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Systemic hemodynamic and regional circulatory effects of centrally administered endothelin-1 are mediated through ET_A receptors

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Abstract

Central endothelin (ET) has been implicated in the regulation of the cardiovascular system. The effect of intracerebroventricular (i.c.v.) administration of ET-1 or IRL 1620 (5, 15 and 45 ng) on the systemic hemodynamics and regional circulation was studied in anesthetized rats using a radioactive microsphere technique. Systemic hemodynamics and regional blood circulation were determined before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. Administration of saline (5 μl, i.c.v.) did not produce any significant cardiovascular effects. The lower doses of ET-1 (5 and 15 ng) did not produce any significant effect on blood pressure (BP), heart rate (HR), cardiac output (CO), stroke volume (SV), total peripheral resistance (TPR) and regional blood circulation. However, the higher dose (45 ng) produced a transient rise (26%) followed by a sustained fall (48%) in BP. The decrease in BP was accompanied by significant decreases in CO (44%) and SV (39%), while HR and TPR were not affected. ET-1 (45 ng, i.c.v.) also produced a significant reduction in blood flow to the brain (75%), heart (49%), kidneys (66%), GIT (40%), portal system (52%) and musculo-skeletal system (38%), while blood flow to the skin was not affected. To determine pharmacological specificity of the central effects of ET-1, studies were performed in rats pretreated with BQ-123, a specific ET_A receptor antagonist. Pretreatment with BQ-123 (10 μg, i.c.v.), 15 min prior to the administration of ET-1, completely antagonized the systemic hemodynamic as well as the regional circulatory effects of ET-1 (45 ng, i.c.v.). In order to determine whether stimulation of central ET_B receptors produces any cardiovascular effects, studies were performed using IRL 1620, a specific ET_B receptor agonist. Administration of IRL 1620 (5, 15 and 45 ng, i.c.v) did not produce any effect on systemic hemodynamics and regional blood circulation in rats. It is concluded that ET_A but not ET_B receptors are involved in the central cardiovascular actions of ET.

Keywords: Endothelin-1; BQ-123, Cyclo (-D-Trp-D-Asp-Pro-D-Val-Leu-); Regional vascular resistance; IRL 1620, N-Suc-(Glu^9,Ala^{11,15})ET-1(8-21); Regional blood flow; Systemic hemodynamics; Sprague–Dawley rat; Central nervous system

1. Introduction

Endothelin (ET) is a 21 amino acid peptide originally isolated from the culture medium of porcine aortic endothelial cells [45], and characterized as a potent vasoconstrictor. There are three distinct isoforms of this peptide, that is, ET-1, ET-2 and ET-3 [15]. It was identified that ET is also produced by the non-endothelial cells, such as neurons and astroglial cells [25,26], and that ET-like immunoreactivity was present in the paraventricular nucleus, supraoptic nucleus [37], hippocampus and cerebellum [17] of porcine and rat brain. In human brain, the distribution of ET immunoreactive neurons was similar to that of ET mRNA [23]. The presence of ET receptors in the CNS has been demonstrated by binding and in situ hybridization studies [8,16,26]. At least two subtypes of ET receptors have been identified and cloned; ET_A and ET_B. The affinity rank order for ET_A is ET-1 = ET-2 > ET-3 [1], and that for ET_B is ET-1 = ET-2 = ET-3 [33]. Studies have revealed that N-Suc-(Glu^9,Ala^{11,15})ET-1(8–21) (IRL 1620) [40] is a specific agonist of ET_B receptors, whereas, cyclo (-D-Trp-D-Asp-Pro-D-Val-Leu-) (BQ-123) [14] is a specific antagonist of the ET_A receptors.

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Several lines of evidence support the view that central ET plays an important role in cardiovascular regulation. In urethane-anesthetized and ventilated rats, intracisternal administration of ET-1 (0.1, 1 or 10 pmol) resulted in complex cardiovascular changes. There was an immediate increase followed by a decrease in blood pressure, heart rate and renal sympathetic nerve activity, after which all the variables returned to, or often exceeded the baseline values [21]. Intracerebroventricular (i.c.v.) administration of ET-1 (10 and 100 pmol) or ET-3 (100 pmol) inhibited the supraspinal micturition reflex and this was accompanied by an increase in blood pressure in urethane-anesthetized rats [22]. Central ET also has several neuroendocrine actions. It stimulates the release of vasopressin [44], catecholamines [27], adrenocorticotropin hormone [27], atrial natriuretic factor [44] and oxytocin [34]. It appears that the central effects of ET-1 are mediated through activation of the sympathetic nervous system and release of arginine vasopressin because the pressor response of intracerebroventricular ET-1 was attenuated by ganglion blockers [18,28] and arginine vasopressin receptor antagonist [18]. ET-1 also produces severe vasoconstriction of the cerebral arteries [4,32]. Topical application of ET-1 to the middle cerebral artery results in ischemia which is distal to and in the cortical territory of the middle cerebral artery [31].

The role of central ET mechanisms in the development of hypertension has been studied in several laboratories. It was found that ET receptors are present in high densities in the cardiovascular regulatory areas of the brain [2,12]. ET receptors were found to be downregulated in the hypothalamus and ventrolateral medulla of spontaneously hypertensive rats [11], implicating the role of central ET in cardiovascular regulation. Although studies have been performed to determine the effect of centrally administered ET on blood pressure and heart rate, no study has been performed to determine the effect of centrally administered ET on the regional blood circulation and to determine the subtype of ET receptors involved in cardiovascular regulation. In the present study, we have investigated the effect centrally administered ET-1 and IRL 1620 on systemic hemodynamics and regional blood circulation of anesthetized rats.

2. Material and methods

2.1. Animals and surgical preparations

Male Sprague–Dawley rats (Sasco-King Animal Co., Oregon, WI) weighing 350–400 g were used in the study. Rats were anesthetized with sodium pentobarbitone (50 mg/kg, i.p.) and the lateral cerebral ventricle was cannulated by placing the rat in a stereotaxic device (David Kopf Instruments, CA) and fixing the cannula (using the coordinates: 4.5 mm lateral, 4 mm caudal to bregma and 6 mm deep from the bone) with dental cement. The animals were allowed to recover from surgery for at least 7 days before the experiments were commenced. The volume of drug injection was 5 μl. After each experiment, methylene blue dye was injected and the placement of cannula was confirmed by observing the site and extent of staining.

The rats were anesthetized with urethane (1.5 g/kg, i.p.) and the left femoral vein was cannulated (PE 50 tubing, Clay Adams, Parsipanny, NJ) for drug administration. The left femoral artery was cannulated (PE 50 tubing) and connected to a Gould P23 ID pressure transducer (Grass Instruments, Quincy, MA) for recording the blood pressure on a P7D polygraph (Grass Instruments, Quincy, MA) through a 7PI preamplifier. The heart rate was recorded through a 7P4B Grass tachograph, triggered from blood pressure signals. In order to keep the blood pO_2, pCO_2 and pH constant, and to avoid the effect of respiration on cardiovascular parameters, animals were kept on constant rate artificial respiration by inserting an endotracheal cannula connected to a rodent ventilator (Model 683, Harvard Apparatus, South Natick, MA). The carotid artery of the right side was exposed and a PE 50 cannula was guided through the common carotid artery into the left ventricle. The presence of the cannula in the left ventricle was confirmed by recording the pressure on a polygraph using a pressure transducer (Statham P23 DC, Grass Instruments, Quincy, MA). When the cannula reached the ventricle, diastolic pressure dropped to zero. The femoral artery of the right side was cannulated and connected to a withdrawal pump (Model 22, Harvard Apparatus, South Natick, MA). A period of 1 h was allowed for stabilization before the injection of drugs.

2.2. Determination of systemic hemodynamics and regional blood circulation

At each measurement, a thoroughly mixed suspension of approximately 100,000 microspheres (15 ± 1 μm diameter) labeled with ^{46}Sc (Scandium), ^{113}Sn (Tin), ^{141}Ce (Cerium), ^{95}Nb (Niobium) or ^{103}Ru (Ruthenium) (New England Nuclear Corporation, Boston, MA) in 0.2 ml saline were injected into the left ventricle and flushed with 0.4 ml saline over a 15-s period. In order to calculate the blood flow, arterial blood was withdrawn at a rate of 0.5 ml/min through the right femoral artery. Blood was withdrawn for 90 s starting about 5–10 s before the microsphere injection. At the end of the experiment, the animals were sacrificed with an overdose of sodium pentobarbitone and the tissues and organs were dissected out, weighed and placed in vials. The following tissues were studied: lungs, heart,

liver, stomach, small intestine, cecum, large intestine, mesentery and pancreas, spleen, left kidney, right kidney, left cerebral hemisphere, right cerebral hemisphere, midbrain, cerebellum, brain stem, skin and the rest of the body consisting of muscles and bones. The radioactivity in the standards, the blood samples and the tissue samples was counted in a Packard Minaxi Auto-Gamma 5000 series gamma counter with preset windows discriminating the isotope energies. The following parameters were calculated: (1) cardiac output, (2) stroke volume, (3) total peripheral resistance, (4) regional blood flow and (5) regional vascular resistance. The data were calculated using the programs described by Saxena et al. [35]. ET-1 or IRL 1620 was administered i.c.v. in cumulative doses of 5, 15 and 45 ng, and its effect on systemic hemodynamics and regional circulation was studied at 30 min after the injection of each dose in normal rats. The effect of ET-1 (5, 15 and 45 ng, i.c.v.) was also studied in BQ-123 (10 μg, i.c.v.) treated rats. Pretreatment with BQ-123 was done 15 min prior to the administration of ET-1. Administration of saline (5 μl, i.c.v.) served as a control for all these experiments. The dose of BQ-123 was selected on the basis of studies conducted by Clozel and Watanabe [3] and in our laboratory where central administration of BQ-123 (6 to 10 μg, i.c.v.) significantly attenuated the cardiovascular effects of centrally administered ET-1.

2.3. Drugs

ET-1 (human, porcine) was purchased from Sigma Chemicals Company, St. Louis, MO. BQ-123 and IRL 1620 were purchased from American Peptide Co. Inc., Sunnyvale, CA.

2.4. Statistics

All data are presented as the mean values ± 1 S.E.M.. Mean blood pressure (BP; mmHg) was calculated using the formula [(Systolic BP-Diastolic BP)/3] + Diastolic BP. Heart rate was recorded as beats/min. The number of animals used in saline group, ET-1 group, ET-1 + BQ-123 group and IRL 1620 group were 6, 8, 7 and 7, respectively. The data were subjected to an analysis of variance followed by a Duncan's test. A level of $P < 0.05$ was considered significant.

3. Results

3.1. Effect of saline, ET-1, ET-1 + BQ-123 or IRL 1620 on systemic hemodynamics

Administration of saline in the lateral cerebral ventricle had no effect on the systemic hemodynamic parameters (Table 1). Lower doses of ET-1 (5 and 15

Table 1
Effect of ET agonists (5, 15 and 45 ng, i.c.v.) on the systemic hemodynamics of rats

Parameter	Baseline	5 ng	15 ng	45 ng
Heart rate (beats / min)				
Control ($n = 6$)	405 ± 7	413 ± 8	414 ± 6	399 ± 10
ET-1 ($n = 8$)	373 ± 9	378 ± 7	398 ± 13	345 ± 17
ET-1 + BQ-123 ($n = 7$)	360 ± 18	368 ± 23	360 ± 24	351 ± 32
IRL 1620 ($n = 7$)	390 ± 20	398 ± 20	395 ± 19	387 ± 19
Blood pressure (mmHg)				
Control ($n = 6$)	99 ± 6	96 ± 8	97 ± 7	95 ± 6
ET-1 ($n = 8$)	86 ± 5	87 ± 6	89 ± 7	45 ± 3 [a]
ET-1 + BQ-123 ($n = 7$)	86 ± 3	77 ± 4	79 ± 5	84 ± 11 [b]
IRL 1620 ($n = 7$)	85 ± 6	75 ± 7	74 ± 8	71 ± 8
Cardiac output (ml / min)				
Control ($n = 6$)	109 ± 11	102 ± 9	102 ± 8	99 ± 9
ET-1 ($n = 8$)	87 ± 7	81 ± 6	79 ± 7	49 ± 6 [a]
ET-1 + BQ-123 ($n = 7$)	99 ± 5	103 ± 10	97 ± 18	107 ± 14 [b]
IRL 1620 ($n = 7$)	126 ± 10	120 ± 10	117 ± 13	98 ± 11
Stroke volume (ml)				
Control ($n = 6$)	0.27 ± 0.03	0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.02
ET-1 ($n = 8$)	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.02	0.14 ± 0.02 [a]
ET-1 + BQ-123 ($n = 7$)	0.27 ± 0.04	0.28 ± 0.03 [b]	0.23 ± 0.05	0.28 ± 0.03 [b]
IRL 1620 ($n = 7$)	0.32 ± 0.02	0.29 ± 0.01	0.29 ± 0.02	0.27 ± 0.01
Total peripheral resistance (mmHg / l / min)				
Control ($n = 6$)	986 ± 163	1006 ± 153	993 ± 119	1026 ± 146
ET-1 ($n = 8$)	1036 ± 113	1114 ± 121	1202 ± 152	1001 ± 109
ET-1 + BQ-123 ($n = 7$)	810 ± 56	833 ± 59	880 ± 111	913 ± 64
IRL 1620 ($n = 7$)	718 ± 51	893 ± 53	785 ± 75	748 ± 70

[a] Indicates significant ($P < 0.05$) difference as compared to baseline. [b] Indicates significant ($P < 0.05$) difference as compared to the ET-1 group.

ng) produced no significant changes in the systemic hemodynamics. The higher dose (45 ng), however, produced marked cardiovascular effects. There was an initial transient increase followed by a long-lasting decrease in BP after administration of ET-1 (45 ng) (Fig. 1). ET-1 (45 ng) decreased BP (48%) [$F(3,28) = 5.62$; $P = 0.0001$], SV (39%) [$F(3,28) = 5.15$; $P = 0.002$] and CO (44%) [$F(3,28) = 6.4$; $P = 0.001$]. The HR and TPR were not significantly affected. BQ-123 pretreatment completely antagonized the initial increase [$F(1,12) = 5.68$; $P = 0.03$] and subsequent decrease [$F(1,12) = 24.06$; $P = 0.0004$] in BP (Fig. 1). It also antagonized the decrease in SV [$F(1,13) = 15.8$; $P = 0.001$] and CO [$F(1,13) = 15.32$; $P = 0.001$] produced by 45 ng of ET-1 (Table 1). On the other hand, IRL 1620 did not produce any effect on the systemic hemodynamic parameters (Table 1 and Fig. 1).

3.2. Effect of saline, ET-1, ET-1 + BQ-123 or IRL 1620 on the brain circulation

The lower doses of ET-1 (5 and 15 ng) did not produce any significant effect on blood flow to the brain, whereas, the higher dose (45 ng) produced a marked decrease [75%, ($F(3,27) = 9.34$; $P = 0.0005$] in blood flow 30 min after its injection (Fig. 2). This decrease was accompanied by an increase [$F(3,27) = 6.93$; $P = 0.002$] in vascular resistance of the brain. Pretreatment with BQ-123 completely antagonized the effect of ET-1 (45 ng) on blood flow [$F(1,12) = 27.32$; $P = 0.0002$] and vascular resistance [$F(1,12) = 6.05$; $P = 0.03$] of the brain. Administration of IRL 1620 (5, 15 and 45 ng, i.c.v.) produced no effect on the brain circulation (Fig. 2).

3.3. Effect of saline, ET-1, ET-1 + BQ-123 or IRL 1620 on the coronary circulation

Administration of lower doses (5 and 15 ng, i.c.v.) of ET-1 produced no effect on coronary blood flow or vascular resistance (Fig. 3). The higher dose (45 ng, i.c.v.) of ET-1 produced a 49% [$F(3,28) = 6.14$; $P = 0.004$] decrease in coronary blood flow without affecting vascular resistance (Fig. 3). Pretreatment with BQ-123 blocked [$F(1,13) = 5.54$; $P = 0.03$] the decrease in coronary blood flow induced by 45 ng of ET-1. IRL 1620 (5, 15 and 45 ng, i.c.v.) had no significant effect on the coronary circulation (Fig. 3).

3.4. Effect of saline, ET-1, ET-1 + BQ-123 or IRL 1620 on the renal circulation

The lower doses (5 and 15 ng, i.c.v.) of ET-1 had no effect on renal blood flow and vascular resistance (Fig. 4). However, 45 ng of ET-1 produced a significant decrease (66%) [$F(3,27) = 14.23$; $P = 0.0001$] in renal blood flow. The decrease in blood flow was accompa-

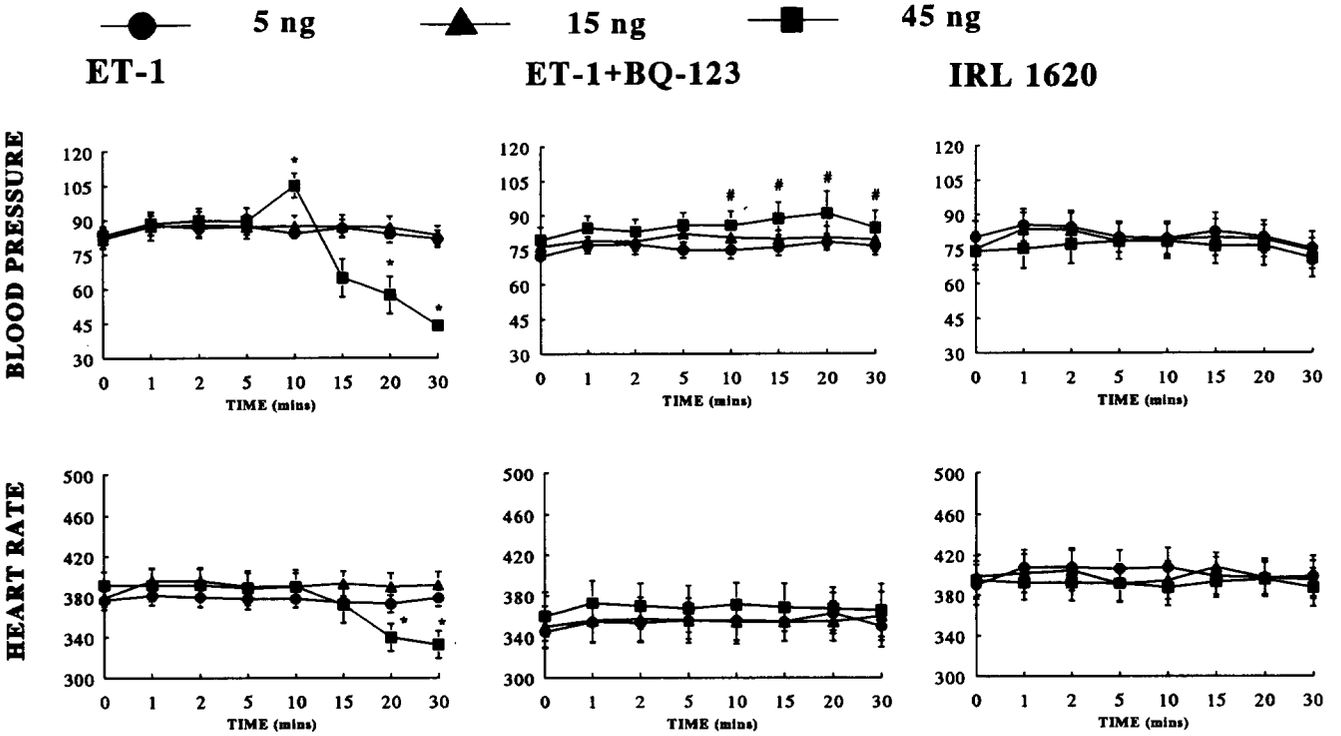

Fig. 1. Time course of effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the blood pressure and heart rate. Measurements (mean ± S.E.M.) were performed for 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

nied by an increase (85%) in renal vascular resistance [$F(3,27) = 4.71$; $P = 0.0005$]. BQ-123 pretreatment completely blocked the decrease in renal blood flow [$F(1,13) = 16.75$; $P = 0.001$] and increase in renal vascular resistance [$F(1,12) = 13.85$; $P = 0.003$] induced by 45 ng of ET-1. Centrally administered IRL 1620 (5, 15 and 45 ng, i.c.v.) had no effect on the renal circulation (Fig. 4).

3.5. Effect of saline, ET-1, ET-1 + BQ-123 or IRL 1620 on the gastrointestinal tract (GIT) circulation

The lower doses (5 and 15 ng, i.c.v.) of ET-1 did not alter the GIT circulation, but the higher dose of ET-1 (45 ng, i.c.v.) reduced blood flow to the GIT by 40% [$F(3,28) = 6.59$; $P = 0.002$] without any change in vascular resistance (Fig. 5). The decrease in blood flow induced by ET-1 was significantly blocked [$F(1,13) = 10.3$; $P = 0.006$] by BQ-123 pretreatment. IRL 1620 (5, 15 and 45 ng, i.c.v.) did not alter the GIT circulation.

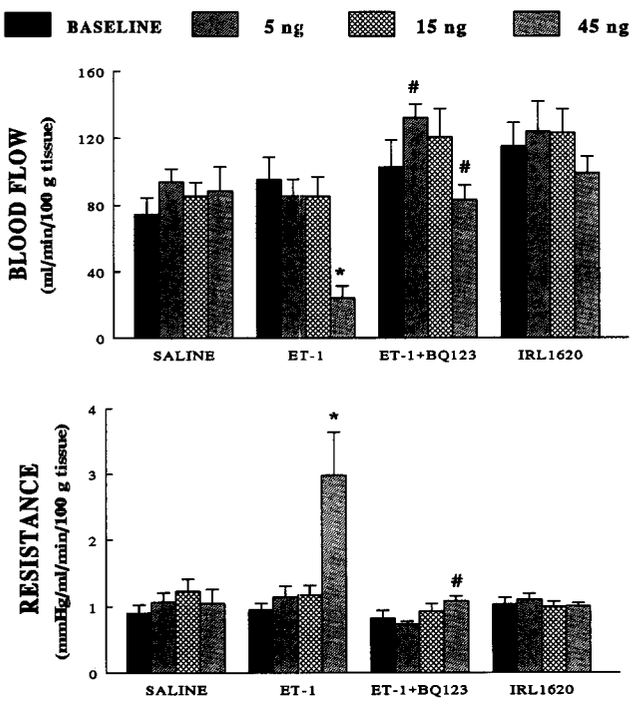

Fig. 2. Effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the cerebral blood flow (ml/min/100 g tissue) and vascular resistance (mmHg/ml/min/100 g tissue). Measurements (mean ± S.E.M.) were performed before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

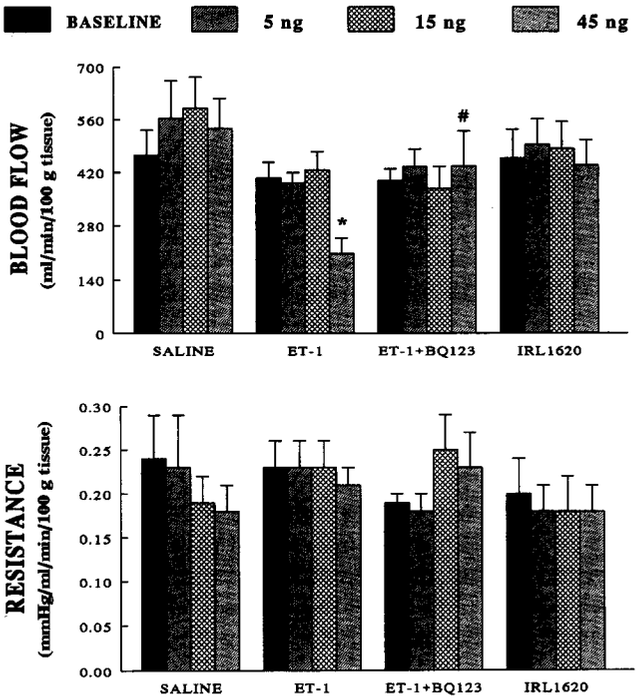

Fig. 3. Effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the coronary blood flow (ml/min/100 g tissue) and vascular resistance (mmHg/ml/min/100 g tissue). Measurements (mean ± S.E.M.) were performed before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

3.6. Effect of saline, ET-1, ET-1 + BQ-123 or IRL 1620 on the portal circulation

ET-1 (5 and 15 ng, i.c.v.) did not produce any change in portal circulation. High dose (45 ng, i.c.v.) of ET-1 decreased the portal blood flow by 52% [$F(3,26) = 16.32$; $P < 0.0001$] without affecting vascular resistance (Fig. 6). This decrease in blood flow was completely blocked [$F(1,13) = 21.67$; $P = 0.0005$] by pretreatment with BQ-123. IRL 1620 (5, 15 and 45 ng, i.c.v.) administration did not affect the portal circulation.

3.7. Effect of saline, ET-1, ET-1 + BQ-123 or IRl 1620 on the musculo-skeletal circulation

Administration of ET-1 (5, 15 and 45 ng, i.c.v.) produced a dose-related decrease in blood flow to the musculo-skeletal system (Fig. 7). However, this decrease was statistically significant [$F(3,28) = 2.46$; $P = 0.02$] only with 45 ng of ET-1. There was no significant change observed in vascular resistance. BQ-123 blocked [$F(1,13) = 6.05$; $P = 0.02$] the decrease in blood flow

produced by 45 ng of ET-1. IRL 1620 (5, 15 and 45 ng, i.c.v.) did not produce any significant effect on the musculo-skeletal blood flow (Fig. 7).

3.8. Effect of saline, ET-1, ET-1 + BQ-123 or IRL 1620 on the skin circulation

ET-1 (5, 15 or 45 ng, i.c.v.) had no effect on blood flow (Fig. 8) to the skin. The 5 ng and 15 ng doses of ET-1 had no effect on vascular resistance of the skin, but the 45 ng dose of ET-1 produced a significant decrease [$F(3,20) = 6.1$; $P = 0.007$] in vascular resistance. In BQ-123 pretreated rats, the skin blood flow was significantly increased by 5 ng [$F(1,12) = 11.07$; $P = 0.006$], 15 ng [$F(1,12) = 8.71$; $P = 0.01$] and 45 ng [$F(1,12) = 7.84$; $P = 0.01$] as compared to ET-1 treated animals. The decrease in vascular resistance of skin produced by ET-1 (45 ng) was blocked [$F(1,11) = 6.89$; $P = 0.02$] by BQ-123 pretreatment. IRL 1620 (5, 15 and 45 ng, i.c.v.) administration produced no change in the skin blood circulation (Fig. 8).

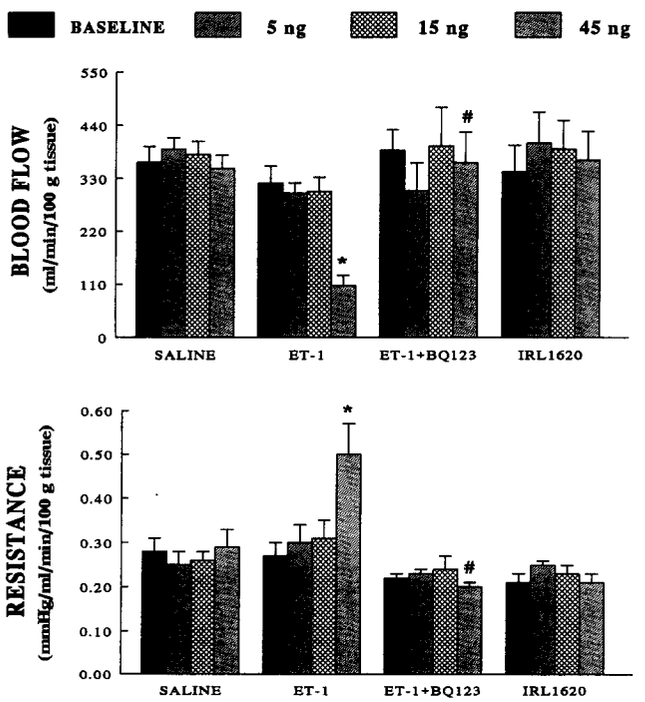

Fig. 4. Effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the renal blood flow (ml/min/100 g tissue) and vascular resistance (mmHg/ml/min/100 g tissue). Measurements (mean ± S.E.M.) were performed before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

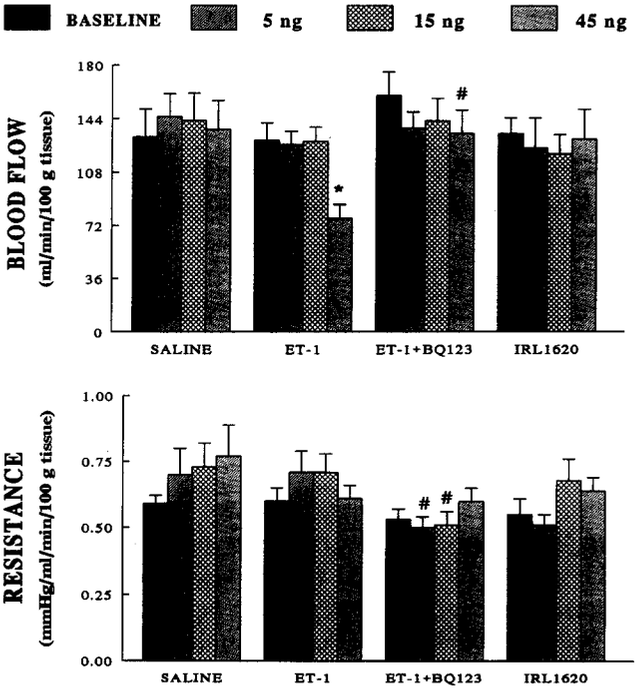

Fig. 5. Effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the gastrointestinal blood flow (ml/min/100 g tissue) and vascular resistance (mmHg/ml/min/100 g tissue). Measurements (mean ± S.E.M.) were performed before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

4. Discussion

In the present study we have examined the effect of intracerebroventricular administration of ET agonists on systemic hemodynamics and regional blood circulation. The lower doses (5 and 15 ng) of centrally administered ET were without any effect but the high dose (45 ng) of ET-1 caused a biphasic change in blood pressure; increase followed by a decrease. Siren et al. [38] also found that lower doses of ET-1 (1, 3 and 10 pmol/kg, i.c.v.) did not produce any effect on blood pressure, heart rate, cardiac index and total peripheral resistance. However, higher dose of ET-1 (30 pmol/kg, i.c.v.) produced a transient increase followed by a decrease in blood pressure. These effects were similar to those observed in the present study using 45 ng (or 18 pmol) of ET-1. The biphasic response observed in our study confirms some of the earlier findings. Ferguson et al. [6] observed that injection of ET-1 (2 pmol) in the area postrema of anesthetized rats produced a significant increase followed by a decrease in blood pressure. Hashim et al. [13] also observed that injection of ET-1 (30 pmol) in the fourth cerebral ventricle of

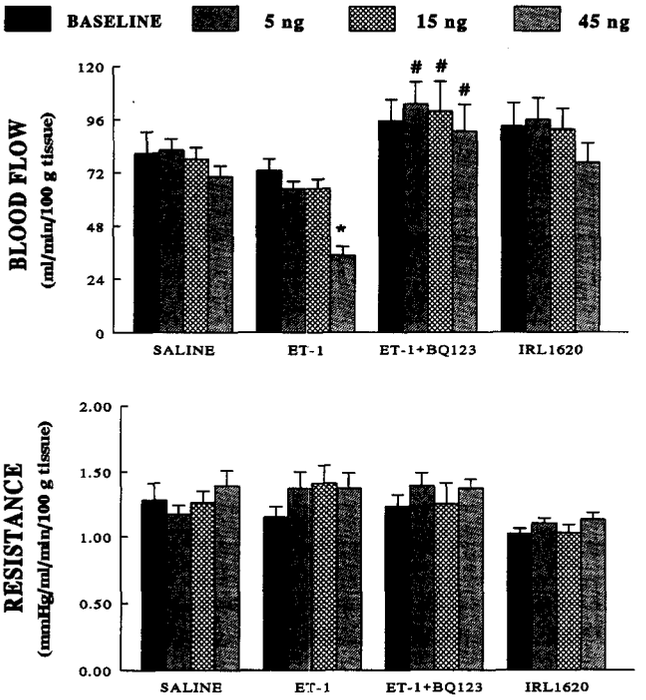

Fig. 6. Effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the portal blood flow (ml/min/100 g tissue) and vascular resistance (mmHg/ml/min/100 g tissue). Measurements (mean ± S.E.M.) were performed before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

anesthetized rats produced a biphasic response in blood pressure. The observation that central ET-1 induced increase in pressor response was suppressed by pretreatment with phenoxybenzamine [30], implicates the participation of sympathetic system in the initial pressor response. Kuwaki et al. [19] reported that the intracisternal (i.c.) administration of the lower doses of ET-1 elicits a pressor response with increase in renal sympathetic nerve activity. However, the higher dose of ET-1 (1 or 10 pmol, i.c.) produced a subsequent depressor response with sympathoinhibition. Similar responses were observed when ET-1 was applied directly to the ventral surface of medulla. The cardiovascular effects of intracisternally administered ET-1 were not altered following precollicular decerebration or vagotomy [21]. These studies clearly indicate that central ET-1 produces cardiovascular effects mainly through the sympathetic nervous system. In addition to the sympathetic involvement there is a possibility that ET-1 may have modulated the release of other neurotransmitters/modulators. It is known that ET-1 inhibits nerve stimulation-evoked release of [^{3}H]norepinephrine [41] and [^{3}H]acetylcholine [42], whereas it stimulates the release of dopamine [7], substance P [46], [^{3}H]D-aspartate [24] and vasopressin [36] in several brain structures. The ability of centrally administered ET-1 at picomolar doses to affect the cardiovascular system further shows the importance of central ET as a neuromodulator.

The cardiovascular effects of centrally administered ET-1 are mediated through ET_A receptors in the CNS because BQ-123, a specific antagonist of ET_A receptors, completely blocked its cardiovascular effects. In order to explore whether stimulation of ET_B receptors in the CNS produces cardiovascular effects, we administered IRL 1620, a specific ET_B agonist, in the lateral cerebral ventricle of rats. It was found that IRL 1620 did not produce any effect on the cardiovascular system, indicating that ET_B receptors in the CNS are not involved in the cardiovascular effects of centrally administered ET-1. Earlier studies [19,20] have demonstrated that both ET-1 and ET-3 produce biphasic responses in BP and HR upon intracisternal injection in anesthetized rats but the dose required for ET-1 was 1 or 10 pmol and that for ET-3 was 100 pmol. At a 100 times higher dose, ET-3 is also likely to produce some actions on ET_A receptors. These findings further sup-

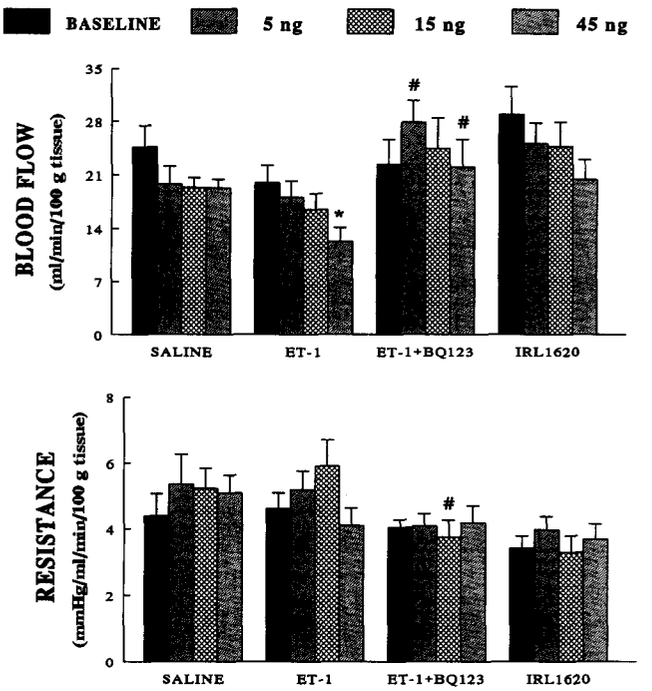

Fig. 7. Effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the musculo-skeletal blood flow (ml/min/100 g tissue) and vascular resistance (mmHg/ml/min/100 g tissue). Measurements (mean ± S.E.M.) were performed before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

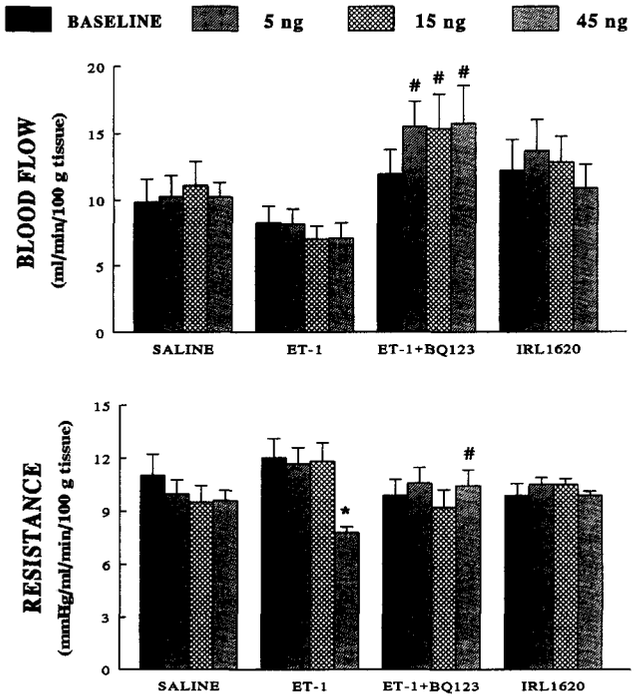

Fig. 8. Effect of saline (5 μl, i.c.v., $n = 6$), ET-1 (5, 15 and 45 ng, i.c.v., $n = 8$) or IRL 1620 (5, 15 and 45 ng, i.c.v., $n = 7$) on the skin blood flow (ml/min/100 g tissue) and vascular resistance (mmHg/ml/min/100 g tissue). Measurements (mean ± S.E.M.) were performed before (baseline) and at 30 min after the injection of each dose of ET-1 or IRL 1620. ET-1 (5, 15 and 45 ng, i.c.v.) was also administered in BQ-123 pretreated rats ($n = 7$). BQ-123 (10 μg, i.c.v.) was administered 15 min before the injection of ET-1. * Indicates significant difference as compared to baseline and # indicates significant difference as compared to ET-1 group.

port our results that ET_A receptors are involved in the cardiovascular effects of centrally administered ET-1. The exact site of ET-1 actions in the CNS in producing cardiovascular effects can not be ascertained from the present studies. Drugs when administered intracerebroventricularly predominantly act upon periventricular structures like the hypothalamus and ventral surface of medulla. Previous studies have implicated subfornical organ, area postrema [6], nucleus tractus solitarius [13], hypothalamus [12] and ventral surface of medulla [11,19] in the cardiovascular actions of ET-1. It appears that central cardiovascular effects of ET-1 are mediated through ET_A receptor activation in areas like hypothalamus and ventral surface of medulla.

ET-1 in higher doses (45 ng, i.c.v.) produced a very severe reduction in blood flow to the brain. The reduction in cerebral blood flow (CBF) by ET-1 was not merely due to the reduction in cardiac output because the reduction in CBF (75%) was much more marked than the decrease in CO (44%). Therefore, the reduction in CBF was also due to, in addition to reduced CO, a direct vasoconstrictor action of ET-1 on the cerebral blood vessels. This was confirmed by the observation that there was an increase in vascular resistance of the brain blood vessels following central ET-1 administration. These findings confirm some earlier reports describing the vasoconstrictor actions of ET in the CNS. For example, intracerebroventricular administration of ET-1 reduces blood flow to the periventricular brain structures and choroid plexus [10]. Topical application of ET-1 to the middle cerebral artery results in ischemia which is distal to and in the cortical territory of the middle cerebral artery [31]. The reduction in CBF by central ET-1 may be clinically relevant because in vasospastic disorders such as cerebral ischemia and subarachnoid hemorrhage the release of ET-1 is augmented [9,43]. It is also known that ET-1 levels are increased in the plasma of patients with ischemic stroke [47] and in the cerebrospinal fluid of patients with subarachnoid hemorrhage [39]. The reduction in CBF by central ET-1 is mediated through the ET_A receptors because BQ-123 antagonized the reduction in CBF and increase in vascular resistance induced by central administration of ET-1. The inability of IRL 1620 to affect the CBF further indicates that the effects observed with ET-1 were not mediated through central ET_B receptors. Earlier investigations showed that the cerebral blood vessels are contracted by ET with a rank order potency of ET-1 > ET-2 ≫ ET-3 [5,32]. Studies are also available showing that ET_A receptor antagonists prevent the occurrence of cerebral vasospasm in several models of subarachnoid hemorrhage [3,29]. It is therefore clear that ET_A but not ET_B receptors in the CNS are involved in the regulation of CBF.

This is the first report on the effect of central administration of ET agonists on the blood circulation of peripheral organs. Central ET-1 reduced blood flow to the peripheral organs, such as, the heart, portal system, gastrointestinal tract and musculo-skeletal system. This may be due to a decrease in cardiac output induced by central ET-1 because the percent decreases in blood flow to most of these organs was found to closely parallel the percent decrease in CO. It is unlikely that the decrease in blood flow to the peripheral organs by centrally administered ET-1 was due to its leakage to the peripheral sites because it has been reported that intravenous administration of 30 pmol [13], or 100 ng/kg [18] of ET-1 had no significant hemodynamic effect. Furthermore, we did not observe any increase in vascular resistance of the peripheral organs, suggesting that there was no direct vasoconstrictor effect on the peripheral blood vessels. Kidneys were the only exception because decrease in the renal blood flow was accompanied by an increase in the renal vascular resistance. Centrally administered ET-1 is known to increase the peripheral release of vasoactive substances [27,28,30,44], and/or to increase the renal sympathetic nerve activity [19]. The increase in vascular resistance of the kidneys could be due to local

release of vasoactive substances or due to an increased responsiveness of renal blood vessels to sympathetic stimulation. The regional circulatory effects of centrally administered of ET-1 appear to be mediated via central ET_A receptors since BQ-123 was able to completely antagonize the reduction in blood flow to peripheral organs induced by ET-1. ET_B receptors are not involved in central ET-1 induced decrease in blood flow to the peripheral organs because central administration of ET_B agonist, IRL 1620, did not affect blood flow to the peripheral organs.

In conclusion, observations presented here indicate that centrally administered ET-1 produces a transient increase followed by a long-lasting decrease in blood pressure. The depressor effect is due to a reduction in stroke volume and cardiac output, which contributes to decrease in blood flow to peripheral organs. The cerebral blood flow is further reduced due to a direct action of central ET-1 on cerebral blood vessels. The cardiovascular effects of centrally administered ET-1 are mediated through ET_A but not ET_B in the CNS.

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