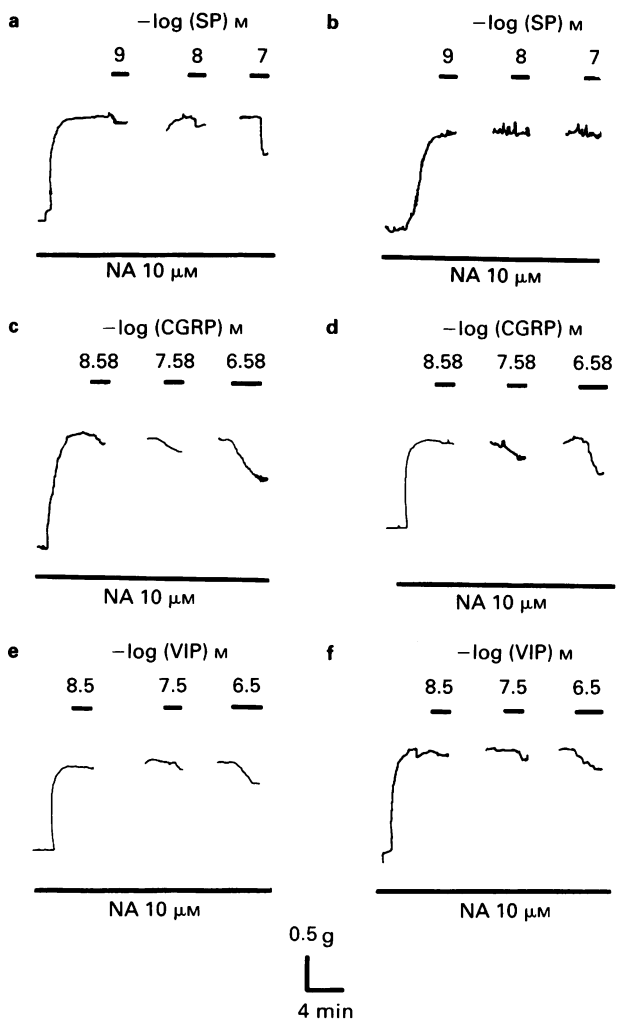

Figure 4 Rabbit isolated hepatic arterial ring preparations preconstricted with noradrenaline (NA) to 75% of the maximal constriction. Responses to single additions of substance P (SP) (a) in the presence and (b) in the absence of endothelium; calcitonin gene-related polypeptide (CGRP) (c) in the presence and (d) in the absence of endothelium; vasoactive intestinal polypeptide (VIP) (e) in the presence and (f) in the absence of endothelium.

1979; Griffith *et al.*, 1981), significantly antagonized relaxations to adenosine (Table 1). The maximum response of the vessel to ATP was reduced in the presence of 8-phenyltheophylline. The EC_{50} value for ATP in vessels with endothelium was not affected by 8-phenyltheophylline while vessels without endothelium were more sensitive to the purine in the presence of 8-phenyltheophylline. Relaxations to adenosine were not affected by the solvent for 8-phenyltheophylline.

Responses to peptides

SP produced a concentration-dependent relaxation of the vessel that was entirely dependent on the presence of an intact endothelium (Figures 4a,b). A maximum response of 45 ± 11.4% ($n = 7$) (Table 2) relaxation of the NA-induced contraction was observed after application of 0.1 μM SP to the organ bath. In the presence of endothelium, CGRP and VIP elicited a maximum relaxation of the preparation of 51 ± 6.6% ($n = 7$) and 34 ± 7.5% ($n = 5$) respectively. In endothelium-denuded preparations, CGRP and VIP induced a vasodilatation with a maximum of 58 ± 12.0% ($n = 6$) and 46 ± 11.2% ($n = 5$) respectively. The magnitude of the responses to CGRP and VIP were not significantly different in

Table 1 Effects of ATP and adenosine on the rabbit isolated hepatic artery in the absence (control) and presence of 8-phenyltheophylline (8-PT) (10 μM) in endothelium intact (+e) and endothelium denuded (−e) preparations

	Maximal relaxation (%)		EC_{50}	
Agonist	*Control*	*+8-PT*	*Control*	*+8-PT*
ATP				
+e	81 ± 6.46 (6)	52 ± 9.34 (6)*	2.7 ± 0.32 (6)	12.2 ± 7.25 (6)
−e	75 ± 7.09 (6)	52 ± 6.22 (7)*	2.1 ± 0.59 (6)	0.91 ± 0.10 (6)*
Adenosine				
+e	90 ± 4.09 (10)	69 ± 9.77 (6)*	6.4 ± 3.06 (10)	89 ± 25.5 (6)***
−e	83 ± 6.75 (6)	64 ± 10.95 (6)*	2.0 ± 0.63 (6)	12.1 ± 4.51 (6)**

All values are given as mean ± s.e.mean with the number of observations (n) in parentheses.
* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ indicate significant differences between control responses and those in the presence of 8-PT.

Table 2 Effects of substance P (SP), calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP) on the rabbit hepatic artery preconstricted with noradrenaline in the presence (+e) and absence (−) of endothelium

Peptide	*% relaxation*		
SP	1 nM	10 nM	100 nM
+e	3.7 ± 2.44 (7)	13 ± 7.0 (7)	45 ± 11.4 (7)
−e	0 (7)	0 (7)	0 (7)
CGRP	2.63 nM	26.3 nM	263 nM
+e	16 ± 9.8 (7)	28 ± 11.6 (7)	51 ± 6.6 (7)
−e	5.7 ± 5.7 (6)	37 ± 6.1 (6)	58 ± 11.9 (6)
VIP	3.0 nM	30 nM	300 nM
+e	3.6 ± 2.2 (5)	9.4 ± 3.2 (5)	34 ± 7.5 (5)
−e	8.3 ± 7.6 (5)	16 ± 10.5 (5)	46 ± 11.2 (5)

All values are expressed as mean ± s.e.mean with the number of observations (n) in parentheses.
Responses are measured as the percentage relaxation of the noradrenaline-induced contraction.

the absence and presence of the endothelium (Figure 4, Table 2).

Immunocytochemistry

All vessels showed positive SP-like immunoreactivity (SP-LI), positive CGRP-LI and positive VIP-LI.

Discussion

In this study, the endothelial dependence of the responses of the hepatic artery of the rabbit to several vasoactive agents was assessed by examining their reactivity before and after the intimal surface was rubbed to remove the endothelium.

The ability of the hepatic artery to constrict in response to NA was not significantly affected by the removal of the endothelium. This result not only demonstrates that hepatic arterial vasoconstriction in response to NA is independent of the endothelium, but also that the mechanical removal of the endothelium did not damage the smooth muscle of the preparation.

The endothelium has been shown to have an obligatory role in the relaxations of isolated arteries to ACh (Furchgott & Zawadzki, 1980; Furchgott *et al.*, 1981; De Mey & Vanhoutte, 1982; Vanhoutte & Rimele, 1983; Peach *et al.*, 1985). Our experiments show that ACh-induced vasodilatation of the rabbit hepatic artery also depends on the presence of an intact endothelium. In most peripheral blood vessels, ATP also induces a relaxation that is endothelium-dependent (Furchgott *et al.*, 1981; De Mey *et al.*, 1982; Rapoport *et al.*, 1984; Martin *et al.*, 1985; Houston *et al.*, 1987). Furthermore, endothelium-dependent relaxation of the perfused rabbit hepatic arterial bed by ATP has also been demonstrated (R. Mathie, personal communication). In this study, however, relaxation to ATP and 2-methylthio ATP was not significantly affected by the removal of the endothelium. In the rabbit portal vein (Kennedy & Burnstock, 1985a) and the rabbit mesenteric artery (Mathieson & Burnstock, 1985), ATP also caused relaxation via P_2-purinoceptors by an endothelium-independent mechanism. The physiological relevance of the different locations of P_{2y}-purinoceptors in the isolated hepatic artery and in the perfused hepatic arterial bed preparation has yet to be established. Vasodilatation of the hepatic artery to adenosine was independent of the endothelium in common with other vessels (Hardebo *et al.*, 1983; Kennedy & Burnstock, 1985b; Mathieson & Burnstock, 1985). 8-Phenyltheophylline, a selective P_1-purinoceptor antagonist (Smellie *et al.*, 1979; Griffith *et al.*, 1981), significantly increased the EC_{50} value for adenosine but not that for ATP, indicating that adenosine but not ATP is acting via P_1-purinoceptors. However, it should be noted that the maximum response to high concentrations of ATP is reduced in the presence of 8-phenyltheophylline. This result implies that ATP may have some action via P_1-purinoceptors as a result of its breakdown to adenosine.

In contrast to ATP, 2-methylthio ATP and adenosine, α,β-methylene ATP did not produce a relaxation of the rabbit hepatic artery in which tone had been induced by NA. Indeed, a concentration-dependent contraction was observed both in the absence and presence of endothelium. Similar results have been reported in other blood vessels (Kennedy *et al.*, 1985; Kennedy & Burnstock, 1985a; Mathieson & Burnstock, 1985; Houston *et al.*, 1987). In 1985, Burnstock & Kennedy proposed a subdivision of the P_2-purinoceptor into P_{2x} and P_{2y} subtypes and suggested that the P_{2x}-purinoceptor mediates vasoconstriction and the P_{2y}-purinoceptor mediates vasodilatation. Brizzolara & Burnstock (1990) have demonstrated that in the hepatic artery of the rabbit, α,β-methylene ATP induces a concentration-dependent contraction and that ATP and NA act as cotransmitters from sympathetic nerves, the purinergic component being mediated by ATP acting through postjunctional P_{2x}-purinoceptors. Thus, it would appear that in the rabbit hepatic artery, three sub-populations of purincoeptor exist on the smooth muscle, namely a P_1-purinoceptor mediating a vasodilatation to adenosine, a P_{2x}-purinoceptor mediating a vasoconstriction to ATP and a P_{2y}-purinoceptor mediating a vasodilatation to ATP.

Immunocytochemical studies have shown a wide distribution of SP, (Edvinsson *et al.*, 1981; Furness *et al.*, 1982; Barja *et al.*, 1983; Goehler *et al.*, 1988), CGRP (Rosenfeld *et al.*, 1983; Hanko *et al.*, 1985; Sasaki *et al.*, 1986; Goehler *et al.*, 1988) and VIP (Larsson *et al.*, 1976; Uddman *et al.*, 1981; Malencik & Andersson, 1983; Varga *et al.*, 1986) in both the central and peripheral nervous systems. In the rabbit hepatic artery, nerve fibres containing SP, CGRP and VIP were identified. CGRP and VIP have been shown to be potent vasodilators of several blood vessels, and in this study, both CGRP and VIP induced a relaxation that was independent of the endothelium. This result is consistent with the results reported for most other vessels where removal of the endothelium does not prevent CGRP- or VIP-induced vasodilatation (Duckles & Said, 1982; Brum *et al.*, 1985; Brain *et al.*, 1985; Girgis *et al.*, 1985; Hanko *et al.*, 1985; Schoeffter & Stoclet, 1985; Varga *et al.*, 1986; Edvinsson *et al.*, 1989).

SP has also been demonstrated to be a powerful vasodilator of several blood vessels and in all cases its action requires the presence of an intact endothelium (Furchgott, 1983; D'Orleans-Juste *et al.*, 1985; Edvinsson *et al.* 1985; Bolton & Clapp, 1986; Stewart-Lee & Burnstock, 1989). The presence of SP-like immunoreactivity has been demonstrated in the hepatic arteries of the rat and human (Burt *et al.*, 1987; Tanikawa *et al.*, 1988) and its potent vasodilator action observed in the hepatic artery of the dog (Withrington, 1987). Removal of the endothelium in this study, completely abolished any response of the rabbit hepatic artery to SP thus demonstrating the obligatory role of an intact endothelium in SP-mediated vasodilatation.

Constant blood flow to the liver must be maintained since the hepatic clearance of many blood-borne drugs and hormones is blood-flow limited. Adenosine has been shown to be an important mediator of the compensatory hyperaemic response of the hepatic artery in response to portal vein occlusion (Lautt, 1981; Mathie & Blumgart, 1990). The results of this study provide further evidence for the involvement of adenosine in the control of hepatic arterial tone via P_1-purinoceptors which are located on the smooth muscle. Whilst there is strong evidence for the role of adenosine in the regulation of hepatic arterial resistance (Lautt, 1981; Mathie & Blumgart, 1990), it does not appear to be the sole mediator of hepatic arterial dilatation. The results from this study provide evidence for the putative role of ATP as a vasodilator of the hepatic artery of the rabbit, acting through postjunctional P_{2y}-purinoceptors located on the vascular smooth muscle. Furthermore, previous studies have demonstrated a

hepatic arterial constriction in response to ATP via smooth muscle P_{2x}-purinoceptors following sympathetic nerve stimulation (Brizzolara & Burnstock, 1990). In most vessels, the P_{2y}-purinoceptor is located on the endothelium (De Mey & Vanhoutte, 1980; 1982; Cocks & Angus, 1983; Furchgott, 1983; Kennedy *et al.*, 1985; Burnstock & Kennedy, 1985; Liu *et al.*, 1989). Although the response mediated via the two P_2-purinoceptors in the hepatic artery are in opposition, the location of both receptors on the smooth muscle may be of physiological significance in the control of vascular tone. It could be that, depending on the tone of the vessel, responses of the artery to ATP may be either constriction or relaxation and that in some pathological situations, one or other of these responses may dominate.

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