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Relaxing actions of corticotropin-releasing factor on rat resistance arteries

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1 Although it well established that corticotropin-releasing factor (CRF) injected i.v. can cause hypotension and vasodilatation, there is no *in vitro* evidence that CRF acts as a vasodilator. We have therefore tested the hypothesis that the hypotensive effect of i.v. CRF is due to a direct vasodilator action by carrying out experiments *in vitro* on rat resistance arteries (i.d. 150–300 μm).

2 Initial *in vivo* experiments confirmed that CRF (1.5 nmol·kg^{-1}) injected i.v. caused hypotension in rats, this being partially antagonized by the CRF analogue CRF_{9-41}.

3 For the *in vitro* experiments, vessels were taken from the mesenteric, cerebral and femoral vascular beds, and mounted as ring preparations in an isometric myograph. The vessels were pre-contracted with one of 3 agonists (prostaglandin $F_{2\alpha}$, arginine vasopressin or noradrenaline) or with a high-potassium solution (K^+).

4 With maximal concentrations of the agonists, CRF caused relaxation of mesenteric and cerebral vessels with 10 nM, and near complete relaxation with 100 nM. Femoral vessels preconstricted with agonists and all vessels preconstricted with K^+ were less affected by CRF. In the mesenteric vessels, with sub-maximal levels of pre-constriction, CRF caused substantial relaxation at 1 nM and could cause complete relaxation at 10 nM.

5 The relaxant effect of CRF on contractions of mesenteric vessels was antagonized by 100 nM CRF_{9-41}. Neither tetraethyl ammonium (30 mM) nor glibenclamide (3 μM) antagonized the relaxant effect of CRF.

6 The relaxant effect of CRF on mesenteric small arteries was found to be unaffected by removal of the endothelium.

7 The results indicate that CRF causes an endothelial-independent vasodilatation of rat resistance arteries under *in vitro* conditions at concentrations which are consistent with this being an important cause of the hypotension observed with i.v. injection of CRF.

Keywords: Corticotropin-releasing factor (CRF); CRF_{9-41}; corticotropin-releasing hormone; resistance arteries; blood pressure; heart rate; vasodilatation

Introduction

Corticotropin-releasing factor (CRF), a 41-residue peptide, is secreted from the hypothalamus and conducted through a portal system to the anterior pituitary, where it causes the release of adrenocorticotropic hormone (ACTH) and other peptides derived from pro-opiomelanocortin (Vale *et al.*, 1981; Taylor & Fishman, 1991; Pralong *et al.*, 1991). CRF thus plays a key role in the hypothalamo-pituitary-adrenal axis which leads to the release of cortisol and catecholamines from the adrenal gland (Vale *et al.*, 1981; Owens & Nemeroff, 1991; Fisher, 1989). However, CRF is not only secreted from the hypothalamus: CRF can be released from the adrenal gland as a result of sympathetic stimulation (Edwards & Jones, 1988) or haemorrhage (Bruhn *et al.*, 1987), but the role of this extra-hypothalamic CRF is not known. Exogenous CRF has been found to have a number of effects: in calf, for example, CRF infused into the adrenal artery can cause the release of cortisol from the adrenal gland (Jones & Edwards, 1990), while i.v. infused CRF can inhibit thermal injury (Kiang & Wei, 1992). CRF has also been found to inhibit the protein leakage caused by substance P (Gao *et al.*, 1991). The cardiovascular effects of exogenous CRF depend on whether the drug is applied centrally or peripherally. Intracerebroventricular (i.c.v.) injection of CRF causes an increase in blood pressure and heart rate, a response dependent on an intact sympathetic nervous system (Brown & Fisher, 1983; Overton & Fisher, 1989), and may thus participate in the defence and stress reactions (Fisher & Brown, 1991). Intravenous (i.v.) injection in rats causes reduction in blood pressure, with a reflex rise in heart rate (Fisher *et al.*, 1983). Similar hypotensive actions of CRF have been seen in other species (Lenz, 1987; Kali *et al.*, 1983; Udelsman *et al.*, 1986), including man (Hermus *et al.*, 1987). The hypotensive action of CRF is caused by vasodilatation (Kiang & Wei, 1992), especially in the mesenteric bed (MacCannell *et al.*, 1986; Lenz, 1987; Gardiner *et al.*, 1988; Grosskreutz & Brody, 1988). The relaxation does not appear to be due to ACTH secretion (Pralong *et al.*, 1991), and it has been suggested that it is due to a direct action of CRF on the vascular smooth muscle (Corder *et al.*, 1992). This possibility has not, however, been tested on vascular preparations *in vitro*. We have therefore tested the hypothesis that CRF has a direct vasodilator action using rat resistance arteries (i.d. 150–300 μm) taken from the mesenteric, cerebral and femoral vascular beds, and mounted as rings in an isometric myograph (Mulvany & Halpern, 1977). The results showed that CRF can indeed have a direct vasodilator action, particularly for the mesenteric and cerebral resistance arteries. The specificity of the effect was tested by use of the CRF analogue, CRF_{9-41}, which has been shown to inhibit both the hypertensive effect of an i.c.v. CRF injection (Brown *et al.*, 1986) as well as the hypotensive effect of i.v. CRF injection (Corder *et al.*, 1992).

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Methods

Male Wistar rats (Møllegaard Breeding Centre, Ll Skensved, Denmark) weight ca. 320 g were used. All animals were housed in temperature and humidity-controlled quarters and provided with food and water *ad libitum*.

In vivo *experiments*

Rats were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}, i.p.) and polypropylene catheters (PE50, Portex) were inserted in the right femoral artery and right femoral vein. Blood pressure and heart rate were recorded from the femoral artery using a BIOMONITOR (Messgerätewerk Zwönitz, former GDR) pressure transducer and cardiotachometer. Data were calculated and analyzed with a computer.

After the surgical manipulations, about 30 min was allowed to stabilize the blood pressure and heart rate baselines before commencing investigations. Intravenous (i.v.) injections of saline (vehicle) or peptides were administered in a volume of 0.2 ml. The rats were divided randomly into three groups which were subjected to one of the following protocols: (a) CRF (1.5 nmol kg^{-1}) 15 min after i.v. injection of vehicle; (b) CRF (1.5 nmol kg^{-1}) 15 min after pretreatment with CRF_{9-41} (15 nmol kg^{-1}); (c) single i.v. injection of vehicle.

In vitro *experiments*

Preparation Segments (ca. 2 mm long) of small arteries were taken from the mesenteric (Mulvany & Halpern, 1977), cerebral (Aalkjaer *et al.*, 1985), and femoral (Nilsson & Mulvany, 1981) vasculatures, one or two vessels of each type, as described previously. The segments were mounted as ring preparations on a double isometric myograph (Mulvany & Nyborg, 1980) and set to an optimal lumen diameter, l_1, equal to 90% of the diameter the vessels were estimated to have when relaxed and under a transmural pressure of 100 mmHg, based on the relaxed wall tension-internal circumference relation (Mulvany & Halpern, 1977). The optimal diameters were 144 μm–306 μm (mesenteric),. 135 μm–245 μm (femoral) and 160 μm–270 μm (cerebral). During dissection and mounting the vessels were kept in oxygenated physiological salt solution (PSS, see below) at 37°C.

After equilibration for 30 min in PSS, arteries were activated in turn with control, activating solution (10 μM noradrenaline (NA) in 125 mM K^+, see below), twice, 10 μM NA, 125 mM K^+, and again with control activating solution, 2 min per activation, 5 min washout between, to test the contractility of the preparations. If the final response to control activating solution corresponded to an effective pressure (equal to wall tension $\times 2 \times l_1^{-1}$) less than 100 mmHg, the vessel was discarded (Mulvany & Halpern, 1977). The vessels were then equilibrated in PSS for a further 10 min.

Protocol CRF concentration-relaxation curves were obtained as follows. Vessels were contracted with one of three agonists (prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), arginine vasopressin (AVP) or NA) or with K^+, in the concentrations indicated for 5 min, after which CRF was applied in a cumulative fashion (10^{-9} M, 10^{-8} M and 10^{-7} M), for a period of 5 min for each concentration. Unless otherwise indicated, responses are given as the wall tension at the end of each period expressed as a percentage of the wall tension just before the first addition of CRF. Vessels were then washed in PSS for 20 min, before a new CRF concentration-relaxation curve was obtained with a different agonist. The order in which the agonists were applied was random. Time control experiments showed that the responses of all vessels to all agonists remained constant to within about 10% between 5 min and 20 min after adding the agonist. The responses were unaffected by the vehicle (see below).

Experiments designed to investigate the possible inhibitory effects of drugs were performed as follows. CRF concentration-relaxation curves, determined as above, were obtained first under control conditions, and then in the presence of the drug indicated. The vessel was exposed to the drug for 5 min before the start of the determination.

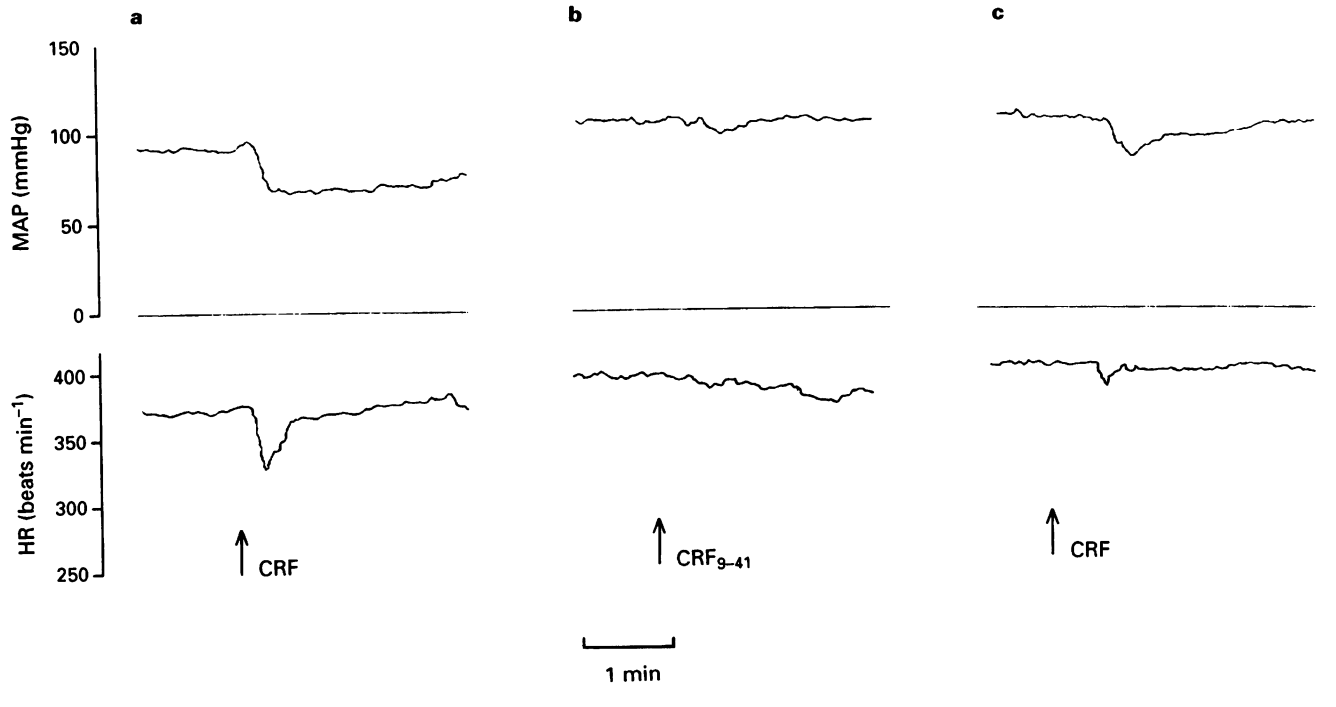

Figure 1 Measurements of mean blood pressure (MAP) and heart rate (HR) in anaesthetized Wistar rats. (a) Hypotensive action of i.v. injection of corticotropin releasing factor (CRF, 1.5 nmol kg^{-1}); (b) lack of effect of CRF_{9-41} on MAP and HR and later in same rat (c) partial antagonism of hypotensive effect of CRF when CRF_{9-41} had been injected 15 min previously. Note that hypotensive effect of CRF is maintained in (a), but is transient in (c).

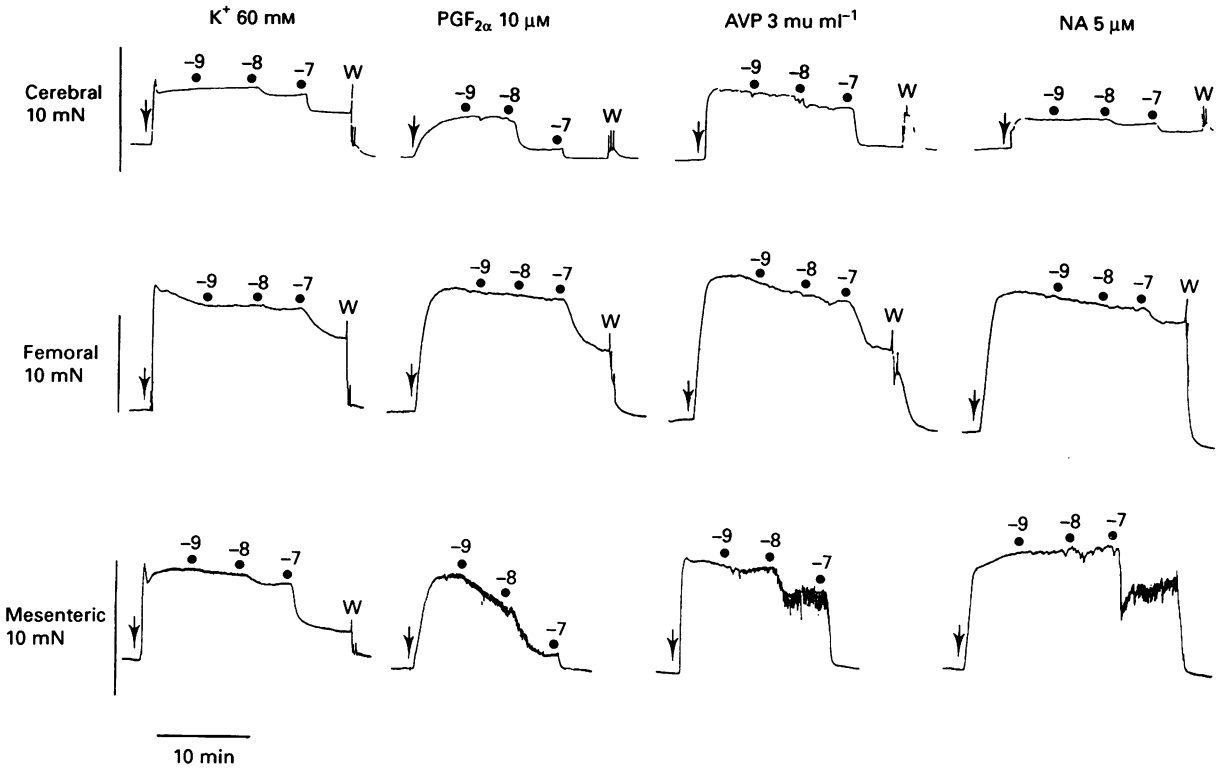

Figure 2 Traces showing relaxing effects of corticotropin releasing factor (CRF) on rat (from top) all cerebral, femoral and mesenteric small arteries precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10 μM) arginine vasopressin (AVP, 3 mu m^{-1}), noradrenaline (NA, 5 μM) or K^+ (60 mM), as indicated. CRF concentrations are given as log concentrations (M). Arrows indicate addition of agonists. Washout with PSS indicated by 'W'.

The possible influence of the endothelium was tested in mesenteric small arteries by obtaining CRF concentration-relaxation curves first under control conditions, and then after the endothelium had been removed by rubbing the inner surface of the artery with a human hair. Functional evidence for endothelial removal was obtained by lack of relaxation of vessels to 10 μM acetylcholine after precontraction with 10 μM NA.

Solutions Physiological salt solution (PSS) contained (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, ethylene-diamenetetraacetic acid 0.026, glucose 5.5. x mM K^+, as for PSS but with the indicated concentration (x) of K^+, obtained by an equimolar exchange of KCl for NaCl. Other substances used were arginine vasopressin (AVP, Sandoz), glibenclamide (Sigma), NA-HCl (NA, Sigma), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Upjohn), tetraethylammonium (TEA, Merck). Corticotropin-releasing factor (human CRF either as trifluoroacetate (CRF-TFA) or HCl (CRF-HCl) salts) and the CRF analogue CRF_{9-41} were kindly prepared by Drs M. Beyermann and E. Krause at the Institute of Molecular Pharmacology, Berlin, Germany. Stock solutions of CRF (0.5 mM) were prepared by dissolving CRF in 50% acetonitrile and 50% water. The CRF-TFA was used in the *in vivo* experiments, and both CRF-TFA and CRF-HCl were used in the *in vitro* experiments. *In vitro* investigations showed that CRF-TFA and CRF-HCl had identical potency and efficacy in relaxing responses due to 3 mu ml^{-1} AVP ($n = 8$), and the two forms were therefore treated as identical.

Statistics Data are expressed as mean ± s.e.mean. The data from the *in vivo* experiments were analyzed by one-way analysis of variance, followed by Dunnett's multiple comparison test. In the *in vitro* experiments, differences were tested for significance by paired two-tailed Student's *t* test. Significance was accepted at $P<0.05$. Degrees of freedom were based on number of rats in the *in vivo* experiments and number of vessels in the *in vitro* experiments.

Results

In vivo *experiments*

Bolus injection (i.v.) of CRF (1.5 nmol kg^{-1}) caused a reduction of mean blood (MAP) pressure, but no change in heart rate (HR) apart from a short-lasting transient drop (Figure 1a). MAP fell within a few seconds from 107 ± 7 mmHg to 79 ± 5 mmHg, the corresponding heart rates being 402 ± 15 beats min^{-1} and 398 ± 16 beats min^{-1} (8 rats). The blood pressure reduction was sustained for about 5–6 min. When the CRF injection was performed in 8 other rats pretreated with CRF_{9-41}, the fall in blood pressure was attenuated (mean blood pressure fell from 114 ± 7 mmHg to 98 ± 7 mmHg), and the fall was transient (Figure 1c). HR was not changed (392 ± 13 beats min^{-1} and 386 ± 14 beats min^{-1}, respectively). CRF_{9-41} (15 nmol kg^{-1}) injection alone did not affect MAP or HR (Figure 1b), nor did injection of vehicle (data not shown).

In vitro *experiments*

CRF caused a concentration-dependent relaxation of rat mesenteric, cerebral and femoral small arteries preconstricted with 10 μM $PGF_{2\alpha}$, 3 mu ml^{-1} AVP, 5 μM NA or 60 mM K^+ (Figures 2 and 3). CRF was least potent with K^+ preconstriction for all vessel types. In the femoral small arteries, CRF also had little effect against the other preconstrictors, substantial relaxations being seen only with 100 nM CRF. In

the cerebral small arteries, CRF caused significant relaxations with 10 nM CRF and close to complete relaxation with 100 nM CRF for all preconstrictors, except K^+. In the mesenteric small arteries, CRF was most potent in vessels preconstricted with $PGF_{2\alpha}$, giving a 50% relaxation with 10 nM CRF. Complete relaxation with 100 nM CRF was seen in vessels preconstricted with either $PGF_{2\alpha}$ or AVP.

The dependence of the relaxant effect of CRF on the type of preconstrictor used was found in mesenteric small arteries also to depend on the level of activation (Figure 4). Thus for low levels of activation (0.15 mu ml^{-1} AVP, 1 μM NA, 25 mM K^+), significant relaxation was observed with 1 nM CRF, while 10 nM CRF gave 50% relaxation with K^+ and complete relaxation with AVP.

The relaxant effect of CRF on contractions of mesenteric small arteries induced by AVP was antagonized by 100 nM CRF_{9-41} (Figures 5 and 6). The dose-ratio for 100 nM CRF_{9-41}

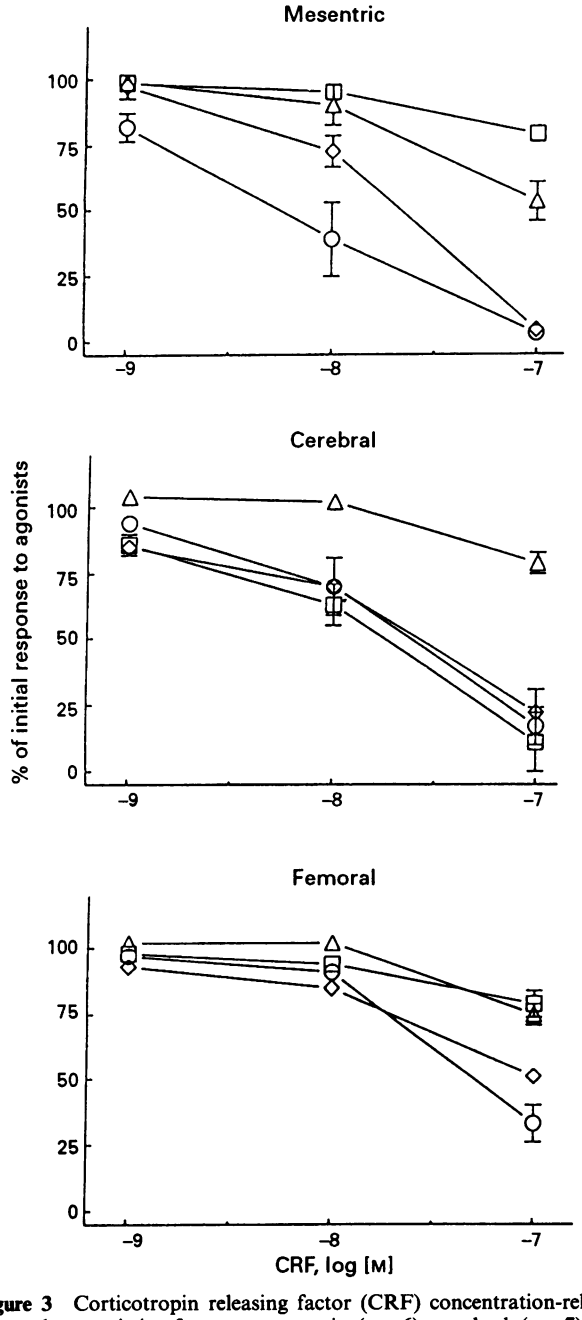

Figure 3 Corticotropin releasing factor (CRF) concentration-relaxation characteristics for rat mesenteric ($n = 6$), cerebral ($n = 7$) or femoral ($n = 6$) small arteries determined in experiments similar to those shown in Figure 2. Arteries activated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10 μM) (○), arginine vasopressin (AVP, 3 mu ml^{-1}) (◇), noradrenaline (NA, 5 μM) (□) or K^+ 60 mM (△). Values show mean ± s.e.mean of steady state tensions, expressed as percentage of initial tensions in the absence of CRF. The initial tensions were as follows. $PGF_{2\alpha}$: 2.3 ± 0.1 Nm^{-1} (mesenteric), 0.8 ± 0.1 Nm^{-1} (cerebral), 2.4 ± 0.3 Nm^{-1} (femoral). AVP: 2.5 ± 0.2 Nm^{-1} (mesenteric), 1.0 ± 0.2 Nm^{-1} (cerebral), 2.8 ± 0.3 Nm^{-1} (femoral). NA: 2.8 ± 0.3 Nm^{-1} (mesenteric), 0.5 ± 0.1 Nm^{-1} (femoral), 2.7 ± 0.4 Nm^{-1} (femoral. K^+: 2.2 ± 0.2 Nm^{-1} (mesenteric), 1.0 ± 0.2 Nm^{-1} (cerebral), 2.2 ± 0.3 Nm^{-1} (femoral).

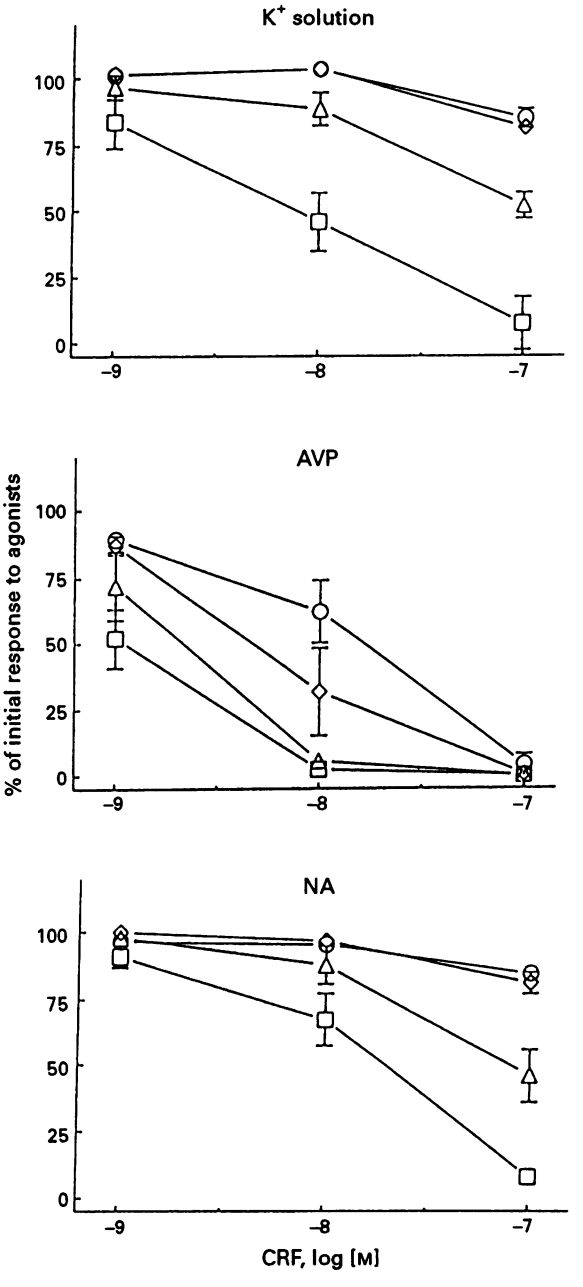

Figure 4 Corticotropin releasing factor (CRF) concentration-relaxation characteristics for rat mesenteric small arteries when activated with arginine vasopressin (AVP, $n = 6$), noradrenaline (NA, $n = 7$) or K^+ ($n = 6$). K^+: 25 (□); 40 (△); 80 (◇); 125 mM (○). AVP: 0.15 (□); 0.3 (△); 0.6 (◇); 3.0 mu ml^{-1} (○). NA: 1 (□); 2 (△); 5 (◇); 10 μM (○). Values show mean ± s.e.mean of steady state tensions, expressed as percentage of initial tensions in the absence of CRF. The initial tensions were as follows. AVP: 1.9 ± 0.3 Nm^{-1} (0.15 mu ml^{-1}), 2.3 ± 0.2 Nm^{-1} (0.3 mu ml^{-1}), 2.4 ± 0.2 Nm^{-1}(0.6 mu ml^{-1}), 2.5 ± 0.2 Nm^{-1} (3 mu ml^{-1}). NA: 1.8 ± 0.2 Nm^{-1} (1 μM), 2.2 ± 0.2 Nm^{-1} (2 μM), 2.5 ± 0.2 Nm^{-1} (5 μM), 2.8 ± 0.3 Nm^{-1} (10 μM). $K^{\pm}$: 0.4 ± 0.1 Nm^{-1} (25 mM), 1.0 ± 0.2 Nm^{-1} (40 mM), 2.5 ± 0.3 Nm^{-1} (80 mM), 2.6 ± 0.3 Nm^{-1} (125 mM).

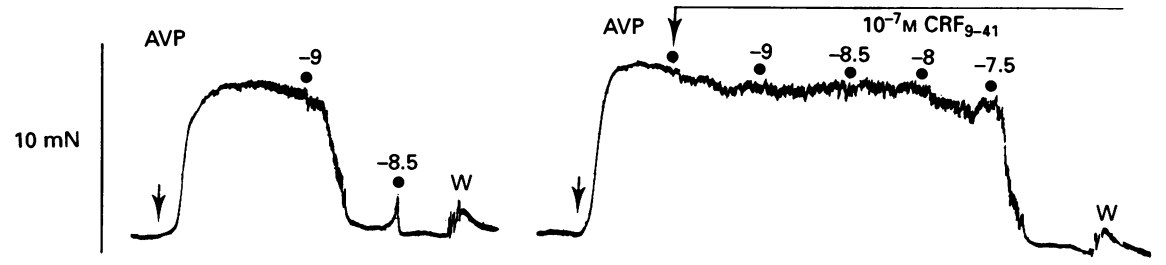

Figure 5 Traces showing inhibitory effects of CRF_{9-41} (100 nM) on the relaxing effects of corticotropin releasing factor (CRF) on rat mesenteric small arteries precontracted with arginine vasopressin (AVP, 0.3 mu ml^{-1}). CRF concentrations are given as log concentrations (M). Arrows indicate addition of agonists. Washout with PSS indicated by 'W'.

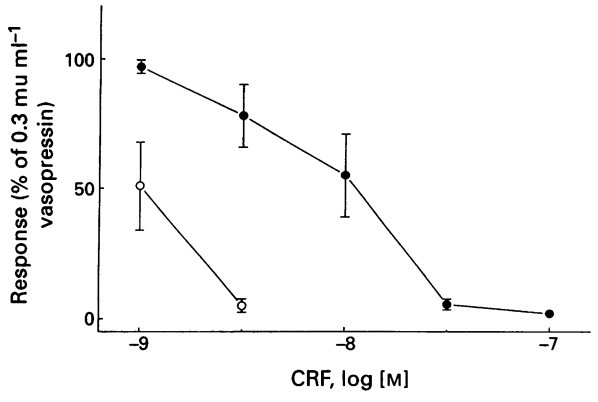

Figure 6 Effect of CRF_{9-41} (0.1 μM, ●) on the corticotropin releasing factor (CRF) concentration-relaxation characteristics of 7 rat mesenteric small arteries pre-contracted with 0.3 mu ml^{-1} AVP, determined as indicated in Figure 5. Control (○) curves show characteristics of the vessels concerned in the absence of CRF_{9-41}. Values show mean ± s.e.mean of steady state tensions, expressed as percentage of initial tensions in the absence of CRF. Initial tension was not significantly affected by CRF_{9-41}.

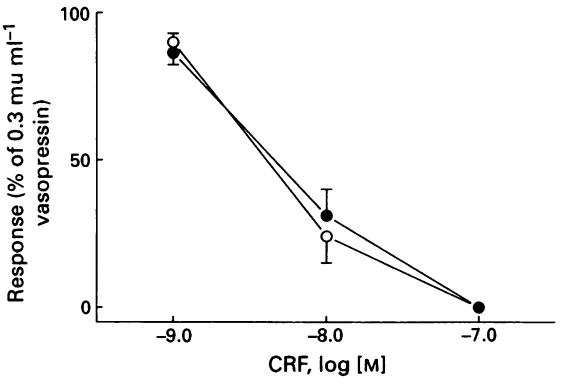

Figure 7 Lack of effect of removal of endothelium on the corticotropin releasing factor (CRF) concentration-relaxation characteristics of 6 rat mesenteric small arteries pre-contracted with 0.3 mu ml^{-1} arginine vasopressin: (●) intact vessels; (○) vessels after removal of the endothelium. Values show mean ± s.e.mean of steady state tensions, expressed as percentage of initial tensions in the absence of CRF. Initital tension was not significantly affected by removal of the endothelium.

Table 1 Effects of glibenclamide and tetraethyl ammonium (TEA) on the steady state wall tensions produced by corticotropin releasing factor (CRF) on rat mesenteric small arteries precontracted with 0.3 mu ml^{-1} (arginine vasopressin (AVP) (expressed as a percentage of precontraction levels)

	Number of vessels	[CRF] (nM) *1*	*10*	*100*
Glibenclamide, 3 μM	6	68 ± 6	12 ± 4	1 ± 1
Control	6	81 ± 6	27 ± 9	1 ± 1
TEA 30 mM	8	67 ± 10	27 ± 12	1 ± 1
Control	8	48 ± 14	14 ± 7	1 ± 1

Values show mean ± s.e.mean of steady state tensions, expressed as a percentage of initial tensions in the absence of CRF, either in the presence of the indicated drug or without drug (control). Glibenclamide reduced the initital tensions by 15% ($P<0.05$), but TEA did not affect the initial tensions significantly. No significant effects of glibenclamide or TEA on the effects of CRF were found.

was about 10. Neither TEA (30 mM) nor glibenclamide (3 μM) antagonized the relaxant effect of CRF (Table 1).

The relaxant effect of CRF on mesenteric small arteries was found to be unaffected by removal of the endothelium (Figure 7).

Discussion

The main result of this investigation is that CRF causes an endothelium-independent vasodilatation of rat resistance arteries under *in vitro* conditions. When precontracted with maximal concentrations of agonist drugs, the threshold for the relaxation of the mesenteric and cerebral resistance arteries was around 1 nM, but around 10 nM for the femoral resistance arteries. The vasodilator effect was inhibited by 100 nM CRF_{9-41}. The results support the hypothesis that the vasodilator action of i.v. CRF injection is mediated through a direct action on the vascular smooth muscle.

Although a number of peptides closely related to CRF, such as urotensin I (Gerritsen *et al.*, 1981; Bolt *et al.*, 1989), have been investigated *in vitro*, the *in vitro* actions of CRF on vascular smooth muscle have not been investigated previously. This investigation is therefore the first to demonstrate that CRF can have a direct vasodilator action. Moreover, the vessels which we have examined are small enough to contribute substantially to the peripheral resistance (Baumbach *et al.*, 1988; Christensen & Mulvany, 1992), and therefore the finding that they are relaxed by CRF is consistent with the reduction in peripheral resistance caused by i.v. CRF injection being due to a direct action on the vasculature (Fisher, 1989). Furthermore, our observation that CRF was more potent in the mesenteric resistance arteries than in the femoral resistance arteries is consistent with previous observations that i.v. CRF injection caused specific vasodilatation of the mesenteric and renal vascular beds compared to the

hindquarter vascular bed (Gardiner *et al.*, 1988; Grosskreutz & Brody, 1988). However, since CRF was also a potent dilator of the cerebral resistance arteries, it may be that CRF is a general vasodilator, the femoral vasculature being exceptional. Further experiments on other vascular beds, as well as different sizes of vessel, both more proximal and more distal, are required to analyze this. It may be noted that urotensin I also causes selective dilatation of rat mesenteric arteries (Bolt *et al.*, 1989), consistent with i.v. injection of this peptide causing selective dilatation of the mesenteric circulation compared to the hindquarters circulation (MacCannell & Lederis, 1983; Lederis *et al.*, 1983).

The CRF dose given in the present experiments (1.5 nmol kg^{-1}) is similar to that used by other investigators to obtain similar hypotensive effects (Gardiner *et al.*, 1988; Corder *et al.*, 1992); Corder *et al.* (1992) used 2 nmol kg^{-1} and measured a resulting plasma CRF concentration of 50 nM. In other experiments, steady infusions of CRF have been given (e.g. 0.5 nmol kg^{-1} min^{-1} which caused a 40% drop in mean blood pressure over 30 min; Pralong *et al.*, 1991). In the present experiments, assuming that CRF is distributed alone in the plasma (volume ca. 30 ml kg^{-1} per rat), the plasma concentration of CRF resulting from a bolus injection of 1.5 nmol kg^{-1} CRF would be ca. 50 nM. Although the assumptions behind this calculation are uncertain, the concentration estimated in this manner corresponds quite well to the concentrations giving relaxation in the present *in vitro* experiments. This is particularly true under conditions where the level of activation in the pre-constriction was not maximal (Figure 4), where significant relaxation was seen with 1 nM CRF with all agonists used, and up to full relaxation could be obtained with 10 nM CRF. It should, however, be noted that lower concentrations of CRF are able to stimulate ACTH secretion in cultured pituitary cells (Rivier *et al.*, 1984; Corder *et al.*, 1992), where under 1 nM CRF gives a half maximal stimulation. The potency of CRF as a vasodilator also appears to be less than urotensin I, where the IC_{50} concentrations of the peptide are less than 1 nM (Lederis *et al.*, 1983).

The mechanism by which CRF causes relaxation is not known. The finding that CRF_{9-41} inhibited the effect of CRF suggests that the effect is specific, this conclusion being supported by the demonstration of specific CRF binding sites in vascular tissue, such as the rabbit aorta (Dashwood *et al.*, 1987). Since we found that the dilator effect was not diminished by removal of the endothelium, the mechanism appears to be endothelial-independent, even though the work of Dashwood and colleagues (1987) indicated that the aortic endothelium contained CRF binding sites. Our finding that CRF was less potent against vessels precontracted with 125 mM potassium, compared to the potency against vessels precontracted with drugs, suggests that the relaxation could be mediated by hyperpolarization (which would be prevented in a high potassium solution), possibly though the activation of K-channels. However, since neither glibenclamide (an inhibitor of ATP-sensitive K-channels) nor TEA (a less specific inhibitor of K-channels) affected the CRF-induced relaxation, this possibility seems less likely. The vasodilator effect of CRF *in vitro* is in contrast to the *in vitro* inotropic effect of CRF on guinea-pig ventricular myocardium, where it was suggested that CRF induces slow inward calcium currents (Saitoh *et al.*, 1990).

The right-ward shift in the CRF concentration-relaxation relation (Figure 6) caused by CRF_{9-41} is similar to the right-shifts caused by this analogue in the dose-response relations regarding the hypotensive effect of i.v. CRF injection (Corder *et al.*, 1992). In primary cultures of rat anterior pituitary cells, 0.5–1 μM CRF_{9-41} caused a right-shift in the EC_{50} of the CRF concentration-ACTH release curve of about 1 log unit (Rivier *et al.*, 1984; Corder *et al.*, 1992), but these data are probably confounded by the intrinsic activity which was also found for CRF_{9-41} (Corder *et al.*, 1992). Corder and colleagues (Corder *et al.*, 1992) also found that the hypotensive action of i.v. injection of 2 nmol CRF was antagonized by prior i.v. injection of a total of 87.5 nmol CRF_{9-41}. The present experiments indicated that 100 nM CRF_{9-41} caused a right-shift in the CRF concentration-relaxation curve of about 1 log unit, which would suggest that the dissociation constant of the CRF binding site for CRF_{9-41} is of the order of 10 nM (dissociation constant = (antagonist concentration)/(dose-ratio − 1)), neglecting any possible intrinsic activity of CRF_{9-41}.

In contrast to the well-established central effects of CRF (Vale *et al.*, 1981; Fisher, 1989; Chrousos & Gold, 1992), the possible physiological or pathophysiological role of plasma CRF remains unclear. Physiological levels of plasma CRF are normally low (e.g. 5 pg ml^{-1} in calves (Edwards & Jones, 1988); under 10 pM in athletes (Hohtari *et al.*, 1992); under 10 pg ml^{-1} in dog (Bruhn *et al.*, 1987)). The plasma concentrations of CRF resulting from the increase in adrenal release of CRF as a result of haemorrhage (Bruhn *et al.*, 1987) have not been reported, but since the adrenal secretion reached only 185 pg min^{-1} the effects on plasma CRF will have been minimal. The only situation in which high levels of plasma CRF have been reported is during human pregnancy, where plasma CRF levels rise dramatically to around 1700 pg ml^{-1} (Campbell *et al.*, 1987) or 2215 pg ml^{-1} (Sasaki *et al.*, 1987) at term, and 4409 pg ml^{-1} (ca. 1 nM) in labour (Sasaki *et al.*, 1987). At these levels, the present data indicate that the direct vasodilator action of CRF could be relevant. The data do not exclude the possibility that lower levels of CRF might modulate the vasoactive actions of other hormones.

In summary, the results indicate that CRF causes an endothelial-independent vasodilatation of rat resistance arteries under *in vitro* conditions. The concentrations causing relaxation are consistent with this direct action of CRF on vascular smooth muscle being an important cause of the hypotension observed with i.v. injection of CRF.

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