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Effects of pre-contraction with endothelin-1 on α_2-adrenoceptor- and (endothelium-dependent) neuropeptide Y-mediated contractions in the isolated vascular bed of the rat tail

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1 The pressor effects to bolus doses of the α_2-adrenoceptor agonist UK-14,304 were studied in the isolated vascular bed of the perfused rat tail before and after increasing the perfusion pressure with infusions of endothelin-1. Those of neuropeptide Y were studied before and after pre-constriction with endothelin-1 or 5-hydroxytryptamine. The pressor effects of neuropeptide Y were studied before and after functional disruption of the endothelium with the detergent CHAPS.

2 Endothelin-1 and the α_1-adrenoceptor agonist phenylephrine induced dose-dependent vasoconstriction, endothelin-1 being some 10^4 times more potent than phenylephrine [log dose (mol) of the ED_{50} for endothelin-1 and phenylephrine: -11.8 ± 0.2 ($n = 7$), -8.2 ± 0.2 ($n = 5$) respectively].

3 Under control conditions, at basal perfusion pressures, UK-14,304 and neuropeptide Y were virtually inactive as vasoconstrictors. Following a sustained increase in perfusion pressure by infusions of endothelin-1 (2.5–10 nM at 0.8 ml min^{-1}), however, both UK-14,304 and neuropeptide Y induced dose-dependent pressor responses and both were some 10^2 times more potent than phenylephrine [log dose (mol) of the ED_{50} for UK-14304 and neuropeptide Y: -10 ± 0.5 ($n = 6$), -10.3 ± 0.4 ($n = 6$) respectively]. Responses to neuropeptide Y also were uncovered when vascular tone was increased with 5-hydroxytryptamine (5–20 nM) [log dose (mol) of the ED_{50} for neuropeptide Y: -10.2 ± 0.2 ($n = 6$)].

4 Pre-constriction-induced pressor responses to UK-14,304 were inhibited by 1 μM rauwolscine whilst those to neuropeptide Y were inhibited by disruption of the endothelium. Removal of the endothelium had no significant effect on the pressor responses to 4 pmol or 8 pmol endothelin-1 and had no effect on the increase in perfusion pressure induced by the endothelin-1 infusions but did decrease the time-course of pressor responses to bolus injections of endothelin-1. Endothelial disruption had no significant effect on the vasoconstriction induced by all but one of the doses of phenylephrine administered [log dose (mol) of the ED_{50} for phenylephrine after CHAPS: -8.6 ± 0.2 ($n = 5$)], indicating that the responsiveness of the vascular smooth muscle was not destroyed by CHAPS. This treatment did, however, slow the onset and prolong the time course of the phenylephrine-induced responses.

5 These results indicate that, in the isolated vascular bed of the rat tail, pressor responses to both α_2-adrenoceptor- and neuropeptide Y receptor-activation are uncovered by agonist-induced preconstriction including that to endothelin-1. Neuropeptide Y-induced vasoconstriction was endothelium-dependent.

Introduction

The pithed rat demonstrates the existence of post-junctional α_1- and α_2-adrenoceptors in the vasculature, both of which contribute to systemic pressor responses (Drew & Whiting, 1979; Docherty *et al.*, 1979; Docherty & McGrath, 1980). Until recently, however, there were few *in vitro* examples of arterial vessels or resistance beds that possess functional populations of postjunctional α_2-adrenoceptors sensitive to the selective α_2-adrenoceptor antagonists yohimbine, rauwolscine and idazoxan and resistant to the selective α_1-adrenoceptor antagonist prazosin. However, in the isolated vascular bed of the rat tail, rauwolscine-sensitive and prazosin-resistant α_2-adrenoceptors were uncovered by increasing the vascular tone with arginine vasopressin (Templeton *et al.*, 1989). The ability to uncover postjunctional α_2-adrenoceptor-mediated responses is not restricted to arginine vasopressin i.e. 'tone' induced by 5-hydroxytryptamine also uncovers responses to UK-14,304 in the isolated perfused rat tail (Templeton, 1988); prostaglandin $F_{2\alpha}$, phenylephrine, acetylcholine, 5-hydroxytryptamine and histamine in the canine portal vein uncover responses to the selective α_2-adrenoceptor agonist B-HT 920 (Furuta, 1988); non-pressor doses of angiotensin II enhance responses to the selective α_2-adrenoceptor agonist UK-14,304 in the rabbit saphenous artery (Dunn *et al.*, 1989) and in the canine plantaris artery, responses to B-HT 920 are uncovered in the presence of the calcium channel activator Bay K 8644 (Sulpizio & Hieble, 1987).

Neuropeptide Y is co-stored with noradrenaline in the large dense cored vesicles of sympathetic nerve endings (Ekblad *et al.*, 1984) and they are co-released from the sympathetic varicosities upon nerve stimulation (Lundberg *et al.*, 1984). Previous *in vitro* studies have shown that, like α_2-adrenoceptor agonists, neuropeptide Y is not a potent vasoconstrictor in its own right (Edvinsson *et al.*, 1987), although low levels of neuropeptide Y can potentiate the vasoconstriction induced by noradrenaline, sympathetic nerve stimulation and other vasoconstrictor agents (Edvinsson *et al.*, 1984; 1987). Furthermore, in cat cerebral vessels and the pig spleen vasculature, it has been shown that neuropeptide Y and α_2-adrenoceptor agonists share the ability to inhibit adenylate cyclase through a pertussis-toxin-sensitive mechanism and to decrease intracellular levels of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Fredholm *et al.*, 1985; Lundberg *et al.*, 1988). We have therefore now investigated and compared the influence of agonist-induced tone on the vascular responses to neuropeptide Y and an α_2-adrenoceptor agonist.

Endothelin-1 is a potent vasoconstrictor in several isolated veins and arteries (Yanagasawa *et al.*, 1988; D'Orleans-Juste *et al.*, 1988) and can facilitate pressor effects of noradrenaline in rabbit ear artery (Wong-Dusting *et al.*, 1989) but little is known about its interaction with peripheral postjunctional α_2-adrenoceptor activity.

We have studied the interactions between endothelin-1, neuropeptide Y and the α_2-adrenoceptor agonist UK-14,304

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in the isolated vascular bed of the rat tail. Vasoconstrictor responses to UK-14,304 were studied in the presence of endothelin-1 induced tone and those to neuropeptide Y were studied in the presence of tone induced by both endothelin-1 and 5-hydroxytryptamine. Previous studies have suggested that neuropeptide Y-induced vasoconstriction is endothelium-dependent (Daly & Hieble, 1987; Hieble *et al.*, 1989) and this was investigated here by disruption of the vascular endothelium of the rat tail vasculature with the detergent CHAPS.

Methods

Male Wistar rats (280–320 g) were killed by stunning followed by cervical dislocation. The ventral surface of the tail was shaved and the skin reflected for 3–5 cm around the proximal end of the tail. The tail artery was exposed with blunt forceps and cleaned of fat and connective tissue and cannulated with a polyethylene cannula. The tail was amputated by cutting through an intervertebral disc and placed on an elevated plastic platform allowing the arterial cannula to be connected to a perfusion circuit. The tail vasculature was perfused at a rate of 0.8 ml min^{-1} with a modified physiological salt solution the temperature of which, at the point of entry into the tail artery, was monitored with a digital thermometer (Digitron Instrumentation Ltd) and maintained at 37°C ± 0.4°C ($n = 12$). The temperature of the perfusate leaving the tail was also monitored and was found to be 26.5°C ± 0.3°C ($n = 12$). After the start of perfusion, blood filled drops were observed from the cut end of the tail, indicating a successful cannulation. The perfusion pressure was allowed to stabilize for 45 min before the start of any experimental procedure.

Measurement of agonist-mediated responses at basal and raised perfusion pressure

Dose-response curves to endothelin-1, neuropeptide Y, phenylephrine and UK-14,304 were constructed by bolus injection of drugs into the perfusing solution via an injection port close to the point of cannulation. The injectate volume was constant at 0.01 μl and doses are expressed as quantity (nmol etc.). Drugs added to the perfusate reservoir are expressed as concentration (nM etc.). In all experiments, the perfusion pressure was allowed to return to baseline before the administration of a subsequent dose of agonist. In those preparations in which endothelin-1 dose-response curves were constructed, the number of doses it was possible to administer in each was variable due to the long recovery period required for the higher doses. The actual doses administered to each tail were therefore variable and the '*n*' values for each dose were correspondingly variable in these experiments (i.e. see Figure 5a).

After establishing control responses to neuropeptide Y and UK-14,304, endothelin-1 (2.5–10 nM) was included in the perfusate until the perfusion pressure was approximately doubled. After a stable perfusion pressure had been maintained over a 10 min period, dose-response curves for the two agonists were repeated. In other experiments, a dose-response curve to neuropeptide Y was determined before and after doubling the vascular perfusion pressure with 5-hydroxytryptamine (5–20 nM). The concentrations of endothelin-1 and 5-hydroxytryptamine used were variable as the dose required to double the vascular perfusion pressure varied between preparations. In those preparations in which UK-14,304 was studied, after establishing the second dose-response curve, 1 μM rauwolscine was added to the perfusate (still containing endothelin-1) and the dose-response curve for UK-14,304 repeated for a third time.

Effect of endothelium removal on neuropeptide Y-, endothelin-1- and phenylephrine-induced responses

In those preparations in which responses to neuropeptide Y were studied in the presence of endothelin-1, after establishing the second dose-response curve, destruction of the endothelium was achieved by perfusion of the vascular bed with 0.3% (w/v) solution of the detergent CHAPS in distilled water at 2 ml min^{-1} for 90 s. The infusion of endothelin-1 was then immediately resumed. After a 30 min re-equilibration period, the dose-response curve for neuropeptide Y (still in the presence of endothelin-1) was repeated for a third time. Removal of the endothelium was confirmed both histologically and pharmacologically. Histologically, the presence or absence of the endothelium was determined by use of cacodylate buffered fixative (Sabatini *et al.*, 1963) on artery samples which were cut on a cryostat. Pharmacologically, in four preconstricted preparations, the effect of CHAPS on acetylcholine-induced vasorelaxation was determined as it is known that removal of the vascular endothelium abolishes endothelium-dependent vasorelaxation to acetylcholine.

In order to establish if treatment with CHAPS affected the responsiveness of the vascular smooth muscle and if the pressor effect of endothelin-1 was affected by such treatment, in those preparations in which dose-response curves for phenylephrine and endothelin-1 had been established, the responsiveness of the vasculature to these agonists was determined after CHAPS treatment.

Drugs and solutions

The composition of the physiological salt solution was (in mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 11 and Na_2EDTA 0.023. Ficoll (2%, molecular weight approximately 70,000) was included to prevent water retention. The following compounds were used: rauwolscine HCl (Roth); UK-14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate, Pfizer); phenylephrine HCl (Sigma); 5-hydroxytryptamine creatinine sulphate, (BDH biochemicals); acetylcholine chloride (Sigma); CHAPS (3-[3-cholamidopropyl)-dimethylammonio]-1- propanesulphonate, Sigma); neuropeptide Y (I.C.I. Pharmaceuticals Division, Cheshire) and endothelin-1 (human, Scientific Marketing Associates).

Statistical analysis

All results are given as the mean ± s.e.mean. The ED_{50}s for the agonists were calculated for each individual preparation (by BBC microcomputer graphical interpolation) and meaned within groups. For all experiments, differences between means were compared by paired Student's *t* test. For those experiments in which the *n* for the first dose-response-curve was greater than that for the subsequent one to be compared, a paired Student's *t* test was applied only to those experiments in which both procedures were conducted, and it is these results which are given below.

Results

Pressor responses to UK-14,304 in the presence and absence of endothelin-1-induced tone, and the effect of rauwolscine

Figure 1 shows the vasoconstrictor responses to UK-14,304 at basal perfusion pressure and in the presence of endothelin-1-induced tone (in the absence and presence of 1 μM rauwolscine). At a basal perfusion pressure of 76 ± 8 mmHg ($n = 6$), UK-14,304 produced a maximum response of only 6 mmHg. During an infusion of endothelin-1 (2.5–10 nM), sufficient to double the perfusion pressure of each individual preparation (162 ± 18 mmHg; $n = 6$), UK-14,304 elicited dose-dependent increases in perfusion pressure. The maximum response was approximately 90 mmHg and the log dose (mol) of the ED_{50} for UK-14,304 was −10 ± 0.5 ($n = 6$). Following an injection of UK-14,304, perfusion pressure returned to baseline after 5–20 min depending on the dose administered. The highest dose of UK-14,304 induced a pressor response

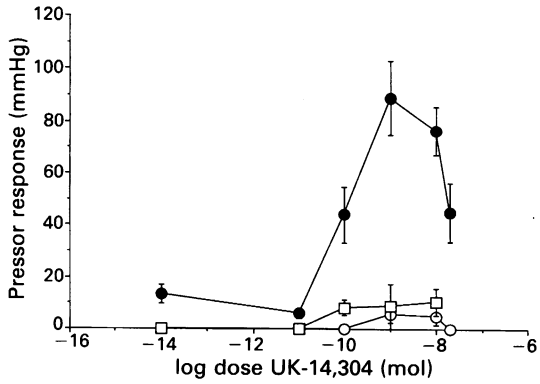

Figure 1 Pressor responses to UK-14,304 in the isolated perfused vascular bed of the rat tail: (○) control responses ($n = 6$); (●) responses in preparations in which the vascular tone has been raised with 2.5–10 nM endothelin-1 [0.8 ml min^{-1}] ($n = 6$) and (□) responses in preparations in which vascular tone has been raised with endothelin-1 and 1 μM rauwolscine included in the perfusate ($n = 4$). The points show the mean and the vertical lines show s.e.mean.

which was significantly less than the maximum response attained ($P < 0.05$). Figure 1 also shows that the responses induced by UK-14,304 were virtually abolished by including 1 μM rauwolscine in the perfusion fluid. Rauwolscine itself did not affect the increase in vascular tone induced by endothelin-1, the perfusion pressure after rauwolscine being 162 ± 18 mmHg ($n = 4$).

Pressor responses to neuropeptide Y in the presence and absence of endothelin-1- or 5-hydroxytryptamine-induced tone and the effect of endothelium removal

Figure 2a shows the vasoconstrictor responses to neuropeptide Y at basal perfusion pressure, after vascular tone was doubled with endothelin-1 and after removal of the endothelium with CHAPS (in the presence of endothelin-1). At a basal perfusion pressure of 72 ± 7 mmHg ($n = 6$), neuropeptide Y failed to elicit a response greater than 14 ± 6 mmHg. After vascular tone had been increased to 157 ± 14 mmHg ($n = 6$), neuropeptide Y induced a dose-dependent increase in perfusion pressure which returned to baseline in 10–40 min depending on the dose administered. The maximum response elicited was 110 mmHg and the log dose (mol) of the ED_{50} for neuropeptide Y was −10.3 ± 0.4 ($n = 6$). In 3 experiments it was also confirmed that neuropeptide Y-induced responses were not affected by 1 μM rauwolscine. Figure 2a shows that removal of the endothelium with CHAPS (still in the presence of endothelin-1) abolished all responses to neuropeptide Y. CHAPS treatment did not significantly affect the increase in vascular tone induced by the endothelin-1 infusion, the perfusion pressure after treatment with CHAPS being 129 ± 16 mmHg ($n = 4$). In 3 experiments it was also confirmed that CHAPS did not affect the ability of the rat tail vasculature to respond to UK-14,304.

Figure 2b shows the vascular responses to neuropeptide Y before ($n = 6$) and after increasing the vascular tone with 5-hydroxytryptamine [5–20 nM] ($n = 6$). At a basal tone of 69 ± 7 mmHg, neuropeptide Y induced a pressor response of 54 ± 11 mmHg only at the highest dose used. After vascular tone was increased with 5-hydroxytryptamine to 148 ± 18 mmHg, neuropeptide Y elicited dose-dependent pressor responses, attaining a maximum response of 210 mmHg with a log dose (mol) of the ED_{50} of −10.2 ± 0.2. From Figure 2b it can be seen that the dose-response curve for neuropeptide Y in the presence of 5-hydroxytryptamine was still rising at the highest dose of neuropeptide Y used (the highest available) and it is possible that even greater pressor responses could have been attained at higher doses. It should also be noted that the ED_{50} for neuropeptide Y may, therefore, not have been calculated from a true maximum and may be higher in value.

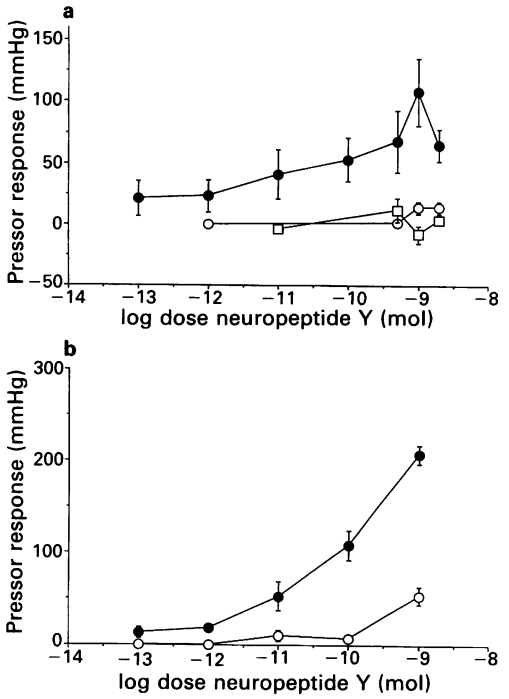

Figure 2 Pressor responses to neuropeptide Y in the isolated perfused vascular bed of the rat tail. (a) Preconstriction with endothelin-1: (○) control responses ($n = 6$); (●) responses in preparations in which the vascular tone has been raised with 2.5–10 nM endothelin-1 (0.8 ml min^{-1}) ($n = 6$) and (□) responses obtained in preparations in which the endothelium had been destroyed with CHAPS (in the continued presence of endothelin-1) ($n = 4$). (b) Preconstriction with 5-hydroxytryptamine: (○) control responses ($n = 6$) and (●) responses in preparations in which the vascular tone has been raised with 5–20 nM 5-hydroxytryptamine ($n = 6$). All points show the mean and the vertical lines show s.e.mean.

In 2 preparations in which vascular perfusion pressure was increased from mean values of 60 mmHg to 200 mmHg with 5 nM 5-hydroxytryptamine, acetylcholine (5.5 nmol) induced a vasorelaxation of 80 mmHg. This was abolished after perfusion of the preparation with CHAPS. Likewise, in 2 preparations in which tone was increased with 1 μM phenylephrine (from means of 60 mmHg to 150 mmHg) acetylcholine (5.5 nmol) induced an initial pressor response followed by a vasorelaxation (Figure 3a). After CHAPS treatment the pressor response remained whilst the vasorelaxation was abolished (Figure 3b). This indicates that the endothelium was successfully removed with CHAPS but the ability of the smooth

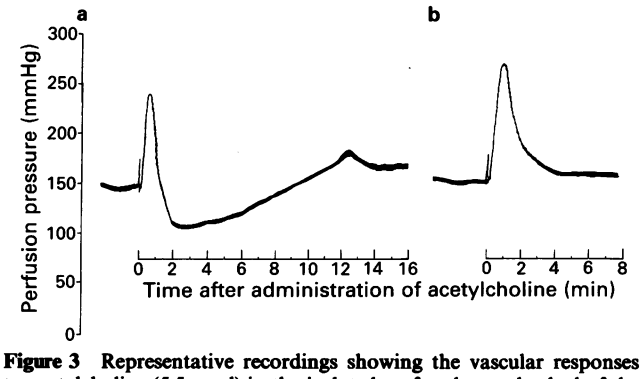

Figure 3 Representative recordings showing the vascular responses to acetylcholine (5.5 nmol) in the isolated perfused vascular bed of the rat tail preconstricted with 1 μM phenylephrine. (a) Control response. (b) Response after disruption of the vascular endothelium with CHAPS.

muscle to contract was not affected. Removal of the endothelium with CHAPS was also confirmed histologically.

Pressor responses to phenylephrine and endothelin-1 and the effect of endothelium removal

Figure 4a shows the vasoconstrictor responses to the selective α_1-adrenoceptor agonist phenylephrine before ($n = 5$) and after perfusion of the tail vascular bed with CHAPS ($n = 5$). Before CHAPS infusion, the basal perfusion pressure was 69 ± 6 mmHg and phenylephrine produced a maximum response of 210 mmHg and its log dose (mol) of the ED_{50} was -8.2 ± 0.2. Following phenylephrine administration, the perfusion pressure recovered after 1–15 min. Treatment with CHAPS did increase the perfusion pressure in some preparations (e.g. see Figure 4b) but overall, had no statistically significant effect on the basal perfusion pressure of the CHAPS-treated group of preparations (93 ± 11 mmHg), and no significant effect on the ability of the vascular bed to respond to phenylephrine (log dose (mol) of the ED_{50} for phenylephrine in the presence of CHAPS was -8.6 ± 0.2) except at the 2 nmol dose. At this one dose, removal of the endothelium with CHAPS significantly decreased the phenylephrine-induced vasoconstriction. Removal of the endothelium slowed the onset and prolonged the time course of the phenylephrine response as depicted in Figure 4b.

Endothelin-1 induced dose-dependent vasoconstriction from a basal perfusion pressure of 73 ± 5 mmHg ($n = 5$–9). Its maximum pressor response was 110 mmHg and the log dose (mol) of its ED_{50} was -11.8 ± 0.2 ($n = 7$) [Figure 5a]. Perfusion pressure returned to baseline some 5–60 min after endothelin-1 administration, depending on the dose administered. Removal of the endothelium with CHAPS had no significant effect on the vascular responses to bolus injections of endothelin-1. With 4 pmol endothelin-1 the response before CHAPS was 51 ± 9 mmHg ($n = 9$) and after CHAPS was 40 ± 8 mmHg ($n = 5$). With 8 pmol endothelin-1 the response before CHAPS was 70 ± 13 mmHg ($n = 7$) and after CHAPS was 127 ± 27 mmHg ($n = 4$). In 2 out of 4 of these preparations, the perfusion pressure was increased by CHAPS treatment and, in all 4, the time course of the endothelin-1 response was altered by removal of the endothelium, being slower in onset and shorter in duration (Figure 5b).

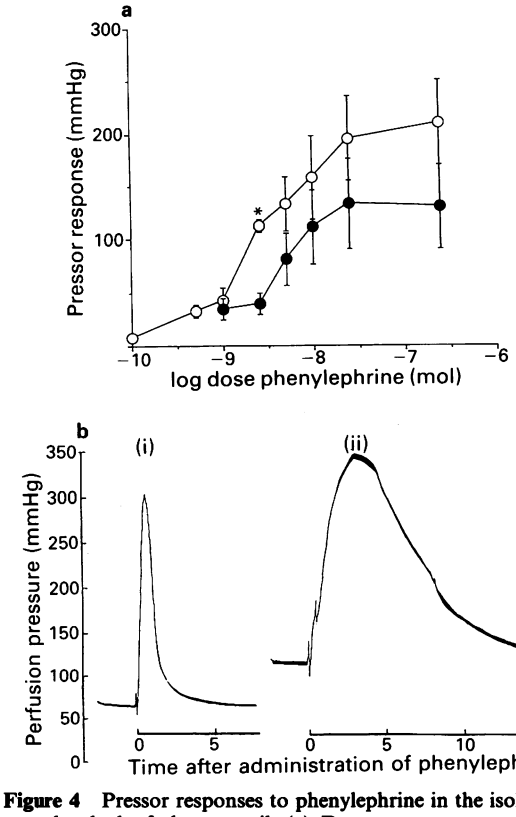

Figure 4 Pressor responses to phenylephrine in the isolated perfused vascular bed of the rat tail. (a) Dose-response curve: (○) control responses ($n = 5$) and (●) the responses obtained in preparations in which the endothelium had been destroyed with CHAPS ($n = 5$). The points show the mean and vertical lines show s.e.mean. * $P < 0.05$ compared with CHAPS-treated group (paired Student's t test). (b) Representative recordings showing the time-course of phenylephrine (10 pmol) induced responses in the isolated perfused vascular bed of the rat tail: (i) before removal of the endothelium; (ii) after endothelium removal with CHAPS.

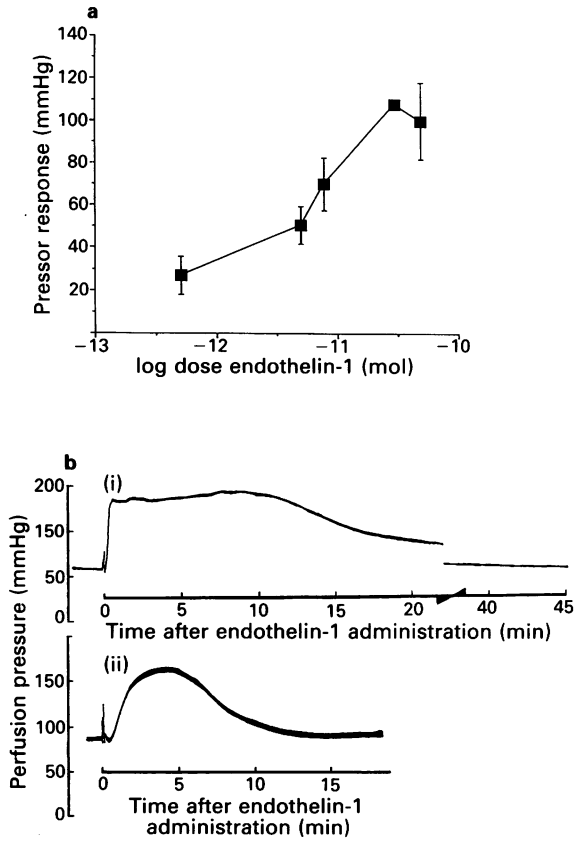

Figure 5 Pressor responses to endothelin-1 in the isolated perfused vascular bed of the rat tail. (a) Dose-response curve ($n = 5$–9). The points show the mean and the vertical lines show s.e.mean. (b) Representative recordings showing the time-course of endothelin-1 (8 pmol)-induced responses in the isolated perfused vascular bed of the rat tail: (i) before removal of the endothelium; (ii) after endothelium removal with CHAPS.

Discussion

The influence of vascular tone on α_2-adrenoceptor- and neuropeptide Y-receptor-mediated vasoconstriction

Under the control experimental conditions used here the temperature of the perfusate was decreased by 10.5°C on passage through the tail indicating that the vasculature was markedly vasodilated. This confirms the importance of the rat tail vasculature in thermoregulation (Rand *et al.*, 1965; Raman *et al.*, 1983). This study shows that, in the isolated perfused vascular bed of the rat tail, at basal vascular tone, the α_2-adrenoceptor agonist UK-14,304 is virtually inactive at inducing pressor responses. Induction of vascular tone with endothelin-1 uncovered a functional population of α_2-adrenoceptors indicated by the ability of the selective α_2-adrenoceptor agonist UK-14,304 to elicit dose-dependent vasoconstrictions which were sensitive to a selective concentration of the α_2-adrenoceptor antagonist rauwolscine. A previous study using a similar preparation has shown that

arginine vasopressin-induced tone will also uncover rauwolscine-sensitive, prazosin-resistant α_2-adrenoceptor-mediated responses and has no effect on vascular responses to phenylephrine (Templeton *et al.*, 1989).

Some previous studies using segments of the isolated tail artery of the rat and selective α_1-adrenoceptor and α_2-adrenoceptor antagonists have indicated postjunctional α_2-adrenoceptor involvement in vasoconstriction induced by nerve stimulation or catecholamines (Medgett & Langer, 1984; 1986; de Moraes *et al.*, 1988). Raising the vascular tone with endothelin-1 not only uncovered responses to UK-14,304 but showed it to be some 10^2 times more potent than the α_1-adrenoceptor agonist phenylephrine (as indicated by its ED_{50} value) though producing maximum responses some 50% of those elicited by phenylephrine. The responses to UK-14,304 were also of longer duration than those to phenylephrine. The highest dose of UK-14,304 induced a pressor response that was significantly less than the maximum response. The reason for this is unclear, though there is some evidence to suggest that in both un-constricted and pre-constricted rat isolated tail arteries, high doses of α_2-adrenoceptor agonists may induce release of an endothelium-dependent relaxing factor which counteracts their direct contractile activity (Matsuda *et al.*, 1985).

At basal vascular tone, neuropeptide Y was virtually inactive at inducing pressor responses. However, in those vascular beds pre-constricted with endothelin-1, neuropeptide Y elicited dose-dependent vasoconstrictions. Like UK-14,304, neuropeptide Y induced a maximum response only 50% that of phenylephrine, was 10^2 times more potent than phenylephrine as indicated by its ED_{50} value and its responses were of a longer duration than those of phenylephrine. Pre-constriction with 5-hydroxytryptamine instead of endothelin-1 had a somewhat different effect on responses induced by neuropeptide Y. Although its ED_{50} value was not changed, in the presence of 5-hydroxytryptamine, neuropeptide Y elicited responses twice the magnitude of those elicited in the presence of endothelin. The results indicate that, in the perfused vascular bed of the rat tail, the magnitude of the response to neuropeptide Y depends upon the pre-constricting substance. Furuta (1988) also demonstrated that, in canine isolated portal vein, the α_2-adrenoceptor agonist B-HT 920 failed to elicit responses at basal vascular tone but could induce concentration-dependent responses, the maxima of which depended both on the pre-contraction levels and on the pre-contracting substance.

Under conditions of increased sympathetic discharge such as occurs in hypertension, haemorrhagic shock and hypoxia, co-release of neuropeptide Y may contribute to the observed increases in regional resistance. That neuropeptide Y overflow is increased under conditions of increased sympathetic discharge is indicated by the observation that circulating neuropeptide Y levels increase following haemorrhage in rats (Morris *et al.*, 1987) and exercise in man (Morris *et al.*, 1986). There is increasing evidence that, in man, circulating levels of endothelin-1 are raised in conditions in which regional vasoconstriction is increased such as in acute renal failure (Tomita *et al.*, 1989) and essential hypertension (Saito *et al.*, 1990). Recalculating the data in these studies shows that the circulating concentration of endothelin-1 is increased to approximately 10 pmol–1 nmol in such patients. This range of endothelin-1 concentration has been shown to be sufficient to induce contraction of human umbilical vessels *in vitro* (Haegerstrand *et al.*, 1989). Thus, combinations of increased sympathetic tone, increased circulating endothelin-1 and increased circulating neuropeptide Y levels may promote increased expression of prolonged α_2-adrenoceptor- and neuropeptide Y-mediated regional vasoconstriction in such disease states.

Acetylcholine-induced vasorelaxations in the perfused rat tail are inhibited by both haemoglobin (an inhibitor of endothelium-dependent relaxing factor, Griffith *et al.*, 1984) and atropine (Templeton, 1988). Perfusion of blood vessels with the detergent CHAPS has been shown to remove the vascular endothelium without any significant effect upon the underlying vascular smooth muscle (Tesfamariam *et al.*, 1984; Hiley *et al.*, 1987). In the tail bed, the vasoconstrictor responses to phenylephrine, endothelin-1 and acetylcholine were not significantly affected, whilst the vasorelaxation induced by acetylcholine was abolished by CHAPS. This proof of selective endothelium removal was supported by histological evidence of endothelial damage. The simplest explanation of these results is that, in the rat perfused tail, perfusion of CHAPS disrupts the vascular endothelium without affecting the underlying smooth muscle.

The neuropeptide Y-induced contractions in the presence of agonist-induced tone were not observed after endothelium removal. This is consistent with previous studies showing that neuropeptide Y-induced potentiation of adrenoceptor-mediated contractions in the rabbit ear artery and canine saphenous vein are endothelium-dependent (Daly & Hieble, 1987; Hieble *et al.*, 1989) but contrary to other studies showing that the direct vasoconstrictor effect of neuropeptide Y on small human skeletal muscle arteries and pig splenic arteries is not endothelium-dependent (Pernow, 1989). This indicates that there may be species or tissue differences in the endothelium-dependency of neuropeptide Y-induced responses. In the rat tail vasculature, it is possible that neuropeptide Y requires the presence of an endogenous endothelium-dependent constricting factor, such as endothelin, to elicit its vasoconstrictor response. The continuous infusion of endothelin-1 did not, however, permit neuropeptide Y-induced responses in the absence of endothelium. This could be because endothelin-1 is not the endogenous endothelium-dependent constricting factor involved in the neuropeptide Y response or that it is crucial for such a factor to be released endogenously from the endothelium to exert its permissive effect on the vascular smooth muscle. The actual endothelial mechanism of the neuropeptide Y response is currently under investigation.

It was of interest to investigate whether or not neuropeptide Y pre-constriction uncovers α_2-adrenoceptor-induced responses. Whilst vascular tone could not be elevated and sustained by neuropeptide Y in the isolated perfused vascular bed of the rat tail, in 2 experiments, infusions of neuropeptide Y (100–200 pmol min^{-1}) did not uncover UK-14,304-induced responses (data not shown). Aubert *et al.* (1988) found that neuropeptide Y potentiated pressor responses to noradrenaline but not to B-HT 933 in the conscious rat. However, Hieble *et al.* (1989) demonstrated that neuropeptide Y potentiated the α_2-adrenoceptor-mediated contraction in canine saphenous vein. This indicates that there may be species or tissue differences in this effect of neuropeptide Y. The potentiation of α_2-adrenoceptor- and neuropeptide Y-mediated responses by preconstriction is probably due to a generalized change such as an increase in $[Ca^{2+}]_i$ regardless of the effector pathway leading to such an increase. It follows from this that responses to both α_2-adrenoceptor agonists and neuropeptide Y would be extremely sensitive to changes in Ca^{2+} availability. Indeed, in the rat isolated tail artery, α_2-adrenoceptor agonist-induced responses are more sensitive than are α_1-adrenoceptor agonist-induced responses to the Ca^{2+} channel antagonists nifedipine and nicardipine, to decreased extracellular Ca^{2+} and to the Ca^{2+} entry promoter Bay K 8644 (Su *et al.*, 1986; Abe *et al.*, 1987). Neuropeptide Y-induced vasoconstriction in human skeletal muscle and renal arteries is sensitive to nifedipine (Pernow, 1988) and in the pithed rat, neuropeptide Y-induced potentiation of α-adrenoceptor activation is also Ca^{2+}-dependent (Dahlöf *et al.*, 1985).

The effect of endothelium removal on pressor responses induced by endothelin-1 and phenylephrine

Removal of the endothelium with CHAPS did not significantly affect the maximum responses to phenylephrine or its ED_{50} and significantly reduced the response at only one indi-

vidual dose. However, there is no positive evidence for endothelium-dependency of the phenylephrine-induced response. The effect of phenylephrine seems to be directly on the vascular smooth muscle and the ability of the vascular smooth muscle to contract is certainly not greatly affected by treatment with CHAPS. In some preparations, removal of the endothelium did increase the basal perfusion pressure. This could indicate a degree of tonic endothelium-derived relaxing factor release from the rat tail vasculature although, as this effect was not seen in all CHAPS-treated preparations, it may simply indicate a less specific 'damage'. More consistently, however, the time-course of the phenylephrine-induced response was markedly prolonged upon removal of the endothelium, usually by a factor of three and the onset of the response was slowed. Garland (1989) reported that, in the rabbit basilar artery, removal of the endothelium inhibited the production of phenylephrine-induced action potentials and that this was associated with a slower development in tension. A similar effect could explain the present increased time-course of the response to phenylephrine after CHAPS. Alternatively, in the rat tail vasculature under normal circumstances, the responses to phenylephrine may be buffered by release of an endothelium-dependent relaxing factor and/or facilitated by an endothelium-dependent constricting factor. This is such a clear effect that it deserves further study.

The most potent agonist used in this study was endothelin-1, being 10^4 times more potent than phenylephrine and 10^2 times more potent than UK-14,304 and neuropeptide Y (even in the presence of vascular tone). This is consistent with its very high potency in most vascular preparations on which it has been tested (e.g. Yanagasawa *et al.*, 1988; D'Orleans-Juste *et al.*, 1988). Removal of the endothelium did not significantly affect the responses to bolus injections of endothelin-1, although there was an increase in endothelin-1 infusion-induced perfusion pressure in some preparations following CHAPS treatment which may be due to the ability of prolonged infusions of endothelin-1 to induce release of endothelium-dependent relaxing factor from the intact endothelium (Warner *et al.*, 1989). As with phenylephrine, however, the evidence suggests that the vasoconstrictor effect of endothelin-1 is via receptors located on the vascular smooth muscle. The time course of the endothelin-1 induced response was reduced by removal of the endothelium and this was associated with slower development of the pressor response. Again, this may indicate that, in the endothelium-intact rat tail vasculature some endothelium-dependent factor may contribute to the development and maintenance of endothelin-1-induced responses.

In conclusion, in the rat isolated tail vasculature, the α_2-adrenoceptor agonist UK-14,304 did not induce vasoconstriction until the vascular tone of the preparation was increased with endothelin-1. Likewise, neuropeptide Y did not cause vasoconstriction until the vascular bed was preconstricted with either endothelin-1 or 5-hydroxytryptamine and the maximum response to neuropeptide Y was higher in the presence of 5-hydroxytryptamine. The neuropeptide Y-induced pressor responses were endothelium-dependent. It is suggested that a preconstriction-induced increase in $[Ca^{2+}]_i$ is necessary for responses to these two agonists. The prolonged, and potent responses to α_2-adrenoceptor agonists and neuropeptide Y, uncovered in the presence of increased vascular tone, may contribute to regional vasoconstriction observed in pathological conditions in which sympathetic nerve activity is increased.

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