Effect of Endothelin on Cortical Microvascular Perfusion in Rats

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We used laser-Doppler flowmetry to study the effects of endothelin-1 on local cortical microvascular perfusion and resistance in 29 pentobarbital-anesthetized rats. Intravenous administration of 10–300 pmol endothelin-1 reduced arterial blood pressure and microvascular resistance and increased microvascular perfusion. However, intracarotid administration of low doses of endothelin-1 increased microvascular perfusion and reduced microvascular resistance and arterial blood pressure, whereas high doses (≥300 pmol) reduced microvascular perfusion and increased microvascular resistance and arterial blood pressure. Only the high dose/low flow response was associated with attenuation of the electrocorticogram. The low dose/high flow and high dose/low flow responses to endothelin-1 were not altered by blockade of muscarinic and adrenergic receptors. In addition, systemic metabolic changes (arterial pH, PaCO2, PaO2, and plasma glucose concentration) did not account for the cerebrovascular effects of endothelin-1. Platelet hyperaggregability also did not appear to be a causative factor in the high dose/low flow response to endothelin-1. In fact, ex vivo rat platelet aggregation was inhibited by intracarotid administration of 300 pmol endothelin-1. In conclusion, the cerebral vasculature exhibits extreme sensitivity to the vasodilator properties of endothelin-1 at low doses. The ischemic vasoconstrictor effects observed at high doses implicate endothelin-1 as an important mediator of cerebral vasospasm and/or postischemic hypoperfusion.


Endothelin (ET-1), a 21-amino acid peptide originally characterized from cultures of porcine aortic endothelial cells, is one in a family of recently described vasoactive isopeptides (ET-1, ET-2, and ET-3).1–3 The most extensively studied member of this family, ET-1 is an extremely potent vasoconstrictor in a variety of in vitro vascular preparations, with veins being more sensitive than arteries.4 In pithed or chemically denervated rats, the systemic administration of ET-1 elicits a slowly developing, prolonged pressor response.1,4 In contrast, in conscious and anesthetized rats the systemic administration of ET-1 produces an immediate hypotensive response, which is associated, to varying degrees, with reductions in carotid, hind limb, renal, and mesenteric vascular resistance but little or no effect on heart rate (HR).5 The decrease in vascular resistance may be related to the release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI2).4,6 Administering high doses of ET-1 (≥300 pmol/kg i.v.) to conscious or anesthetized rats often results in a biphasic arterial blood pressure response, that is, hypotension followed by hypertension.4

Little is known about the cerebrovascular effects of ET-1. However, the localization of specific, high-affinity ET-1 binding sites in small arteries and paravascular nerves,7 together with the recent demonstration of prolonged basilar artery vasospasm following intracisternal administration of ET-1,8,9 suggest its important role in the physiologic or pathophysiologic regulation of cerebral blood flow. The purpose of our study was to determine the effects of circulating ET-1 on local cortical microvascular perfusion (CP) in vivo using laser-Doppler flowmetry.10,11

Materials and Methods

Twenty-nine male Sprague-Dawley rats weighing 370–390 g were housed in a thermally controlled (25°C), 12-hour light-cycled (8 AM to 6 PM) laboratory animal facility with free access to food and water until the day of experimentation. Surgical anesthesia was induced with 2.5% isoflurane in a gas mixture of 40% O2 and 60% N2 delivered by means of an open-circuit face mask at 2 l/min. The left femoral artery and vein were cannulated with polyethylene tubing (0.97 mm o.d., 0.58 mm i.d.) for continuous
FIGURE 1. Schematic diagram illustrating basic principles of laser-Doppler flowmetry used for measuring local cortical microvascular perfusion (CP). Efferent optical fibers in needle probe deliver low-power (2 mW) HeNe laser light (632.8 nm) to cortical surface, where limited penetration and random photon scattering occur. Some backscattered light is conveyed to photodetectors via afferent optical fibers in probe. Voltage generated by photodetectors is analyzed for its AC and DC components. Further processing relates Doppler-shifted frequency component of AC signal (detected light deflected from moving particles in sample medium) to mean velocity (v) of moving particle (primarily erythrocytes [rbc's]). Amplitude of AC signal relative to DC component is proportional to number of moving particles (i.e., rbc's) contributing to Doppler-shifted frequencies. Ideally, CP is equal to rbc's x v within hemisphere of tissue sampled.

FIGURE 2. Representative polygraph tracings demonstrating effects of endothelin (ET-1) on arterial blood pressure (AP, mm Hg) and local cortical microvascular perfusion (CP, units) following intravenous administration of (A) 100 and (B) 300 pmol i.v. ET-1 in rats. Tracings in A and B were obtained from separate animals.
Each rat was placed prone in a stereotaxic instrument (DKI, Tujunga, California) and secured in the flat skull position. A small thermostatic heating pad was then placed beneath the rat's abdomen to maintain rectal temperature at 37–38°C for the remainder of the experiment. The right parietal bone was exposed, and a cortical window 4 mm in diameter was prepared with the meninges intact. The center of the cortical window was located 1 mm lateral and 2 mm caudal to the bregma. At this site primary evoked potentials were reliably recorded following electrical stimulation of the contralateral sciatic nerve (the hind limb somatosensory area of the parietal cortex). When preparation of the cortical window was completed, the rat was paralyzed with 1 mg/kg i.v. tubocurarine and ventilated artificially with a rodent respirator (Model 683, Harvard Apparatus, South Natick, Massachusetts) delivering 75 strokes/min at 4.0 ml of 100% O2 per stroke. After 15 minutes for equilibration, 0.3-ml arterial blood samples were obtained for automated blood gas and plasma glucose analysis. When necessary, ventilation parameters were adjusted to maintain Paco2 and arterial pH within the ranges 33.5–38.0 mm Hg and 7.35–7.45, respectively.

Laser-Doppler flowmetry (Perimed PF3, Stockholm, Sweden) was used to continuously monitor CP from the surface of the exposed parietal cortex. The principles of the technique are summarized in Figure 1. In practice, a micromanipulator was used to place the laser needle probe (1 mm o.d.) ≤100 μm above an area on the cortical surface devoid of obvious blood vessels when magnified sixfold. A sampling site within the cortical window was included in the study if resting CP was between 100 and 220 units and if a brisk vasodilation (≥30% diameter increase) could be recorded following the introduction of 5% CO2 into the inspired gas mixture for 1 minute. Only one site per rat was studied.

In all rats a 15-minute control period followed the identification of an appropriate sampling site and preceded the administration of ET-1; mean arterial blood pressure (MAP), HR, and CP were monitored.
FIGURE 4. Representative polygraph tracings demonstrate effects of endothelin (ET-1) on parietal electrocorticogram (ECoG, μV), local cortical microvascular perfusion (CP, units), and arterial blood pressure (AP mm Hg) following intracarotid administration of 100 (A) and 300 (B) pmol ET-1 in rats. Note differences in ECoG and CP responses observed during (A1 and B2) and 20 minutes after (A2 and B3) administration of ET-1; also note changes in record speed (B3). B1, control period before ET-1 administration. C, effects of intravenous injection of saturated KCl solution. Tracings B and C were obtained from same animal.

continuously for the remainder of the experiment. The apparent local cortical microvascular resistance (CVR) was calculated as MAP+CP.

We studied five groups. In Group 1 (n=5), three increasing doses of ET-1 were administered intravenously at 30–40 minute intervals. In Group 2 (n=5), similar doses of ET-1 were administered via the internal carotid artery (i.c.). In three rats in Group 2 the parietal electrocorticogram (ECoG) was monitored with a bipolar electrode positioned adjacent to the CP sampling site; the electrode did not alter CO₂ responsiveness at the sampling site. Group 3 (n=4) was similar to Group 2; however, 0.2-ml arterial blood samples were taken for blood gas and glucose analyses during the maximum response to ET-1. Group 4 (n=4) received 1–2 mg/kg i.v. labetalol and 200 μg/kg i.v. atropine sulfate at the beginning of the control period; this combined adrenergic (α₁, β₁, and β₂) and muscarinic blockade abolished the MAP and HR effects elicited by ganglionic stimulation with 125 μg/kg i.v. 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). Except for lower MAP and HR, Group 4 was similar to Group 2. In Group 2, the effects of ET-1 on platelet aggregation (stimulated with 1.25, 2.5, and 5.0 μM adenosine diphosphate [ADP]) during the control period and 3 minutes after administration of 300 pmol i.c. ET-1 were studied in five rats surgically prepared as in Group 2. Platelets were prepared for ex vivo aggregation studies as described previously. Platelet aggregation was also evaluated in five time-matched controls treated with saline instead of ET-1.

All drugs were prepared in saline and administered by the intravenous route in volumes of ≤0.30 ml or by the intracarotid route in volumes of 0.1 ml. ET-1 doses were administered on a per-rat basis, and vehicle administration had no significant effect on any parameter monitored. ET-1 (human/porcine endothelin) was obtained from Peninsula Laboratories (Belmont, California), and all other drugs were obtained from common commercial sources (Sigma, St. Louis, Missouri; RBI, Wayland, Massachusetts).

In each group, multiple comparisons with values during the control period were evaluated by applying Bonferroni’s inequality to the paired two-tailed $t$ test. All summary values are expressed as mean±SEM, and differences were considered significant at $p≤0.05$.

Results

In Group 1, the intravenous administration of ET-1 reduced measured MAP and calculated CVR and increased measured CP (Figure 2). Onset of the MAP and CVR responses occurred within seconds after ET-1 administration and were maximal within 0.5–1.0 minute following injection (Figure 3, A and C). In contrast, CP increased during the decrease in MAP and reached a maximum 1–2 minutes following...
ET-1 administration (Figure 3B). It is noteworthy that the maximal increase in CP was not related to the maximal decrease in MAP. All physiologic responses recovered completely and uneventfully during 30–45 minutes following each ET-1 dose. ET-1 did not cause significant changes in HR in Group 1 rats.

In Group 2, when delivered intracarotidly, the effects of 10 and 100 pmol ET-1 on CP and CVR differed qualitatively from those after 300 pmol (Figures 4 and 5). Specifically, 10 and 100 pmol i.c. ET-1 increased CP and decreased CVR, whereas the opposite response was observed following 300 pmol i.c. ET-1 (Figure 5). No changes in ECoG were noted following 10 and 100 pmol i.c. ET-1; however, 300 pmol i.c. ET-1 caused a slowly developing reduction in ECoG voltage during the period of reduced CP (Figure 4). In addition, rats receiving 300 pmol i.c. ET-1 displayed a modest, delayed overshoot in MAP that was clearly dissociated from the CP response 3 minutes after administration (Figure 5). It is noteworthy that at 10 pmol i.c., ET-1 elicited significant cerebral vasodilation without altering MAP, thus distinguishing the low-dose effects of ET-1 from cerebrovascular autoregulatory processes.

In Group 3, arterial blood samples taken during the maximal CP response elicited by 10, 100, and 300 pmol i.c. ET-1 demonstrated a small reduction in arterial pH and base excess in the blood and an increase in plasma glucose concentration relative to control following the 300 pmol dose (Figure 6). These results suggest that the effects of intracarotid ET-1 on CP and CVR were not secondary to systemic metabolic effects, despite evidence of mild metabolic acidosis following the high dose.

In Group 4, pretreatment with atropine sulfate and labetalol abolished the MAP and HR effects elicited by ganglionic stimulation with DMPP; as expected, MAP and HR during the control period in this group (108±6 mm Hg and 314±5 beats/min, respectively) were significantly lower than those in Group 2 (138±4 mm Hg and 383±21 beats/min, respectively). However, administration of 10, 100, and 300 pmol i.c. ET-1 elicited MAP, CP, and CVR responses virtually identical to those observed in Group 2 (compare Figures 7 and 5). These results suggest that peripheral muscarinic and adrenergic mechanisms do not play a prominent role in mediating the cerebrovascular effects of ET-1.

In humans, platelet hyperaggregability is often associated with acute cerebral ischemia. This hemostatic condition may cause or contribute to thromboembolic processes responsible for cerebral vascular occlusion and ischemia. To examine the potential contribution of platelet hyperaggregability in the high dose/low flow response to ET-1, we evaluated ex vivo ADP-induced platelet aggregation. In Group 5, ET-1 significantly inhibited platelet aggregation induced by 1.25 and 2.5 μM ADP; however, aggregation elicited by 5.0 μM ADP was unchanged relative to that during the control period (Figure 8). There were also no significant changes in platelet aggregation of the time-matched controls. In vitro, the addition of 1–100 nM ET-1 alone to rat PRP did not induce platelet aggregation. These results suggest that the maximum high dose/low flow effects of ET-1 were not caused by thromboembolic processes associated with platelet hyperaggregability.
Discussion

ET-1 has potent vasodilator and vasoconstrictor properties.4-6 Our results indicate that in vivo the cerebrovasculature was sensitive to both properties of ET-1; however, the predominant cerebrovascular response depended on the route and dose administered.

Intravenous administration of 10, 100, and 300 pmol ET-1 significantly increased CP and decreased in MAP and CVR, indicating cerebral vasodilation. However, the maximum increase in CP did not appear to be related to the maximum reduction in MAP. These results suggest a differential sensitivity of the cerebral vasculature to the vasodilator and vasoconstrictor properties of ET-1. This impression was confirmed by evaluating the cerebrovascular response to equivalent doses of ET-1 administered at the origin of the internal carotid artery. Direct delivery of ET-1 to the forebrain vasculature circumvented systemic dilution and a substantial first-pass clearance in the lung4 and resulted in two distinct cerebrovascular responses. First, 10 and 100 pmol ET-1 increased CP, reduced CVR, and elicited no change in ECoG. This low dose/high flow response was independent of MAP and contrasted markedly to the reduced CP and increased CVR observed following the administration of 300 pmol i.c. ET-1 (high dose/low flow response). The high dose/low flow response was also independent of MAP but was slower in onset and lasted longer (15-45 minutes) than the low-dose response. A slowly developing reduction in ECoG voltage was also observed during the high dose/low flow response. These changes in ECoG were most likely secondary to the reduction in CP because ET-1 fails to enter the brain parenchyma following systemic administration18 and, therefore, would not be expected to exert direct neuronal effects in the central nervous system.

We also conducted experiments designed to investigate the potential indirect cerebrovascular effects of intracarotidly administered ET-1. In this portion of our study, muscarinic and adrenergic receptor blockade did not alter the response pattern elicited by 10, 100, and 300 pmol i.c. ET-1. In addition, the cerebrovascular effects of ET-1 were not causally related to changes in arterial blood gases despite metabolic acidosis and hyperglycemia following the 300-pmol doses. These metabolic changes are consistent with ischemic injury. Since platelet hyperaggregability may contribute to thromboembolic processes responsible for cerebral vascular occlusion and ischemia,16,17 it was also important to examine the effects of 300 pmol i.c. ET-1 on ex vivo platelet aggregation during the high dose/low flow response. In Group 5, 300 pmol i.c. ET-1 did not enhance ex vivo rat platelet aggregation; in fact, ET-1 inhibited ADP-induced platelet aggregation. Similar effects on platelet aggregation have also been reported in rabbits and may be related to the release of PGI2.19 It is also noteworthy that in vitro, ET-1 alone did not induce rat platelet aggregation. Overall, these results rule out obvious indirect effects associated with the autonomic nervous system, arterial blood gases, and platelet aggregation and suggest that direct ET-1-induced cerebral vasodilation and cerebral vasoconstriction underlie the phenomena of the low dose/high flow and high dose/low flow responses, respectively. In this regard, direct in vivo
Figure 7. Graph of effects of muscarinic (200 μg/kg i.v. atropine sulfate) and adrenergic (1–2 mg/kg i.v. labetalol) receptor blockade on magnitude and time course of changes in (A.) mean arterial blood pressure (MAP), (B.) local cortical microvascular perfusion (CP), and (C.) local cortical microvascular resistance (CVR) elicited by intracarotid administration of 10 (•), 100 (○), and 300 (●) pmol endothelin (ET-1) in four rats. * p≤0.05 different from 0 minutes by paired two-tailed t test and Bonferroni’s inequality.

Evidence of cerebral vasoconstriction has recently been described in angiographic studies of cat and dog basilar arteries following the intracisternal administration of ET-1.8,9 In both studies, prolonged (12–72 hours) reductions in basilar artery caliber were observed.

Comprehensive studies of the mechanisms involved with ET-1-induced cerebrovascular relaxation and constriction have not been reported. However, a number of diverse vascular preparations have already been employed. It is generally agreed that ET-1 acts abluminally to cause prolonged, dose-related and endothelium-independent constriction of vascular smooth muscle by avidly interacting with specific cell surface receptors on smooth muscle.20-21 When stimulated, ET-1 membrane receptors are capable of mobilizing intracellular calcium for contraction via the phospholipase C signal transduction pathway.22-25 In addition, ET-1 receptor activation causes membrane depolarization by opening nonselective cation channels in the plasma membrane. Sufficient depolarization by this mechanism can cause an influx of extracellular Ca2+ by activating voltage-sensitive L-type calcium channels.26 The vasorelaxant effects of low-dose ET-1 have been less well described, and relatively little attention has been devoted to the study of its mechanism of action.4,6 It has been shown that ET-1 causes release of potent vasodilators (EDRF and PGI2) in vitro,4,6 but the role of these mediators in ET-1-induced vasodilation in vivo remains to be determined. In fact, ET-1 does not appear to release EDRF in isolated rabbit aorta,21-25 rabbit vena cava,21 or canine basilar artery (unpublished observations).

In conclusion, the cerebral vasculature exhibits differential sensitivity to the potent vasodilator and vasoconstrictor effects of ET-1. At 10–100 pmol i.c. ET-1, only cerebral vasodilation was observed, whereas cerebral vasoconstriction was the predominant response after ≥300 pmol ET-1. Neither high-nor low-dose effects of ET-1 appear to be related to changes in arterial blood gases, to systemic metabolic
effects, to thromboembolic processes, or to the autonomic nervous system. The sites, mechanisms, and regulation of the action of ET-1 in the cerebral vasculature are currently being investigated in an effort to determine the physiologic and/or pathophysiological relevance of our present results.

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References


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