Central and peripheral administration of endothelin-1 induces an increase in blood pressure in conscious trout

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Le Mével, Jean-Claude, Catherine Delarue, Dominique Mabin, and Hubert Vaudry. Central and peripheral administration of endothelin-1 induces an increase in blood pressure in conscious trout. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1010–R1017, 1999.—The central and peripheral cardiovascular effects of endothelin (ET)-1 and ET-3 were investigated in conscious rainbow trout. Both intracerebroventricular and intra-arterial injections of ET-1 (6.25–25 pmol) but not ET-3 (25 pmol) caused a dose-dependent increase in mean dorsal aortic blood pressure and a concomitant decrease in heart rate. The hypertensive response was elicited by the first injection of ET-1 without a change in plasma cortisol level. The present data demonstrate that intracerebroventricular injection of ET-1 did not significantly modify plasma cortisol level. The present data demonstrate that intracerebroventricular and intra-arterial administration of very low doses of ET-1 produces hypertension in conscious trout. The lack of effect of ET-3 indicates that the hemodynamic actions of ET-1 are mediated both centrally and peripherally through ETA receptors.

The term endothelins (ETs) designates a family of biologically active peptides initially isolated from the culture medium of porcine endothelial cells (33). The three ETs characterized so far (ET-1, ET-2, and ET-3) exhibit vasoconstrictor activity, ET-1 being the most potent isoform. ET-1 causes contraction of arterial and venous preparations in vitro (33) and exerts a positive inotropic effect on isolated mammalian cardiac tissue (11). Intravenous injection of ET-1 in conscious rats produces a transient depressor effect followed by a sustained and potent pressor response that is associated with a decrease in heart rate (HR) (28).

There is now evidence that ETs act as central regulators of mammalian cardiovascular functions. The occurrence of ETs and ET receptors has been demonstrated in the central nervous system of human and rat (9, 15). In addition, intracerebroventricular or intrarectheal administration of ET-1 induces a marked increase in arterial blood pressure generally associated with a decrease in HR in conscious rats (10, 25, 28).

In lower vertebrates, a series of peptides called sarafotoxins that share sequence similarities with ETs have been isolated from the venom of the snake Actuctasps engaddensis (30). Recently, the occurrence of ET-1-like immunoreactivity has been demonstrated in the central and peripheral nervous system of fish (12, 21, 35). The existence of specific binding sites for sarafotoxin-b and ET-1 has been reported in the heart and brain of tilapia and torpedo (35), and the presence of ET$_A$-like receptors has been demonstrated in the gill of the rainbow trout (20). Although immunoreactive ETs have been detected in the plasma of amphibians and fish (32), very few studies have been conducted to investigate the effects of ETs in lower vertebrates. In vitro, ET-1 stimulates corticosterone and aldosterone secretion from the frog adrenal gland (5) and causes constriction of isolated blood vessels in fish (23, 31), amphibians, and reptiles (26). In nonanesthetized fish, intra-arterial injection of ET-1 produces vascular responses that are similar to those observed in mammals (23). However, to date, central cardiovascular effects of ET-1 have not been reported in nonmammalian vertebrates.

The aim of the present study was to investigate the cardiovascular effects of intracerebroventricular and peripheral injections of picomolar doses of ET-1 in the rainbow trout, which has proven to be a very appropriate experimental model to determine the hemodynamic responses to regulatory peptides (18, 19, 23). We have also investigated the mechanisms involved in the cardiovascular activity of ET-1 in conscious trout.

MATERIALS AND METHODS

Chemicals

Synthetic ET-1 and ET-3 were purchased from Neosystem (Strasbourg, France). Norepinephrine was obtained from Sigma (St. Louis, MO). Bosentan or Ro-47–0203 (4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenox)-2, 2′-bipyrimidin-4-yl]-benzenesulfonamide sodium salt) was a generous gift from Hoffmann-La Roche (Basel, Switzerland). ET-1 and ET-3 were stored in stock solution (10$^{-4}$ M) at -25°C. Peptides and norepinephrine were diluted in Ringer buffer (vehicle) just before use. Bosentan was initially dissolved in

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distilled water and diluted to the desired concentration with Ringer buffer. The composition of the Ringer solution was (in mmol/l) 124 NaCl, 3 KCl, 0.75 CaCl₂, 1.30 MgSO₄, 1.24 KH₂PO₄, 25 NaHCO₃, 10 glucose (pH 7.8). All solutions were sterilized by filtration through 0.22-µm filters (Millipore, Molsheim, France) before injection.

Animals

Adult rainbow trout (Oncorhynchus mykiss, 290 ± 3.8 g) were purchased locally and maintained in a cylindrical tank containing 1,000 liters of refrigerated water (12 ± 1°C), dechlorinated, and aerated tap water. Fish were maintained under a standard photoperiod (lights on 0900–2000) for at least 3 days before the beginning of the experiments. Animal manipulations were performed according to the recommendations of the French Ethical Committee and under the supervision of authorized investigators.

Surgical Procedures

The surgical procedures for dorsal aorta cannulation and intracerebroventricular guide placement have been described previously in detail (19). Briefly, trout were anesthetized by immersion in tricaine methanesulfonate (3-aminobenzoic acid ethyl ester; Sigma); 60 mg/l of tap water, and the dorsal aorta was cannulated with a PE-50 catheter (Clay Adams). A 25-gauge needle fitted with a PE-50 catheter was inserted into the third ventricle of the brain, so that the injection of test substances occurred at the level of the preoptic nuclei. The intracerebroventricular injector was made from a 33-gauge stainless steel cannula connected with a PE-10 tubing to a 10-µl Hamilton syringe. In a few experimental fish (n = 11), the cardiac output (Q˙) was also measured. A midline incision was made through the skin and muscle immediately anterior to the base of the pectoral fins at a position overlying the ventral aorta, as previously described (8). A cuff-type Doppler probe (2.0 mm I.D.; Iowa Doppler Products, Iowa City, IA) was placed around the ventral aorta. As previously reported (8), bleeding was usually minimal during the operation. The incision was sutured and the leads from the flow probe were secured to the skin sutures. The trout was allowed to recover from the anesthesia and placed into an experimental 6-liter aquarium, which was painted black and supplied with partially recycled and aerated tap water (11–13°C). Oxygen tension in the water tank (YSI model 57 oxygen probe; 20.0 kPa) and pH (7.50–7.80) were continuously recorded and maintained at constant levels. A small horizontal aperture was made along the upper edge of the aquarium for connection of the dorsal aorta cannula and intracerebroventricular injections of substances without disturbing the trout.

Experimental Protocols

General controls of homeostasis mechanisms before injections. Operated trout were allowed 24 h to recover from surgery and to be accustomed to their new environment. Each day, small amounts of blood were taken from the dorsal aorta to ascertain that the general homeostasis mechanisms of fish were not impaired. One hundred microliters of blood was taken from the dorsal aorta in heparinized tubes (25 U/100 µl). Blood was centrifuged (12,000 g, 4°C, 15 min), and a 50-µl aliquot of plasma was stored at −20°C. Hematocrit was determined by a microcapillary method (microhematometer, Hawksley). A 10-µl sample of plasma was also used to determine osmolality (vapor pressure osmometer, Wescor 5500). Plasma cortisol level was determined at random on 23 experimental fish during the 10 mo of the experiment (from September to June). After extraction in absolute ethanol, cortisol concentration was determined by radioimmunoassay (16). The sensitivity threshold of the assay was 2.5 pg, and values were corrected for recovery (70.3%).

Test for cardiovascular normality and vascular reactivity. The pressure pulsatility (ratio of the pulse pressure to the mean blood pressure) was determined before each experimental session and used as an index of cardiovascular normality (19). When the pulsatility was <0.10, the trout was discarded. A single intra-arterial injection of norepinephrine (0.62 nmol) was used to check the usual cardiovascular reactivity to exogenous adrenergic agents. Only trout that displayed an immediate increase in diastolic blood pressure (DP) and systolic blood pressure (SP) of ~5% over baseline and a concomitant fall in HR of ~10% under baseline were selected for further experiments. Over 90% of the experimental animals fulfilled these criteria. Once baseline levels of mean dorsal aortic blood pressure (PₒD) and HR were stabilized (~2 h), the experimental session for intracerebroventricular or intra-arterial injections started. For all protocols, fish received a control injection of vehicle and, 30 min later, an injection of peptide. The animals usually received a single injection of peptide per day. When two injections were made, a delay of at least 6 h was observed between the injections to avoid tachyphylaxis.

Intracerebroventricular administration of ET-1 or ET-3. A total of 39 fish received intracerebroventricular injection of ET-1 or ET-3. The injector was preloaded with distilled water. A small bubble was created at the level of the PE-10 tubing, and the injector was loaded with vehicle or ET solution. The injector was inserted into the intracerebroventricular guide and, once the cardiovascular parameters had stabilized, the recording session started for 30 min. After 5 min of recording (baseline), 0.5 µl of vehicle (Ringer buffer) or 0.5 µl of ET-1 solution was injected over 30 s into the third ventricle. Pilot experiments showed that intracerebroventricular injection of ET-1, at doses >50 pmol, caused rapid death of the animals. Therefore, in the present study, ET-1 was tested at doses of 6.25, 12.5, and 25 pmol (equivalent to ~21.5, 43, and 86 pmol/kg, respectively). These picomolar doses are in the same range as those applied previously for intracerebroventricular injection in rats (27, 28). They are lower than the doses generally applied intracerebroventricularly to test the hypertensive effects of other neuropeptides in fish (18). ET-3 was only tested at a dose of 25 pmol (equivalent to ~86 pmol/kg).

Intracerebroventricular administration of bosentan. The nonpeptide antagonist of ET receptors Ro-47–0203 (bosentan) (4) was used to test the specificity of the PₒD and HR responses to intracerebroventricular injections of ET-1. A total of 27 fish received an injection of 17.5 nmol of bosentan into the third ventricle. In rat, intracerebroventricular injection of ET receptor antagonists is usually performed at least 15 min before the administration of ETs (27). Thus, in the present study, intracerebroventricular administration of ET-1 (6.25, 12.5, and 25 pmol; equivalent to ~21.5, 43, and 86 pmol/kg, respectively) was performed 30 min after the injection of bosentan.

Intra-arterial administration of ET-1 or ET-3. A total of 31 fish received intra-arterial injection of ET-1 or ET-3. Five minutes after the beginning of the recording session, 50 µl of vehicle or ET-1 (6.25, 12.5, and 25 pmol; equivalent to ~21.5 pmol/kg, respectively) or ET-3 solution (25 pmol; equivalent to ~86 pmol/kg) was injected through the dorsal aorta and immediately flushed with 150 µl of vehicle. To prevent the recording of the pressure artifact due to the injection, the computer was stopped for 10 s during intra-arterial injections.
Intra-arterial administration of bosentan. A total of 19 fish received intra-arterial injection of 1.75 µmol of bosentan. In rat, intra-arterial injection of bosentan at the same dose (3 mg/kg) is usually performed 5 min before the administration of ETs (4). In addition, pilot experiments showed that intra-arterial injection of bosentan 30 min before the intra-arterial administration of ET-1 did not modify the cardiovascular effects evoked by the peptide. So, bosentan was injected through the intra-arterial cannula 5 min before the intra-arterial administration of ET-1 (6.25, 12.5, and 25 pmol; equivalent to ~21.5, 43, and 86 pmol/kg, respectively).

Measurement of Plasma Cortisol Level

The effect of peripheral administration of ET-1 on cortisol secretion was investigated in 10 trout. A sample of 100 µl of blood was taken just before the recording session and replaced by the same volume of Ringer buffer. The animals then received an intra-arterial injection of ET-1 (25 pmol; equivalent to ~86 pmol/kg). Samples of blood (100 µl each) were taken 12.5 and 25 min after the injection of the peptide and replaced by the same volume of Ringer buffer. Plasma cortisol levels were measured by radioimmunoassay as described above.

Recording of PDA, HR, and Q, and Processing of Data

The heparinized aortic cannula was connected to a pressure transducer P23 ID (Gould Electronique, Ballainvilliers, France). The transducer was calibrated every day, with a static water column. The leads from the Doppler flow were attached to a Doppler flowmeter (University of Iowa, Iowa City, IA). The zero flow level was set electronically, and the range gate controls of the Doppler unit were adjusted to record the highest signal on the output. Thereafter, the mean signal was continuously recorded as kilohertz of Doppler shift (ΔkHz). The existence of a linear correlation between the Doppler signal and the mean blood flow has been previously demonstrated in fish (1). SP, DP, pulse pressure (pulse pressure = SP − DP), HR, and Q were processed by a digital oscilloscope (Gould 1604), and the data were transferred every 2 s to a 486 personal computer. The $P_{\text{DA}}$ ($P_{\text{DA}} = (\text{SP} + \text{DP})/2$), the pressure pulsatility (pulsatility = pulse pressure/ $P_{\text{DA}}$), HR, Q, and systemic vascular resistance (SVR = $P_{\text{DA}}/Q$) were also calculated offline by the computer for the preinjection period (control period, 0–5 min) and the postinjection period (5–30 min). The mean maximum value of these parameters was determined during the postinjection period (5–30 min). Central venous blood pressure was assumed to be zero for the calculation of SVR (13). The barostatic gain, corresponding to the change in the HR per unit change in $P_{\text{DA}}$ (8), was also calculated for preinjection and for postinjection maximal values of HR and $P_{\text{DA}}$. Results for cardiovascular parameters are expressed either as absolute values (HR in beats/min, $P_{\text{DA}}$ in mmHg), arbitrary units (Q in ΔkHz, SVR in mmHg/ΔkHz), or percentages of the control values.

Statistical Analysis

All data are expressed as the mean (±SE) for 8–24 experiments. Data were analyzed by Student’s paired $t$-test or by one-way analysis of variance with repeated measures or by two-way analysis of variance. When appropriate, the multiple-range test of Student-Newman-Keuls was used subsequently to determine significant differences within and between groups. The criterion for statistical significance was $P < 0.05$.

RESULTS

Preinjection values for hematocrit and plasma osmolality were 24.6 ± 1.5% and 285 ± 1.3 mosmol/kg H$_2$O, respectively. The mean plasma cortisol level was 39.8 ± 3.4 ng/ml. The baseline value of the $P_{\text{DA}}$ ranged between 22 and 26 mmHg and the spontaneous HR was 55–68 beats/min.

Effect of Intracerebroventricular Administration of ET-1 or ET-3 on Cardiovascular Parameters

The effect of intracerebroventricular injection of ET-1 (25 pmol) on $P_{\text{DA}}$ and HR is illustrated in Fig. 1. The
peptide provoked a gradual increase in \( P_{DA} \), which reached a maximum (8.2 ± 1.9 mmHg above baseline level) within 15 min after the end of ET-1 administration (Fig. 1A). Then, \( P_{DA} \) remained significantly elevated during the whole recording period. Concurrently, intracerebroventricular injection of ET-1 significantly decreased HR 5 min after ET-1 administration and throughout most of the recording (Fig. 1B). Both \( P_{DA} \) and HR returned to preinjection values within 2 h after ET-1 administration (data not shown). No mortality was observed after intracerebro-ventricular injection of 25 pmol of ET-1. At the end of the recording session, some of the animals exhibited tail-flip activity. In contrast, intracerebroventricular injection of ET-3 (25 pmol) or vehicle did not cause any significant effect on \( P_{DA} \) or HR (Fig. 1) and did not affect the behavior of the animals.

Intracerebroventricular injection of graded doses of ET-1 (6.25–25 pmol) induced a dose-related increase in \( P_{DA} \) (Fig. 2A). A significant decrease in HR occurred only after administration of the highest dose (25 pmol) of ET-1 (Fig. 2B). The peak values for both parameters were observed within 5–15 min after the injection, depending on the dose. Intracerebroventricular administration of bosentan (17.5 nmol), 30 min before the injection of ET-1, significantly attenuated the increase in \( P_{DA} \) induced by an intracerebroventricular injection of 12.5 and 25 pmol of ET-1 (Fig. 2A). In addition, bosentan significantly attenuated the bradycardia provoked by ET-1 (\( F_{1,74} = 11.37; P < 0.05 \)). In particular, bosentan totally abolished the bradycardia evoked by 12.5 pmol of ET-1 (Fig. 2B). It was also noticed that intracerebroventricular administration of bosentan caused by itself a rapid and transient increase in \( P_{DA} \) that peaked 2 min after injection of the antagonist (2.35 ± 1 mmHg above baseline; \( P < 0.05 \)). In contrast, intracerebroventricular administration of bosentan did not significantly affect HR.

In another set of experiments, \( P_{DA} \), HR, and \( Q \dot{} \) were simultaneously monitored after intracerebroventricular injection of ET-1 (25 pmol). The hematocrit of the fish equipped with a Doppler probe (24.4 ± 2%) was not significantly different from that of other experimental animals (27.1 ± 2.5%). Central administration of the peptide caused a significant (\( P < 0.05 \)) increase of SVR (63%) but had no significant effect on \( Q \) (Table 1).

**Effect of Intra-Arterial Administration of ET-1 and ET-3 on Cardiovascular Parameters**

Intra-arterial injection of ET-1 (25 pmol) provoked an immediate and sustained increase in \( P_{DA} \) (Fig. 3A). The maximum effect was reached within 10 min after the injection of the peptide (3.9 ± 0.6 mmHg above baseline level). Intra-arterial injection of ET-1 also decreased HR 5 min after ET-1 administration and throughout most of the recording (Fig. 3B). Both \( P_{DA} \) and HR returned to preinjection values within 90 min after ET-1 administration (data not shown). In contrast,

### Table 1. Effect of intracerebroventricular or intra-arterial injection of ET-1 on hemodynamic responses

<table>
<thead>
<tr>
<th></th>
<th>( P_{DA} ), mmHg</th>
<th>HR, beats/min</th>
<th>( Q \dot{} ), kHz</th>
<th>SVR, mmHg/kHz</th>
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</thead>
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<tr>
<td>Intracerebroventricular injection</td>
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<tr>
<td>Preinjection values</td>
<td>21.66 ± 0.53</td>
<td>61.83 ± 2.34</td>
<td>1.15 ± 0.14</td>
<td>22.50 ± 3.29</td>
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<td>Maximum postinjection values</td>
<td>32.25 ± 1.87*</td>
<td>51.33 ± 1.89*</td>
<td>1.39 ± 0.21</td>
<td>36.70 ± 7.26*</td>
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<tr>
<td>Intra-arterial injection</td>
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<tr>
<td>Preinjection values</td>
<td>26.22 ± 1.13</td>
<td>62.57 ± 1.67</td>
<td>1.24 ± 0.15</td>
<td>25.35 ± 3.52</td>
</tr>
<tr>
<td>Maximum postinjection values</td>
<td>31.80 ± 1.42*</td>
<td>52.22 ± 2.48*</td>
<td>0.99 ± 0.48</td>
<td>35.26 ± 4.13*</td>
</tr>
</tbody>
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Values are means ± SE (\( n = 11 \) trout). \( P_{DA} \), mean dorsal aortic blood pressure; HR, heart rate; \( Q \dot{} \), cardiac output; SVR, systemic vascular resistance; ET-1, endothelin-1. *\( P < 0.05 \) vs. preinjection values (Student's paired t-test).
Intra-arterial injection of ET-3 (25 pmol) or vehicle did not significantly affect $P_{DA}$ (Fig. 3A) or HR (Fig. 3B).

Intra-arterial administration of graded doses of ET-1 (6.25–25 pmol) produced a dose-dependent increase in $P_{DA}$ (Fig. 4A) and a dose-related decrease in HR (Fig. 4B). The peak values for both parameters were observed within 5–15 min after the injection, depending on the dose. A two-way analysis of variance applied to the data shown in Figs. 2 and 4 demonstrated that intracerebroventricular injection of ET-1 induced a greater increase in $P_{DA}$ ($F_{1,76} = 6.76; P < 0.05$) than did intra-arterial injection of the same dose of ET-1. In particular, a significant twofold greater increase in $P_{DA}$ was observed after intracerebroventricular administration of ET-1 at doses of 12.5 and 25 pmol compared with the intra-arterial injection of the same doses of the peptide. In addition, after administration of ET-1 at doses of 12.5 and 25 pmol, the barostatic gain was reduced 2.5–3 times compared with that calculated after intra-arterial injection of the same doses of the peptide (for the 12.5 pmol dose: $-1.18 \pm 0.54$ vs. $-2.97 \pm 0.34$ beats·min$^{-1}$·mmHg$^{-1}$, $P < 0.05$; for the 25 pmol dose: $-0.89 \pm 0.16$ vs. $-2.46 \pm 0.45$ beats·min$^{-1}$·mmHg$^{-1}$, $P < 0.05$).

Intra-arterial administration of bosentan (1.75 µmol), 5 min before the injection of ET-1, markedly attenuated the effect of the peptide on $P_{DA}$ (Fig. 4A). The bradycardia induced by intra-arterial injection of 6.25 and 25 pmol of ET-1 was also significantly attenuated after intra-arterial pretreatment of trout with bosentan (Fig. 4B). Intra-arterial administration of bosentan alone induced a biphasic effect on $P_{DA}$, i.e., an immediate and significant ($P < 0.05$) hypotensive phase ($2.9 \pm 1.3$ mmHg under baseline), followed by a brief and significant ($P < 0.05$) hypertensive response ($1.9 \pm 1.2$ mmHg above baseline). In contrast, intra-arterial administration of bosentan did not significantly affect basal HR.

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**Fig. 3.** Effect of intra-arterial injection of 50 µl of vehicle (●), 25 pmol of ET-1 (●), or 25 pmol of ET-3 (▲) on $P_{DA}$ (A) and HR (B) in conscious trout. Arrowhead indicates onset of injection. Each curve represents mean (±SE at selected times) of individual recordings from 10 or 11 fish. *$P < 0.05$ vs. baseline values (0 and 5 min time points) or vehicle at given time.

**Fig. 4.** Histograms showing maximum changes in $P_{DA}$ (A) and HR (B) after intra-arterial injection of vehicle (50 µl) or graded doses of ET-1 (6.25–25 pmol) in control trout (open bars) or in trout treated with 1.75 µmol of bosentan 5 min before ET-1 injection (solid bars). Values are expressed as percentages of preinjection period (0–5 min) and are presented as mean ± SE of 10 or 11 individual recordings from different fish. *$P < 0.05$ vs. vehicle. +$P < 0.05$, pretreated trout vs. control trout.
In another set of experiments, $P_{DA}$, HR, and $Q$ were simultaneously monitored after intra-arterial injection of ET-1 (25 pmol). Peripheral administration of the peptide did not affect $Q$ but caused a significant increase in SVR (+39%; Table 1).

**Effect of Intra-Arterial Administration of ET-1 on Plasma Cortisol Concentration**

The mean cortisol level in trout plasma before administration of ET-1 was 39.2 ± 5.4 ng/ml. Plasma cortisol concentration was unchanged 12.5 and 25 min after intra-arterial injection of ET-1 (47.8 ± 9.8 and 46.6 ± 9.0 ng/ml, respectively).

**DISCUSSION**

The cardiovascular effects of neuropeptides largely depend on the general physiological status and behavior of the animals under experimentation. The present study was conducted in nonanesthetized trout to avoid the effects of anesthetic agents on cardiovascular functions. The hematocrit and the plasma osmolality levels were within the usual range measured in conscious and catheterized trout (19, 29). Cardiovascular parameters were also in the range of values reported in previous studies on rainbow trout (23); in particular, HR level indicated tonic parasympathetic drive to the heart. This observation suggests that the animals had recovered well from the surgical procedures and were adapted to the new environmental situation (8). In addition, the basal plasma cortisol level of the experimental trout was in the same range as that previously determined in cannulated trout within 1–5 days after surgery (2), indicating that the animals had recovered satisfactorily from the surgical stress.

The present study has demonstrated that intracerebroventricular injection of very low doses of ET-1 provoked a dose-dependent increase in $P_{DA}$. These data provide the first evidence for a hypertensive effect of centrally administered ETs in a nonmammalian vertebrate. At the highest dose tested (25 pmol), ET-1 also induced a significant reduction of HR. Several lines of evidence indicate that the cardiovascular effects observed after intracerebroventricular injection of ET-1 are attributable to a direct central action of the peptide rather than a peripheral action. 1) The existence of a blood-brain barrier has been demonstrated in fish (24), and several reports indicate that this blood-brain barrier is impermeable to peptide diffusion. For instance, intracerebroventricular injection of high doses of $\alpha$- or $\beta$-melanocyte-stimulating hormone (up to 0.67 µg) in conscious killifish does not elicit any darkening of the skin, indicating that the peptides do not cross the blood-brain barrier (14). Similarly, intracerebroventricular injection of 500 pmol of melanin-concentrating hormone to trout does not induce any pallor of the body surface of the animals up to 2 h after the injection, whereas intra-arterial administration of the same dose of the peptide induces marked bleaching of the skin within 10 min (personal unpublished data). 2) Intracerebroventricular administration of 12.5 and 25 pmol of ET-1 induced an increase in $P_{DA}$ that was two times higher than that provoked by intra-arterial injection of the same doses of the peptide. The fact that intracerebroventricular injection of ET-1 provoked a barostatic gain that was reduced by 2.5- to 3-fold compared with that calculated after intra-arterial administration of the peptide suggests that, in trout, ET-1 may act centrally to blunt baroreceptor-mediated cardioinhibitory reflex. The delay (5–15 min) observed between the administration of ET-1 and the maximum effect on $P_{DA}$ in trout was similar to that previously reported in conscious rat after intracerebroventricular injection of similar doses of ET-1 (8–66 pmol) (25, 27). Changes in arterial pressure may result from variations of either $Q$ or SVR (or both). The use of a miniature Doppler flow probe for measurement of $Q$ made it possible to demonstrate that the hypertensive response evoked by intracerebroventricular injection of ET-1 could be accounted for mainly by an increase in SVR.

The minimum effective dose of ET-1 applied centrally in trout compares favorably with the threshold doses reported in rat, i.e., −8–20 pmol (25, 27, 28). It has been observed that intracerebroventricular injection of higher doses of ET in rat causes rotational behavior and convulsions (25, 28), followed by cardiovascular collapse and death (28). We also found that, in trout, intracerebroventricular administration of doses of ET-1 >50 pmol caused the death of the animals. The lethal effect of ET-1 can be likely ascribed to cerebral ischemia because intracerebroventricular injection of ET-1 causes reduction of cerebral blood flow (27). However, it has been reported that, in rat, the initial pressor response evoked by centrally administered ET-1 (30 pmol) is not a consequence of cerebral ischemia (10), suggesting that the hypertensive response induced by intracerebroventricular injection of ET-1 in trout is not secondary to local cerebral vasoconstriction. Although no mortality was observed after injection of 25 pmol of ET-1, at the end of the recording session the trout exhibited tail-flip activity.

The effects of ETs are mediated by at least three types of G protein-coupled membrane receptors that exhibit differential affinities for the various isoforms of ETs (30): the $ETA$ receptor type possesses a higher affinity for ET-1 and ET-2 than ET-3; the $ETB$ receptor does not discriminate between the three isoforms; and the $ETC$ receptor exhibits high affinity for ET-3. The observation that the hypertensive response to intracerebroventricular administration of ET-1 was inhibited by the mixed $ETA/ETB$ receptor antagonist bosentan (4) indicated that the effect of the peptide was mediated through $ETA$ and/or $ETB$ receptors. Because intracerebroventricular administration of ET-3 did not affect $P_{DA}$ or HR, we conclude that the central effect of ET-1 can be ascribed to activation of an $ETA$ receptor subtype. In fact, competition studies have shown the occurrence of selective ET-1 binding sites (presumably corresponding to $ETA$ receptors) in the fish brain (35). Studies conducted in mammals have shown that the cardiovascular responses evoked by intracerebroventricular administration of ETs are mediated by $ETA$ receptors (27). It
Peripheral administration of picomolar doses of ET-1 provoked a clear dose-related increase in PDA associated with a decrease in HR. The threshold dose inducing a significant increase in PDA is in the same range as those previously reported for other vasoactive peptides, such as arginine vasotocin (19) and urotensin II (18). In a previous study, Olson et al. (23) reported that bolus intra-arterial injection of 500 ng/kg body wt (=200 pmol/kg body wt) of ET-1 in trout only produced a transient decrease in PDA, whereas, at doses of 1,500 and 5,000 ng/kg body wt (=600 and 2,000 pmol/kg body wt), ET-1 provoked a triphasic pressor-depressor-pressor response. We now demonstrate that intra-arterial injection of ET-1, at doses 3–30 times lower than those used by Olson et al. (23), only caused a pressor effect and a concomitant bradycardia. The observation that the hypertensive response to ET-1 was accompanied by a reduction of Q indicates that the rise in PDA can be accounted for by systemic vasoconstriction. Consistent with this notion, in vitro studies have demonstrated that, in fish, ET-1 causes a dose-dependent contraction of vascular smooth muscles (23, 31). It has been suggested that in vivo administration of ET-1 could be responsible for coronary spasm (7), which may affect cardiac performance. However, ET-1 does not exert any inotropic or chronotropic effects in trout (23). It thus appears that the reduction in HR and Q observed in the present study can be likely ascribed to activation of cardioinhibitory baroreflexes.

Administration of bosentan 5 min before the intra-arterial injection of ET-1 markedly reduced the pressor and chronotropic responses to ET-1, indicating that the peptide exerted its effect through a classical ETA or ETB receptor. In addition, intra-arterial injection of bosentan caused by itself a consistent depressor/pressor response, suggesting the existence of a physiological endothelinsergic system regulating the basal PDA in trout. Because intra-arterial injection of the selective ETB agonist ET-3 did not affect blood pressure or HR, it appears that the hypertensive and bradycardic effects of ET-1 are mediated by an ETA receptor subtype. Consistent with these data, an ETA-like receptor has previously been characterized in the gills of the rainbow trout (20). Similarly, in rat, ETA receptors are involved in the hypertensive effect induced by intra-arterial administration of ET-1 (30).

In vitro studies have shown that, in amphibians, subnanomolar concentrations of ET-1 stimulate the secretion of corticosteroids through activation of ETA receptors (3, 5). Assuming that the plasma volume of the trout is ~20 ml/kg body wt (6), a bolus injection of 25 pmol of ET-1 would yield a circulating concentration of the peptide of ~4 nM. In fact, Olson (22) has shown that, in fish, ET is readily extracted from the circulation, suggesting that the plasma level of ET-1 after a single intra-arterial injection of the peptide could be much lower. The present study has demonstrated that intra-arterial administration of 25 pmol of ET-1 does not induce a significant increase in plasma cortisol level, suggesting that, in trout, ETs do not play a significant role in the control of adrenal steroidogenesis. In support of this hypothesis, it has been reported that prolonged infusion of ET-1 does not affect urine flow and electrolyte concentrations in trout (23).

In conclusion, the present study has demonstrated that central and peripheral administration of picomolar doses of ET-1 causes a substantial rise in PDA and a concomitant decrease in HR. Both the central and peripheral effects of ET-1 can be ascribed to activation of ETA receptors.

Perspectives

The results of the present study have shown that in trout the hypertensive action of ET-1 in the periphery is matched by a similar qualitative effect of the peptide in the brain. The high potency of ET-1 in evoking the hypertensive response is indicative that the peptide has a physiological role in cardiovascular regulation in trout. Significant pressor effects were observed after both intra-arterial and intracerebroventricular injection of 12.5 pmol of ET-1. In contrast, in the same system, a dose of 300 pmol of trout galanin was required to elicit significant hypertension following intracerebroventricular injection, and a dose of 150 pmol was required to produce significant hypotension following intra-arterial injection (17). The relevance of the data to trout cardiovascular physiology is also emphasized by the recent preliminary report describing the purification and structural characterization of trout ET (J. M. Conlon, personal communication). A single molecular form of the peptide was identified in an extract of trout kidney whose amino acid sequence was the same as human ET-1 in 17 out of 21 residues. It is significant that a peptide corresponding to human ET-3, which did not exhibit biological activity in the trout under the conditions used in this study, was not present in trout tissues.

ET is released by the constitutive rather than the regulated secretory pathway, and it is conceivable that ET may exercise its effects in the periphery by a classical hormonal mechanism. Centrally, ET may have a neuromodulatory or a neurotransmitter function acting on nuclei involved in neuroendocrine or neuronal control of cardiovascular functions. Further studies are clearly required to determine the localization of ET-producing elements as well as the distribution of ET-binding sites and to reveal under which circumstances the endothelinsergic system of fish is triggered to participate in cardiovascular homeostasis.

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