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Endothelin-1 and the periaqueductal gray area of the rat: an autoradiographic and functional pharmacological study

[1,*†‡]Michele D'Amico, †Michael R. Dashwood & ‡Timothy D. Warner

*Institute of Pharmacology and Toxicology, 2nd University of Naples, Italy; †Department of Physiology, Royal Free Hospital, Rowland Hill Street, London and ‡The William Harvey Research Institute, Charterhouse Square, London

1 Endothelin-1 (ET-1) injected centrally induces pressor effects and associated haemodynamic changes. Here we have evaluated the effects on systemic and regional cardiovascular parameters of injection of ET-1 into the periaqueductal gray (PAG) area of anaesthetized rats. In addition, we have used the ET_A receptor-selective antagonist, FR 139317, the ET_B receptor-selective antagonist, BQ-788, and the ET_A/ET_B receptor non-selective antagonist, SB 209670, to identify the receptor(s) mediating these effects. We have also used *in vitro* autoradiography to identify binding sites for ET-1 in the PAG.

2 *In vitro* autoradiography showed dense binding of [^{125}I]-PD 151242 (for ET_A receptors) in the PAG area, with the binding sites being homogeneously distributed within the dorsal, lateral and ventral subregions. Tissues incubated with [^{125}I]-BQ 3020 (for ET_B receptors) had little binding.

3 Injection of ET-1 (0.1, 1 and 10 pmol per rat) in the dorsolateral PAG area significantly increased, in a dose-dependent manner the mean arterial blood pressure (MAP). The highest dose of ET-1 (10 pmol) also decreased the heart rate by $18 \pm 1\%$, $n=6$ ($P<0.05$). Increases in blood pressure induced by ET-1 (1 pmol; 31 ± 6.6 mmHg, $n=6$) were greatly reduced by pre-administration to the PAG area of FR 139317 (5 nmol per rat) or SB 209670 (3 nmol per rat) (97 and 94%, respectively), but were unaffected by BQ-788 (5 nmol per rat). Similarly, FR 139317 and SB 209670 prevented the decrease in heart rate induced by ET-1 while BQ-788 did not affect it.

4 Injection of ET-1 to the PAG area caused falls in renal blood flow (RBF) as measured by an ultrasonic flow probe, and increased renal vascular resistance (RVR). Pre-treatment of the PAG with FR 139317 or SB 209670, but not with BQ-788, prevented this ET-1-induced effect.

5 Injection of ET-1 (10 pmol) also increased total peripheral resistance (TPR; control, 2.39 ± 0.2 mmHg ml^{-1} min 100 g body weight) by $100 \pm 9\%$ ($n=5$) and reduced the cardiac output (CO; control, 94.7 ± 3.1 ml min^{-1}) by $30 \pm 3\%$ ($n=5$), as determined by radioactive microspheres. Vascular resistances were increased in other organs, such as skeletal muscle ($88 \pm 5\%$, $n=4$), the colon ($55 \pm 7\%$, $n=4$) and the stomach ($47 \pm 3\%$, $n=4$). Pretreatment of the PAG area with FR 139317 or SB 209670 reduced the increases in TPR and vascular resistance, and the reduction in CO caused by ET-1. BQ-788 did not affect the responses to ET-1.

6 Thus, there are predominantly ET_A binding sites within the PAG area and injection of ET-1 into the PAG area causes complex haemodynamic changes which are sensitive to ET_A receptor antagonism. ET_A receptors are, therefore, the predominant mediators of the actions of ET-1 in the PAG of the rat.

Keywords: Endothelin-1; periaqueductal gray; autoradiography; haemodynamic changes; ET_A/ET_B receptors

Introduction

Endothelin-1 (ET-1) has been implicated as a regulator of blood pressure via local actions within the circulation (see Rubanyi & Polokoff, 1994). However, ET-1 also has marked central effects, and when injected into the periaqueductal gray (PAG) area of rats induces marked pressor responses (D'Amico *et al.*, 1994; 1995). Currently it is not known which of the two ET receptor subtypes, ET_A and ET_B (Arai *et al.*, 1990; Sakurai *et al.*, 1990), mediates this pressor response. To investigate this we have examined the effects of the endothelin receptor antagonists, FR 139317 (ET_A receptor selective, Sogabe *et al.*, 1992), SB 209670 (ET_A/ET_B receptor non-selective, Ohlstein *et al.*, 1994) and BQ-788 (ET_B receptor selective antagonist, Ishikawa *et al.*, 1994) on the responses following administration of ET-1 to the PAG. In particular we have evaluated changes in regional blood flows, with special regard to the renal vascular bed, following these manipulations. We have also used *in vitro* autoradiographic studies to identify binding sites for ET-1 in the PAG.

[1] Author for correspondence at: Institute of Pharmacology and Toxicology, Faculty of Medicine and Surgery, 2nd University of Naples, Via Costantinopoli 16, 80138 Naples, Italy.

Methods

Autoradiographic study

Autoradiography was performed in four rats which were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, *i.p.*) and then perfused intracardially with ice-cold 100 mM phosphate buffer (pH 7.4) containing 300 mM sucrose and lightly fixed with 0.1% formaldehyde in phosphate/sucrose buffer. The brains were removed and 10 μm sections were cut in a cryostat at -20°C and thaw-mounted on to gelatinized microscope slides. Endogenous peptide levels were reduced by pre-incubating slide-mounted tissue in 50 mM tris buffer. [^{125}I]-ET-1 binding was determined in the presence of 100 or 150 pM of radioligand (Dashwood *et al.*, 1994) and receptor subtypes were identified with [^{125}I]-PD 151242 (ET_A, Davenport *et al.*, 1994) and [^{125}I]-BQ 3020 (ET_B, Molenaar *et al.*, 1992). Non-specific binding was established by incubating sections in the presence of 1 μM ET-1. Autoradiographs were generated by exposing sections to Hyperfilm ^{3}H and LM-1 nuclear emulsion (Amersham International) as described previously (Moody *et al.*, 1990). Tissue sections were stained with haematoxylin and eosin or neutral red for subsequent histology. Autoradiographs and tissue sections were then viewed on a Nikon macro system and photographed where appropriate.

In vivo *experiments*

Surgical procedure Male Wistar rats (250–300 g) were anaesthetized with urethane ethyl carbamate (1.2 g kg^{-1}, *i.p.*) and catheterised through the femoral artery for measurement of blood pressure (pressure transducer, Elcomatic type 750). The animals, while spontaneously breathing, were then placed in a stereotaxic head frame and the dorsal surface of the brain exposed by a craniotomy to permit intracerebral microinjections using a Hamilton 10 μl syringe supported in a stereotaxic micromanipulator. The co-ordinates of the atlas of Paxinos and Watson (1986) (measured in mm from the bregma: posteriorly, −7.8; laterally, 0.8; vertically, 4.5) were used to position the needle tip of the microsyringe. Another catheter was inserted into a jugular vein for the systemic administrations of agents, and for the administration of saline (1.5 ml h^{-1}) to compensate for any fluid loss. The left kidney was exposed *via* a mid-line laparotomy and the renal artery was isolated. An ultrasonic flow probe (internal diameter = 1 mm), embedded in a silicone cuff to provide optimal alignment, was placed around the left renal artery for measurement of total renal blood flow (RBF) using a Transonic T206 Flowmeter (Transonic Systems Inc., New York, U.S.A.). A small amount of acoustical couplant (100 mg Nalco 1181, mixed with 10 ml distilled water; Nalco Chemical Co., IL, U.S.A.) was deposited in the acoustic window of the probe adjacent to the artery, in order to displace all air. Renal vascular resistance (RVR) was calculated by dividing MAP by RBF. Under these conditions rectal temperature was monitored and maintained between 37 and 38°C by means of a heating blanket (Bioscience, Sheerness, Kent). Intracerebral injections were given in a total volume of 1 μl over a period of 10 s. After some experiments, the positioning of the injection site was checked histologically. Five minutes before the rat was killed with a high dose of sodium pentobarbitone (200 mg kg^{-1}, *i.v.*), 100 nl methylene blue (0.2%) was injected intracerebrally. Each animal was perfused intracardially with 50 ml of phosphate-buffered saline followed by 50 ml of a 10% formalin solution in phosphate-buffered saline. The brain was removed and immersed in saturated formalin for 24 h. The injection site was verified using 2 consecutive sections (40 μm), one stained with cresyl violet to identify nuclei and the other unstained to determine the dye diffusion.

Regional blood flows Regional blood flows were evaluated in a separate series of experiments using rats prepared as described above with regard to exposure of the PAG area. In addition, the animals were catheterised into the right femoral artery for measurement of systemic blood pressure while the left femoral artery was cannulated and connected to a syringe pump (Perfusor VI, Braun, Melsungen AG, Germany) for the later withdrawal of a reference blood sample. For the injection of microspheres, a catheter was inserted into the left ventricle via the carotid artery. Intra-catheter pressure was monitored throughout to indicate when the left ventricle had been properly entered. The rats were then placed in the stereotaxic frame and i.c injections given as described above. 60,000–80,000 ^{57}Co labelled microspheres (15±3 μm diameter) were suspended in 0.3 ml 0.9% w/v saline containing 0.01% w/v polyoxyethylene 80 sorbitan mono-oleate (Tween 80), a detergent added to prevent microsphere aggregation. The microsphere suspension was drawn into a syringe and injected into the left ventricle over a 20 s period at the peak of the hypertensive response to ET-1 (1 pmol). The cannula was then flushed through with a further 0.3 ml of vehicle. A reference blood sample was concurrently withdrawn at a rate of 0.5 ml min^{-1} during, and for 70 s following, the injection period. After the reference blood sample had been removed the animals were immediately killed with an air embolism and the tissues were dissected out, weighed and placed in vials. Organs with a low blood flow per unit mass (e.g. skeletal muscle) had multiple samples taken to ensure that a minimum of 400 microspheres were counted for each tissue, thus compensating for any random variability in microsphere distribution. The reference blood sample and the injection syringe and cannula were also placed in vials. All the vials were then counted for radioactivity in a gamma counter (Nuclear Enterprises, NE 1600) for 5 min on the same day to avoid count variations due to radioactive decay. The amount of radioactivity, and thus the number of microspheres, injected into the rat were calculated by subtracting the counts for the waste (i.e. microspheres caught in the injection syringe and cannula etc.) from the total starting radioactivity.

Cardiac output (CO) was calculated using the reference blood sample method described by McDevitt and Nies (1976). The fraction of cardiac output received by an organ and organ vascular resistance were calculated as described by Thomas *et al.* (1988).

Experimental protocol After a 30 min stabilisation period ET-1 was injected into the PAG area in consecutive doses of 0.1, 1, 10 pmol, each injection being made when the blood pressure had returned to its basal value, to construct dose-response curves. After this, antagonist studies were performed using submaximal doses of ET-1 (1 pmol per rat) and two different doses, 0.5 and 5 nmol for FR 139317 and BQ-788, or 0.3 and 3 nmol for SB 209670, of each antagonist. In these studies, the reproducibility of the pressor response to ET-1 was established using consecutive injections of ET-1. If the pressor response was reproducible the lowest dose of antagonist was microinjected, followed 10 min later by another injection of ET-1. If the response to ET was unaffected, the highest dose of antagonist was given. During all experiments the arterial blood pressure was continually monitored. In addition, heart rate was monitored in some experiments.

Materials

ET-1 was purchased from Peptide Institute (Osaka, Japan), and urethane ethyl-carbamate from Sigma Chemical Co (Poole, Dorset, UK). The microspheres were obtained from Du Pont, NEN research products, (Boston, U.S.A.). [^{125}I]-ET-1 (specific activity 2000 Ci $mmol^{-1}$), [^{125}I]-PD 151242 ((*N*-[(hexahydro-1-azepinyl)carbonyl]L-Leu-(1-Me)D-Trp-D-Tyr)), [^{125}I]-BQ 3020 ([Ala^{11,15}]Ac-ET-1$_{(6-21)}$), Hyperfilm ^{3}H, LM-1 nuclear emulsion were all purchased from Amersham International (Amersham, Bucks, U.K.). For the *in vivo* experiments, FR 139317 ((*R*-2-[(*R*)-2-[(*S*)-2-[[1-hexahydro-1H-azepinyl)] carbonyl] amino-4-methylpentanoyl]- amino-3-(2-pyridyl)propionic acid) (Parke Davis Pharmaceutical Research) (an ET_A receptor selective antagonist, Sogabe *et al.*, 1992), SB 209670 ((+)-(1*S*,2*R*,3*S*)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid) (Banyu Pharmaceutical Co., Japan) (an ET_A/ET_B receptor non-selective antagonist, Ohlstein *et al.*, 1994) and BQ-788 (*N*-*cis*-2,6-dimethylpiperidinocarbonyl-L-γ-metLeu-D-1-methoxy-carbonylTrp-D-Nle) (Hoechst AG, Frankfurt, Germany) (an ET_B receptor selective antagonist, Ishikawa *et al.*, 1994) were dissolved in 0.9% NaCl. ET-1 was reconstituted in 0.1% v/v acetic acid and then dissolved in 0.9% w/v saline containing 0.1% w/v bovine serum albumin and 10 mM sodium bicarbonate. Control injections were carried out with saline containing the same amount of solvent as the drug solutions. These did not produce any changes in blood pressure.

Statistics

All results are expressed as mean±standard error (s.e.), with $P<0.05$ being considered significant. Cardiovascular changes were compared by analysis of variance (ANOVA) and Newman Keuls test for multiple comparisons (Tallarida & Murray, 1987). In experiments with microsphere injections groups were compared by unpaired Student's *t* test or Mann Whitney U test as appropriate. $P<0.05$ was taken as significant.

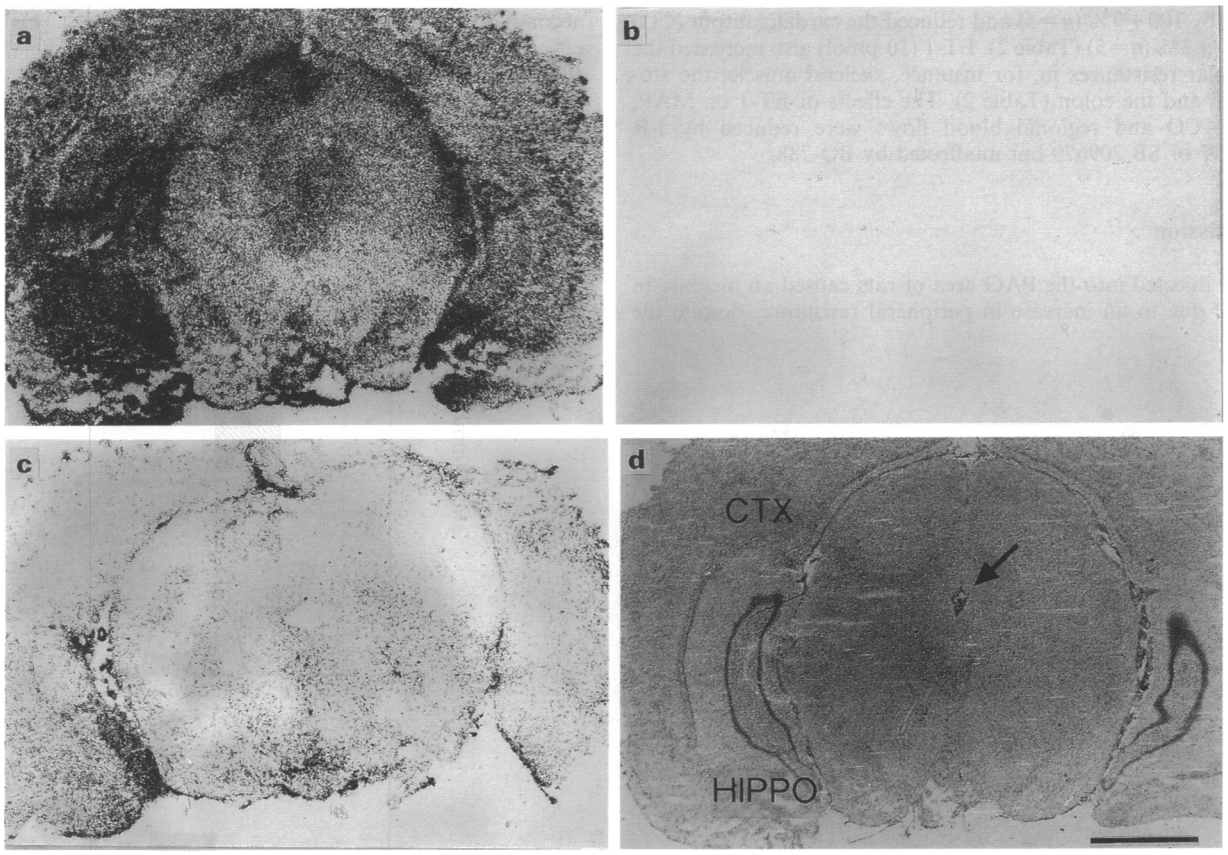

Figure 1 Autoradiographic localization of endothelin-1 (ET-1) binding in rat brain. (a) Film autoradiograph of [^{125}I]-PD 151242 (ET_A sites) binding to coronal section of rat brain. (b) Binding to section incubated with [^{125}I] PD 151242 in the presence of 1 μM unlabelled ET-1 (non-specific binding). (c) Autoradiograph from brain section incubated with [^{125}I]-BQ 3020 (ET_B sites). (d) Representative brain section (mm from the bregma: posteriorly, −7.8; laterally, 0.8; vertically, 4.5; level according to atlas of Paxinos & Watson, 1986) stained with neutral red. Arrow indicates aqueduct, CTX = cortex, HIPPO = hippocampus. Scale bar = 250 μm.

Results

Autoradiographic study

There was a dense binding of [^{125}I]-ET-1 to various areas of the rat brain, as described previously (Koseki *et al.*, 1989). Binding was reduced by >90% when sections were incubated in the presence of excess unlabelled peptide (non-specific binding <10%) as assessed in a gamma counter. ET-1 binding to transverse sections of the brain, cut at the level of the PAG area, was predominantly to ET_A sites ([^{125}I]-PD 151242 binding) (Figure 1a). Binding was associated with the PAG (dorsal, lateral and ventral subregions) as well with the hippocampus and other regions such as the cortex and amygdala. Only weak binding of [^{125}I]-BQ 3020 (ET_B) was detected at this level, and was predominantely confined to the amygdala and ventral PAG area (Figure 1c).

Effects of ET-1 injections into the PAG area on blood pressure and heart rate

Injection of ET-1, 0.1, 1 and 10 pmol per rat, in the dorsolateral PAG area significantly increased, in a dose-dependent manner, the mean arterial blood pressure (MAP) from a basal level of 104±7.8 mmHg (n=13). The durations of the pressor responses to these doses of ET-1 were, 11±2 min, 25±7 min and 41±8, respectively (n=13). ET-1 at the highest dose used also decreased the heart rate (Figure 2b; saline, 470±30 b.p.m., n=6). Increases in MAP induced by ET-1 (1 pmol) were greatly reduced by FR 139317 (5 nmol) or SB 209670 (3 nmol), but were unaffected by BQ 788 (5 nmol) (Figure 3a). FR 139317 and SB 209670 also prevented the decrease in heart rate induced by ET-1 (10 pmol), which was unaffected by BQ-788 (Figure 3b). BQ-788, FR 139317 and SB 209670 had no effects on heart rate or systemic parameters when injected into the PAG alone (n=6). Lower doses of antagonists (SB 209670, 0.3 nmol per rat; FR 139317 and BQ-788, 0.5 nmol per rat) did not affect the ET-1-induced responses.

Effects of ET-1 on the renal vasculature as measured by flow probe

Injection of ET-1 to the PAG caused a fall in left renal blood flow (RBF) and an associated increase in renal vascular resistance (RVR) (Table 1). The peak decrease in RBF coincided with the peak increase in MAP (8±2 min, n=13). However, following injection of 10 pmol ET-1 the change in RBF lasted up to 80 min, which was longer than the accompanying change in MAP. Pretreatment of the PAG with FR 139317 or SB 209670 but not BQ-788 prevented the ET-1-induced (1 pmol) effects on RBF (Table 1).

Effects of ET-1 on systemic measurements and regional blood flows as measured by radioactive microspheres

Injection of ET-1 into the PAG area caused systemic and regional haemodynamic changes, as measured by microsphere injection 8 min after ET-1 application. For instance, ET-1 (10 pmol) increased the MAP by 37±3 mmHg (n=6) and

TPR by 100±9% ($n=5$) and reduced the cardiac output (CO) by 30±3% ($n=5$) (Table 2). ET-1 (10 pmol) also increased the vascular resistances in, for instance, skeletal muscle, the stomach, and the colon (Table 2). The effects of ET-1 on MAP, TPR, CO and regional blood flows were reduced by FR 139317 or SB 209670 but unaffected by BQ-788.

Discussion

ET-1 injected into the PAG area of rats caused an increase in MAP due to an increase in peripheral resistance, despite the accompanying decrease in cardiac output (CO). This latter effect probably being related to reductions in stroke volume (Beyer *et al.*, 1994) and heart rate, following central activation of the baroreflex arc. In particular, we found that ET-1 reduced renal blood flow, which is in contrast to the increase in

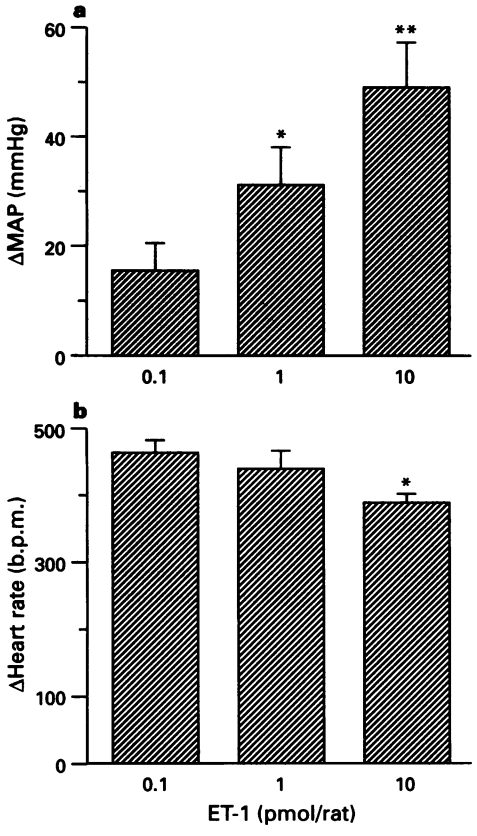

Figure 2 (a) Changes in mean arterial blood pressure (ΔMAP) after microinjection of endothelin-1 (ET-1, 0.1, 1 and 10 pmol) into the dorsolateral periaqueductal gray (PAG) area of rats. Each column represents the mean of 13 observations ±s.e. Significant differences from vehicle-treated animals are shown by asterisks (*$P<0.05$ and **$P<0.01$). (b) Changes in heart rate (b.p.m.) after microinjection of ET-1 (0.1, 1 and 10 pmol) into the dorsolateral PAG area of rats. Each column represents the mean of 6 observations ±s.e. Significant differences from groups treated with vehicle are shown by *$P<0.05$.

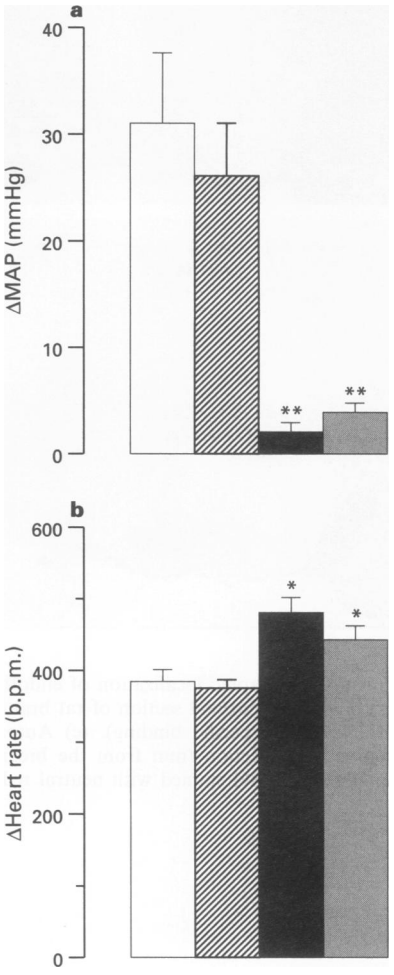

Figure 3 (a) Changes in mean arterial blood pressure (ΔMAP) after microinjection of endothelin-1 (ET-1, 1 pmol, open column) into the dorsolateral periaqueductal gray (PAG) area of rats treated, 10 min before, with BQ 788 (5 nmol, hatched column), FR 139317 (5 nmol, solid column) or SB 209670 (3 nmol, stippled column). Each column represents the mean of 6 observations±s.e. Significant differences from groups treated with ET-1 are shown as **$P<0.01$. (b) Changes in heart rate (b.p.m.) after microinjection of ET-1 (10 pmol) in presence of antagonists (columns as for a). Each column represents the mean of 6 observations±s.e. Significant differences from groups treated with ET-1 are shown as *$P<0.05$.

Table 1 Effects of endothelin-1 (ET-1) on renal blood flow

	RBF (ml min^{-1})	*Change in RBF* (%)	*RVR* (mmHg ml min^{-1})	*Changes in RVR (%)*
Control	9.9±0.8		11.7±1.4	
ET (0.1)	8.6±0.7	−13±1	11.9±1	2±1
ET (1)	7.9±0.3*	−20±5	14.1±0.4*	20±4
ET (10)	7.3±1.1*	−27±9	14.8±0.9*	27±3
ET (1)+FR 139317	9.4±1	−5±1	12.0±1	3±1
ET (1)+SB 209670	8.9±0.7	−10±1	12.9±1.3	10±2
ET (1)+BQ-788	8.0±0.4*	−20±3	14.2±0.9*	21±4

Changes in renal blood flow (RBF) and renal vascular resistances (RVR), as evaluated with ultrasonic flow probe, after injection of ET-1 (0.1–10 pmol) into the PAG area of anaesthetized rats, treated or not, 10 min before the ET-1 injection, with FR 139317 (5 nmol), SB 209670 (3 nmol) or BQ-788 (5 nmol). Data represent the mean of 13 observations. Significant differences versus control group are shown by an asterisk (*$P<0.05$).

Table 2 Effects of endothelin-1 on cardiovascular parameters

	Control	*ET-1*	*ET-1 + BQ-788*	*ET-1 + SB 209670*	*ET-1 + FR 139317*
MAP (mmHg)	99±5	136±4**	132±5**	116±7°	101±4°°
CO (ml ml^{-1})	94.7±3.1	66.6±8.2**	69.3±7.2**	75.9±4.9°	93±2.3°°
TPR (mmHg ml^{-1} min) per 100 g body weight	2.4±0.3	5.4±0.4**	4.8±0.9*	3.8±0.7°	2.5±0.1°°
Organ vascular resistances (mmHg min ml^{-1})					
Skeletal muscle	3.55±0.4	7.66±2.2*	7.27±2.0*	4.16±0.6°	3.54±0.8°
Stomach	30.59±0.9	44.50±3.9**	42.68±5.8*	30.75±3.1°	30.03±2.0°°
Right kidney	27.65±1.6	125.25±13.2**	127.41±11.9**	94.45±8.2°	27.19±1.8°°
Left kidney	24.44±1.1	112.13±11.6**	113.87±10.5**	84.27±7.2°	25.94±1.6°°
Colon	32.82±3.5	51.45±7.4*	48.54±8.9*	39.20±1.6°	30.73±1.8°°

Cardiovascular parameters after injection of endothelin-1 (ET-1; 10 pmol) or vehicle into the periaqueductal gray (PAG) area of anaesthetized rats, treated, 10 min before the ET-1 injection, with FR 139317 (5 nmol), SB 209670 (3 nmol), BQ-788 (5 nmol) or vehicle (control). CO is the cardiac output as calculated by the microsphere method, TPR is the total peripheral resistance calculated from the cardiac output and MAP is the mean arterial pressure assuming central venous pressure to be zero. Values are given as the mean±s.e.mean of 4–5 determinations. Significant differences versus control group are shown as $^{*}P<0.05$ and $^{**}P<0.01$ while significant differences compared to ET-1 are shown as $^{\circ}P<0.05$ and $^{\circ\circ}P<0.01$.

renal blood flow that follows injection of ET-1 into areas such as the nucleus tractus solitarius and the cerebral ventricles (Hashim & Tadepalli, 1992). Interestingly, when lower doses of ET-1 were employed the decrease in renal blood flow had the same time of onset as the increase in MAP, and reached its lowest value at the same time as the maximum increase in MAP. In contrast, at the highest dose used ET-1 caused a fall in renal blood flow which lasted longer than the accompanying increase in MAP. Such prolonged reductions in renal blood flow can lead to hypoxic insult and so a compromise in renal function (Myers & Moran, 1986). However, an answer to the question of whether renal decompensation and failure can be precipitated by centrally active ET-1 would require further investigation.

Together with autoradiography our data from functional experiments using selective and non-selective antagonists allow us to identify the receptor(s) mediating the ET-1-induced effects discussed above. Thus, *in vitro* autoradiography showed the presence of ET-1 binding sites in the rat brain, as described previously (Koseki *et al.*, 1989), and particularly revealed ET_A receptor binding at the level of the PAG area, indicated by [^{125}I]-PD 151242. Binding of [^{125}I]-BQ 3020 was low, indicating little involvement of ET_B receptors at this region. From this we might suggest that, although both ET_A and ET_B receptors can mediate vasoconstriction following systemic administration of ET-1 (Warner *et al.*, 1993; Fukuroda *et al.*, 1994), at the level of the PAG area cardiovascular changes induced by ET-1 are principally mediated by ET_A receptors. This suggestion is supported by our observations that the ET_A receptor antagonist FR 139317 or the ET_A/ET_B non-selective receptor antagonist SB 209670 reduced the cardiovascular changes induced by ET-1 injected in the PAG area, while BQ-788 was without effect. This cannot be explained by BQ-788 being given in an insufficient dose for BQ-788 has a pA_2 at ET_B receptors of 7.2 (Ishikawa *et al.*,1994) while SB 209670, which was effective in our experiments, has a pA_2 at these receptors of 6.7 (Ohlstein *et al.*, 1994). Thus, ET_A receptors are most probably the predominant receptor type mediating the actions of ET-1 in the PAG area of the rat.

It is not clear where the ET_A receptors that mediate the responses we have recorded are situated. Recently, however, we have suggested that endogenously produced ET-1 acts within the PAG area to modulate the cardiovascular changes induced by exogenously applied NMDA (D'Amico & Warner, 1995). Thus, the effects of ET-1 that we have observed on arterial blood pressure and regional blood flow may be secondary to an activation of neuronal pathways involving the glutamatergic system leading to an increase in sympathoadrenal drive. This latter contention being supported by the observation that activation of glutamatergic neurones in the PAG area increases sympathetic tone (Maione *et al.*, 1992). In conclusion, therefore it may well be that endogenously produced ET-1 acting on ET_A receptors present with the PAG area is an important modulator of sympathoadrenal drive.

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