Contrasting cardiovascular effects following central and peripheral injections of trout galanin in trout

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Le Mével, Jean-Claude, Dominique Mabin, Ann M. Hanley, and J. Michael Conlon. Contrasting cardiovascular effects following central and peripheral injections of trout galanin in trout. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1118–R1126, 1998.—Little is known about the role of galanin (Gal) in fish. In the present study, cardiovascular effects of central and peripheral administrations of a synthetic replicate of trout Gal (tGal) were investigated in the unanesthetized trout. Intracerebroventricular injection of 0.1, 0.5, 1.0, and 3.0 nmol/kg body mass of the peptide demonstrated that the two highest doses tested produced a significant (P < 0.001) and equivalent increase in mean dorsal aortic blood pressure (PDA) without changing heart rate (HR). At a dose of 1.0 nmol/kg, the systemic vascular resistance (Rsys) increased, but no change was detected in cardiac output compared with that produced by intracerebroventricular injection of vehicle only. In contrast, intra-arterial injections of 0.1, 0.5, and 1.0 nmol/kg body mass of tGal produced a dose-dependent decrease in PDA with a threshold dose for significant effects observed at a dose of 0.5 nmol/kg. None of the doses tested changed HR. At a dose of 1 nmol/kg, a significant decrease in Rsys (P < 0.001) was the factor responsible for the fall in PDA. Intra-arterial injection of porcine Gal (1 nmol/kg) produced a change in PDA similar to that of the same dose of tGal, but HR increased slightly. Pretreatments of trout with the cyclooxygenase inhibitors indomethacin and meclofenamate did not inhibit the vasodepressor effects of tGal. However, after intra-arterial injection of Nω-nitro-arginine methyl ester, an inhibitor of nitric oxide synthase, the hypotensive action of Gal was reduced threefold, suggesting the possible involvement of the nitric oxide system in mediating the vasodilatory effect of Gal. In conclusion, our results have shown that tGal may have contrasting cardiovascular regulatory functions in trout depending on whether its site of action is the brain or the peripheral circulation.

neuropeptide; intracerebroventricular injection; intra-arterial injection; vasoconstriction; vasodilatation

GALANIN (Gal) is a 29- or 30-amino acid residue neuropeptide that was first isolated from porcine intestine on the basis of its α-amidated COOH-terminal amino acid (29). Subsequently, Gal was purified and characterized from several vertebrate species, and data demonstrated that the 14 amino acids at the NH2 terminus of the molecule are fully conserved from fish to human (1, 34). In mammals, Gal is present within the central nervous system, within the gastrointestinal tract, and in cardiac sympathetic neurons (reviewed in Ref. 4). The major actions of Gal in mammals include inhibition of acetylcholine, glutamate, and insulin release, stimulation of feeding, stimulation of pituitary hormone release, and inhibition of spinal nociceptive reflexes (reviewed in Ref. 4). Gal was shown to elicit a variety of cardiovascular effects that are species dependent. Intravenous Gal had little or no effect on blood pressure (BP) in dogs (23) but slightly decreased BP and inhibited cardiac vagal action in cats (27). In rats, central administration of Gal induced hypotensive and tachycardic effects (9), but renal vasodilatation, without significant effects on blood pressure or heart rate, could also be observed in this species (8). Gal neurons originating from the paraventricular nucleus inhibit the baroreflex response (3).

In fish, Gal immunoreactivity was detected in nerve fibers of the gastrointestinal tract (10, 18), within intracardiac axons innervating the atrium, and in nerve fibers in lung (10). Gal-IR neurons are also present in the brains of several species of teleost (reviewed in Ref. 1) and in the brain of the elasmobranch fish Scyliorhinus canicula (30). In addition, binding sites for 125I-labeled Gal have been identified in the brain of the Atlantic salmon Salmo salar (11). This widespread distribution of Gal and its receptors throughout the peripheral and central nervous system suggests that Gal may have some neuroregulatory functions in fish. However, there have been few physiological studies in fish, and all the previous work was conducted using a mammalian sequence of Gal. In vitro, Gal increased the dilution potential within epithelial cells (18) and contracted isolated vessels and arteries of teleost (16) and elasmobranch fish (26). In vivo, the cardiovascular effects of Gal are inconsistent and appear to be species dependent. Intra-arterial injection of the peptide in the conscious lungfish Neoceratodus forsteri induced no change in mean dorsal aortic blood pressure (PDA) but reduced heart rate (HR) (10), whereas, in an anesthetized elasmobranch, the effects after intra-arterial injection of Gal were conflicting (26).

The recent elucidation of the amino acid sequence of trout galanin (tGal) (1) prompted us to investigate the effects of intracerebroventricular and intra-arterial injection of a synthetic replicate of the peptide on the hemodynamic parameters in the unanesthetized trout.
Peptide Synthesis

tGal (Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu\textsuperscript{16}-Leu-Gly-Pro-His-Gly-Ile-Asp-Gly-His-Arg\textsuperscript{20}-Thr-Leu-Ser-Asp-Lys-His-Gly-Leu-Ala-NH\textsubscript{2}) was synthesized by solid-phase methodology on a 0.025-mmol scale using an Applied Biosystems synthesizer. Fluorenylmethoxycarbonyl-labeled amino acids were coupled as their hydroxybenzotriazole-active esters following the manufacturer's standard protocols. The peptide was cleaved from the resin using trifluoroacetic acid-water-ethanedithiol-thioanisol (900:30:30:40 by volume) and purified to near homogeneity by reversed-phase HPLC. Identity of the peptide was confirmed by amino acid analysis, and electrospray mass spectrometry (observed molecular mass 3043.0 Da; calculated molecular mass 3043.4 Da).

Chemicals

Porcine Gal (pGal), N\textsubscript{6}-nitro-arginine methyl ester (L-NAME), meclofenamate, and indomethacin were obtained from Sigma Chemical (L'Isle d'Abeau, France). tGal and pGal were stored in stock solution at −25°C. Peptides, L-NAME, and meclofenamate were diluted in Ringer buffer (vehicle) just before use. Indomethacin was dissolved in 0.1 M NaHCO\textsubscript{3}-ethanol (3:1, vol/vol). This vehicle for indomethacin had no noticeable effect on baseline levels of HR and P\textsubscript{DA} and did not modify the usual responses observed after intra-arterial injection of Gal. The composition of the Ringer solution was (in mM) 124 NaCl, 3 KCl, 0.75 CaCl\textsubscript{2}, 1.30 MgSO\textsubscript{4}, 1.24 KH\textsubscript{2}PO\textsubscript{4}, 25 NaHCO\textsubscript{3}, and 10 glucose, pH 7.8. All solutions were sterilized by filtration on 0.22-μm filters (Millipore, Molsheim, France) before injection.

Animals

Adult rainbow trout (Oncorhynchus mykiss; 305 ± 4.2 g body mass) were purchased locally and maintained in a round tank containing 1,000 liters of dechlorinated, aerated tap water under a standard photoperiod (lights on: 0900–2000). Fish were maintained under these controlled conditions for at least 8 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 given previously in detail (20). In brief, trout were anesthetized by immersion in tricaine methanesulfonate (3-aminobenzoic acid ethyl ester; Sigma; 60 mg/l tap water) and the dorsal aorta was cannulated with PE-50 polyethylene tubing (Clay Adams, Parsippany, NJ). A small bone flap was removed, and, under stereomicroscopic control, a 25-gauge needle fitted with a PE-50 catheter was inserted into the third ventricle of the brain so that the injection of the test substances occurred at the level of the preoptic nuclei. The absence of cerebral cortex in fish allowed direct and accurate placement of the intracerebroventricular guide within the third ventricle without the need of dye injection or postmortem examination. The intracerebroventricular injector was made from a 33-gauge stainless steel cannula connected via PE-10 polyethylene tubing to a 10-μl Hamilton syringe. In some fish (n = 14), cardiac output (Q = ventral aortic blood flow) was also measured using a cuff-type Doppler probe (2.0-mm internal diameter; Iowa Doppler Products, Iowa City, IA) placed around the ventral aorta through a midline incision immediately anterior to the base of the pectoral fins. The incision was sutured, and the leads from the flow probe were secured to the skin sutures. The trout were allowed to recover from the anesthesia and were placed into an experimental 6-liter aquarium that had been painted black. The trout were supplied with partially recycled and aerated tap water (11–13°C). Oxygen tension in the water tank (20.0 kPa) and pH (7.60–7.80) were continuously recorded and maintained at these constant levels. A small aperture was made along the upper horizontal 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 and cardiovascular reactivity before injections of Gal. Operated trout were allowed 24 h to recuperate from the surgical procedures and to become accustomed to their new environment. Each day, small quantities of blood were taken from the dorsal aorta to ensure that the general homeostasis mechanisms of the fish were not impaired. Hematocrit was determined by a microcapillary method (microhematometer, Hawkslay, UK). Plasma (10 μl) was used to determine osmolality (vapor pressure osmometer, Wescor 5500, Logan, UT). All animals used in the study responded to a bolus intra-arterial injection of norepinephrine (2 nmol/kg) with an immediate rise (−5% above baseline) in P\textsubscript{DA} and a concomitant fall in HR (−10% below baseline). After baseline levels of P\textsubscript{DA} and HR were stabilized (−2 h), the experimental session involving intracerebroventricular or intra-arterial injections started.

Intracerebroventricular administration of Gal. The injector was preloaded with distilled water. A small bubble was created in the PE-10 polyethylene tubing, and the injector was loaded with vehicle or tGal at the appropriate concentration. The injector was inserted into the intracerebroventricular guide, and the cardiovascular parameters were allowed to stabilize. The recording session was then started for 30 min, and, after 5 min of recording (baseline), 0.5 μl of vehicle (Ringer solution) or 0.5 μl of tGal was injected over 30 s into the third ventricle of the brain. tGal was tested at doses of 0.1, 0.5, 1, and 3 nmol/kg body mass.

Intra-arterial administration of Gal. Five minutes after the beginning of the recording session, 50 μl of vehicle or tGal at the appropriate concentration was injected through the dorsal aorta and immediately flushed by 150 μl of vehicle. tGal was tested at doses of 0.1, 0.5, and 1 nmol/kg body mass. pGal was also injected within the systemic circulation, but at only one dose of 1 nmol/kg. To prevent the recording of the pressure artifact due to the injection, the computer was stopped for 10 s during intra-arterial injections.

Effects of Peripheral Administration of Cyclooxygenase Inhibitors and Nitric Oxide Synthase Inhibitor on P\textsubscript{DA} and HR Responses to Intra-Arterial Injections of Gal

Four groups of trout were used for this study. In the first group (control trout, n = 11), trout were injected peripherally with vehicle (50 μl). In the second group (indomethacin-pretreated trout, n = 9), trout were injected with indomethacin (5 mg/kg). In the third group (medofenamate-pretreated trout, n = 12), trout were injected with medofenamate (3 mg/kg). In the fourth group (L-NAME-pretreated trout, n = 17), trout were injected with L-NAME (1 mg/kg). Thirty minutes after these initial pretreatments, all groups of trout received an intra-arterial injection of tGal (1 nmol/kg). The
doses of indomethacin and meclofenamate used in the present study have been previously shown to abolish the vascular effect of prostaglandin precursors in teleosts (35) or to inhibit release of prostaglandins in dogs (33), respectively. The efficiency of l-NAME at a dose of 1 mg/kg to inhibit nitric oxide synthase (NOS) in rats was demonstrated by Walder et al. (32).

Recording of \( P_{DA} \), HR, and Q and Processing of Data

The heparinized (80 U/ml) aortic cannula was connected by means of a three-way T to a pressure transducer (P23 ID, Gould Electronique, Ballainvilliers, France). Calibration of the transducer was made each day against a static column of water. The leads from the Doppler flow probe were attached to a Doppler flowmeter (The University of Iowa, Iowa City, IA). The zero-flow level was set electronically, and the range-gate control of the Doppler unit was adjusted to record the highest signal on the output. Thereafter, the mean signal was continuously recorded as kilohertz of Doppler shift (\( \Delta kHz \)). The Doppler flowmeter measures velocity, but there is a direct relation between blood velocity and instantaneous flow. In situ calibration of Doppler flowmeter in fish has demonstrated that there is a good linear correlation between Doppler signal and mean volume flow (2). Systolic blood pressure (SP), diastolic blood pressure (DP), pulse pressure (pulse pressure = \( SP - DP \)), HR, and Q were processed with a digital oscilloscope (Gould 1604), and the data were transferred every 2 s to a 486 personal computer. \( P_{DA} \) = \( (SP + DP) / 2 \) (5), the pressure pulsatility (pulsatility = \( P_{DA} / R_s \)), HR, Q, and the systemic vascular resistance (\( R_s \) = \( PDA/Q \)) were also calculated offline by the computer for the preinjection (control period, 0–5 min) and the postinjection period (5–30 min). Central venous blood pressure was assumed to be zero for the calculation of \( R_s \) (13, 17). In a recent study, however, it was calculated that central venous pressure in trout is \( 2.8 \pm 0.3 \) mmHg (24). Results for cardiovascular parameters are expressed in Figs. 1–5 and text as either absolute values (HR in beats/min, \( P_{DA} \) in kPa), arbitrary units (Q in \( \Delta kHz \), \( R_s \) in kPa/\( \Delta kHz \)), or percent changes of the control values.

Statistical Analysis

All data are expressed as means ± SE for 9–17 experiments. Data were statistically evaluated using one-way ANOVA with or without repeated measures, followed by Dunnett’s test. In addition, and when appropriate, changes in the cardiovascular parameters were also compared using Student’s paired t-test. The criterion for statistical significance was set at \( P < 0.05 \).

RESULTS

Preinjection values for hematocrit and plasma osmolality were \( 24.2 \pm 1.3\% \) and \( 282 \pm 0.8 \) mosmol/kgH2O, respectively. The baseline \( P_{DA} \) was between 2.7 and 3.2 kPa, and HR was between 55 and 65 beats/min. In previous experiments, it was observed that intracerebroventricular or intra-arterial injection of vehicle (Ringer solution) did not induce significant change in cardiovascular parameters, and, therefore, for clarity of the time-course curves, the effects of vehicle were omitted in Figs. 1, 3, and 5.

Preliminary experiments demonstrated that the intracerebroventricular injection of tGal at doses of 0.1 and 0.5 nmol/kg produced no change in the hemodynamic parameters of the conscious trout. Figure 1 shows the changes in the time course curves of HR, \( P_{DA} \), Q, and \( R_s \) after intracerebroventricular injection of Gal (1 nmol/kg). tGal induced a rapid and significant increase in \( P_{DA} \) that reached a maximal value (+0.49 ± 0.15 kPa) by 8 min after the injection of Gal and remained significantly elevated throughout the recording period. During this hypotensive period, there was no change in HR or Q, but the calculated \( R_s \) increased significantly. The histogram in Fig. 2 summarizes the overall effects of intracerebroventricular injection of tGal on the cardiovascular parameters. tGal elicited a 12 ± 1.3% increase in \( P_{DA} \) and a 13 ± 2.2% increase in \( R_s \). These changes are significant (\( P < 0.001 \)) when compared with the values after intracerebroventricular injection of vehicle only.

The effect of intracerebroventricular injection of the highest dose of tGal (3 nmol/kg) on the changes in \( P_{DA} \) (11 ± 1.6%) was not significantly different from the effect of 1 nmol/kg, and HR did not change significantly (2 ± 0.5%).

Hemodynamic Effects of Intra-Arterial Injection of Gal

Figure 3 shows an example of the changes in the time course curves of HR, \( P_{DA} \), Q, and \( R_s \) after intra-arterial injection of tGal (1 nmol/kg). tGal potently evoked a rapid decrease in \( P_{DA} \). This decrease is highly significant within 5 min after the injection of the peptide and reached its nadir ~10 min after the injection (~0.46 ± 0.09 kPa). This level remained below baseline for at least 1 h (not shown). There was no variation in HR during this hypotensive period. After intra-arterial injection of tGal, Q did not change. The calculated \( R_s \) significantly decreased after intra-arterial injection of the peptide.

The histogram in Fig. 4 summarizes the overall effects of intra-arterial injection of tGal on the cardiovascular parameters. tGal elicited a 12 ± 1.4% decrease in \( P_{DA} \) and a 17.5 ± 1.1% fall in \( R_s \). All changes are significantly different from those observed after intra-arterial injection of vehicle only.

A series of experiments analogous to those presented in Fig. 3 was carried out using graded doses of tGal, and the results are presented in Table 1. Intra-arterial administration of tGal (0.1–1 nmol/kg) elicited a dose-dependent decrease in \( P_{DA} \) with a threshold dose for significant effects observed at a dose of 0.5 nmol/kg. In this dose range, HR did not change after intra-arterial administration of tGal. In addition, Table 1 depicts the effects of intra-arterial injection of pGal at a dose of 1 nmol/kg. pGal induced a decrease in \( P_{DA} \) similar to that induced by tGal, but pGal significantly increased HR compared with vehicle injection.
Effects on Gal-Evoked Hypotension of Pretreatment of Trout With Inhibitors of Cyclooxygenase and With an Inhibitor of NOS

Pretreatment of trout with indomethacin and meclofenamate did not significantly change the baseline level of HR (Table 2). Although these inhibitors of cyclooxygenase induced transient changes in $P_{DA}$, their overall effects on $P_{DA}$ remained nonsignificant (Table 2). Pretreatment of trout with L-NAME had no effect on the baseline values of HR and $P_{DA}$ (Table 2).

Figure 5 shows the changes in the time-course curves of HR and $P_{DA}$ after intra-arterial injection of tGal in control trout or in pretreated trout. Pretreatment of trout with either indomethacin or meclofenamate did not change the prolonged hypotensive effect of tGal observed after intra-arterial injection of the peptide in control trout. In trout pretreated with L-NAME, the magnitude and duration of this hypotensive phase was significantly reduced. Table 3 gives a summary of the observed effects of intra-arterial administration of tGal after the preceding pretreatments. The data indicate that pretreatment of trout with inhibitors of the...
cyclooxygenase products did not have any effect on the tGal-induced fall in $P_{DA}$ because the decrease in $P_{DA}$ was the same as that observed in control trout. Although the Student's paired t-test revealed that the overall change in $P_{DA}$ after intra-arterial administration of tGal in L-NAME-pretreated trout was still significant, the magnitude of this change was significantly reduced (3 times) compared with that in control trout.

DISCUSSION

The present experiments were conducted in nonanesthetized trout to obviate the effects of an anesthetic agent on cardiovascular functions. The hematocrit and the plasma osmolality levels were within the usual ranges for values determined in conscious and catheterized trout in this and other laboratories (19, 20, 28). It has previously been shown that surgical procedures, especially the invasive technique for placement of the Doppler probe, lead to chronic stress that significantly decreases $P_{DA}$ and increases HR (7). However, in the

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**Fig. 3.** Time-course curves for effects of intra-arterial (IA) injection of trout Gal (1 nmol/kg) on HR, $P_{DA}$, $Q$, and $R_s$ in conscious trout. Arrowhead indicates onset of intra-arterial injection. Each curve represents mean ± SE (at selected times) of $n = 8$ individual recordings. *$P < 0.05$, **$P < 0.01$ vs. 0- or 5-min time points.

**Fig. 4.** Histograms showing overall changes in HR, $P_{DA}$, $Q$, and $R_s$ during the 25-min period after intra-arterial injection of vehicle (50 µl) or trout Gal (1 nmol/kg) in conscious trout. Values are expressed as percentages of preinjection period (0-5 min) and are presented as means ± SE of 8–10 individual recordings. ***$P < 0.001$ vs. vehicle injection.
present study, HR and PDA for trout equipped with a Doppler probe were not significantly different from those measured in trout fitted only with a dorsal aortic cannula. Resting HR and PDA were in the same ranges previously observed for trout maintained at 11–13°C in our laboratory.

$tGal$ injected within the third ventricle of the brain of the conscious trout produced a rapid and significant increase in $PDA$ without changing HR, but only for the two highest doses tested (1 and 3 nmol/kg). The maximal effect on $PDA$ was observed for the dose of 1 nmol/kg, suggesting that, unlike the effect of centrally administered picomolar doses of arginine vasotocin (20) or angiotensin II (21), the action of $tGal$ observed for much higher doses was not dose dependent. However, the cardiovascular effects of centrally administered $tGal$ resembled those obtained for trout uterotonin II (19). The responses to the two neuropeptides were not dose dependent, and the two neuropeptides induced a sustained and comparable increase in $PDA$ without changing HR. In the present study we demonstrated that intracerebroventricular injection of $tGal$ produced elevation of $PDA$ from enhancement of $R_s$ (vasoconstriction).

In mammals, despite the fact that Gal immunoreactivity and Gal receptors are present in brain areas involved in cardiovascular control, including the hypothalamus, locus coeruleus, and dorsal medial medulla, only a few reports have examined the central effects of Gal on cardiovascular regulation. Intracisternal injections of 3 nmol (~12 nmol/kg) of Gal in $\alpha$-chloralose-anesthetized rats induced a weak hypotensive effect (9). This hypotensive response was associated with a slight tachycardia that was clearly developed after intracisternal injection of 10 nmol of Gal (~40 nmol/kg). Renal vasodilatation at a dose that had no significant effect on blood pressure or HR was also observed after intracisternal injection of Gal (1–5 nmol, i.e., ~3–16 nmol/kg) in rats under methohexital sodium anesthesia (8). In pentobarbital sodium-anesthetized rats, microinjection of Gal within the nucleus tractus solitarii (100 pmol, i.e., ~400 pmol/kg) inhibited the baroreceptor reflex response to transient hypertension induced by phenylephrine (3).

The neuroanatomic pathways underlying the central cardiovascular action of Gal cannot be ascertained from the present study. We used intracerebroventricular injection to mimic possible endogenous release of Gal. Because the peptide was injected into the third ventricle, Gal may act predominantly on periventricular structures, possibly at the hypothalamic level. Within the preoptic nuclei, Gal may activate neuroendocrine cells to increase the release of vasoconstrictive neuropeptides such as arginine vasotocin. Immunohistochemical studies have revealed that, in the brain of teleosts, Gal cell bodies are mainly localized within the anterior preoptic region and the mediobasal hypothalamus (1, 12). Another explanation may be that Gal could excite preoptic neurons that are known to project toward cardiovascular autonomic nuclei of the medulla oblongata and also toward the spinal cord (31), where they could activate sympathetic preganglionic fibers. However, the density of Gal-IR fibers originating from the preoptic nuclei and projecting within the posterior brain is rather low in the Atlantic salmon and in the rainbow trout (1, 12), suggesting that the Gal preoptic neurons themselves might not be involved in this pathway. Alternatively, Gal may have diffused to the fourth ventricle and thus could act directly on brain.

Table 1. Overall changes in heart rate and mean dorsal aortic blood pressure of conscious trout in response to intra-arterial injection of vehicle or graded doses of $tGal$ or $pGal$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Preinjection</th>
<th>Postinjection</th>
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<tr>
<td></td>
<td></td>
<td>HR, beats/min</td>
<td>$PDA$, kPa</td>
</tr>
<tr>
<td>Vehicle (control)</td>
<td>11</td>
<td>61.53 ± 2.47</td>
<td>2.98 ± 0.16</td>
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<tr>
<td>$tGal$ (0.1 nmol/kg)</td>
<td>9</td>
<td>65.87 ± 3.2</td>
<td>2.87 ± 0.14</td>
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<tr>
<td>$tGal$ (0.5 nmol/kg)</td>
<td>9</td>
<td>62.52 ± 1.9</td>
<td>2.84 ± 0.12</td>
</tr>
<tr>
<td>$tGal$ (1 nmol/kg)</td>
<td>10</td>
<td>65.49 ± 2.37</td>
<td>2.75 ± 0.09</td>
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<tr>
<td>$pGal$ (1 nmol/kg)</td>
<td>7</td>
<td>61.24 ± 2.8</td>
<td>3.12 ± 0.21</td>
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</table>

Values are means ± SE; n = no. of tests. $HR$, heart rate; $PDA$, mean dorsal aortic blood pressure; $tGal$, trout galanin; $pGal$, porcine galanin. Preinjection period, 0–5 min; postinjection period, 5–30 min. $aP < 0.01$, $bP < 0.001$ vs. preinjection values (paired t-test). $cP < 0.05$, $dP < 0.01$, $eP < 0.001$ vs. control trout (Dunnett’s test or Student’s t-test).

Table 2. Overall changes in baseline level of heart rate and mean dorsal aortic blood pressure of conscious trout in response to intra-arterial injection of inhibitors of either cyclooxygenase products or nitric oxide synthase.

<table>
<thead>
<tr>
<th>Pretreatment</th>
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<th>Preinjection</th>
<th>Postinjection</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>HR, beats/min</td>
<td>$PDA$, kPa</td>
</tr>
<tr>
<td>Vehicle (control)</td>
<td>11</td>
<td>58.32 ± 2.9</td>
<td>3.12 ± 0.16</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>9</td>
<td>63.55 ± 2.02</td>
<td>2.81 ± 0.10</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>12</td>
<td>61.02 ± 2.0</td>
<td>3.01 ± 0.08</td>
</tr>
<tr>
<td>L-NAME</td>
<td>17</td>
<td>63.46 ± 3.3</td>
<td>2.81 ± 0.09</td>
</tr>
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</table>

Values are means ± SE; n = no. of tests. Indomethacin (5 mg/kg) and meclofenamate (3 mg/kg) inhibit cyclooxygenase products, whereas N⁶-nitro-L-arginine methyl ester (L-NAME; 1 mg/kg) inhibits nitric oxide synthase. No changes observed after intra-arterial injection were significant.
stem nuclei or on fibers projecting caudally and involved in systemic blood pressure regulation. It is known that the brain stem area and spinal cord of salmon contain a high level of Gal binding sites (11). Whatever the mechanisms of action of Gal may be, our functional study has revealed for the first time that Gal may be a component of the “cocktail” of neuropeptides that may be involved in the central cardiovascular regulation in fish.

In contrast to the central hypertensive effect of tGal observed after intracerebroventricular administration, intra-arterial injection of the peptide induced a highly significant and dose-dependent hypotensive effect without change in HR. This decrease in $P_{DA}$ is mediated through a fall in $R_s$. These results are the opposite of those we previously obtained for arginine vasotocin and for angiotensin II, two well-known vasopressor peptides in trout (19). They are also quite different from results observed for the effect of Gal in other fish. Gal induced vasoconstriction with an increase in blood pressure in the Atlantic cod Gadus morhua (16) and in three elasmobranch fish, Heterodontus portusjacksoni, Hemiscyllium ocellatum, and Rhinobatos typus (26). In the Australian lungfish Neoceratodus forsteri, intra-arterial injection of Gal reduced HR and increased vascular resistance in the lung but reduced vascular resistance in the coeliacomesenteric artery. The $P_{DA}$ did not change (10). Our results in trout are consistent with previous data obtained in the anesthetized cat (27), demonstrating that Gal induces a decrease in blood pressure. In dogs, however, Gal was devoid of effect on blood pressure (23). The demonstration that intra-arterial injection of pGal in trout induced the same, or even larger, fall in $P_{DA}$ as tGal suggests that the vasodepressor effect of tGal in trout is not a consequence of structural differences in the peptide compared with the mammalian analogs. The vasodepressor effects of tGal in trout resembled those previously published for some tachykinins such as scyliorhinin I or trout substance P in the spiny dogfish, because intra-arterial injection of these two peptides at the dose of 0.3 nmol/kg induced a long-lasting fall in $P_{DA}$ without change in HR (13).

Several mechanisms might be involved in the vasodepressor action of peripherally administered Gal in trout. First, Gal might have direct vasodilatory action on arterial vessels of the trout. However, there have been no studies to date showing the effect of Gal on isolated vessels of trout. Previous studies on isolated vessels of fish demonstrated that Gal contracts arterial strips from the intestine of the Atlantic cod Gadus morhua (16) and segments of mesenteric arteries of elasmobranchs (26). Second, Gal might produce vasodilatory effects by an indirect mechanism mediated partly by prostaglandins. However, our results provide in vivo evidence that vasodilator cyclooxygenase products are not involved in the vasodepressor effects of tGal. This conclusion is based on the similar decrease in $P_{DA}$ observed after intra-arterial injection of tGal in control trout or in trout pretreated with two structurally dissimilar cyclooxygenase inhibitors, indomethacin and

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Fig. 5. Time-course curves for cardiovascular effects of intra-arterial injection of trout Gal (1 nmol/kg) 30 min after pretreatment of trout with vehicle (A), indomethacin (Indo, 5 mg/kg; B), meclofenamate (Meco, 3 mg/kg; C), or N$^\omega$-nitro-L-arginine methyl ester (L-NAME, 1 mg/kg; D). Arrowheads indicate onset of intra-arterial injection. Each curve represents mean ± SE (at selected times) of n = 9–17 individual recordings. *P < 0.05, **P < 0.01 vs. 0- or 5-min time points.
medofenamate. Third, the vasodilatory effect of Gal might be mediated by an endothelium-derived relaxing factor such as nitric oxide (NO). In the rainbow trout, NO is present within the enteric nervous system (22), and NO is also known to be a vasodilator of preconstricted small arteries from the gut (14). However, in a study examining isolated peripheral arteries and veins of the rainbow trout Oncorhynchus mykiss, Olson and Villa (25) were unable to find NO-dependent mechanisms in trout vasoregulation, and vasoactive intestinal peptide-induced relaxation of small arteries in this teleost does not involve NO (14). The present study, however, suggests the possible involvement of an NO system mediating the peripheral vasodilatory effect of Gal, because, in trout pretreated with L-NAME, an inhibitor of NOS, the hypotensive action of Gal was reduced three times compared with that in control trout.

In conclusion, our results have shown that tGal may have neuroregulatory functions within the brain of the trout because intracerebroventricular injection of the peptide induces significant central hypertensive effects without changing HR. Peripheral vasoconstriction accounts for this effect. Within the systemic circulation, tGal induces opposite effects, because intra-arterial injection of the peptide causes a significant and long-term hypotensive action. Vasodilator mechanisms that are responsible for this response do not appear to be mediated by prostanoids, but an NO-dependent vasodilatory system might be involved. Finally, in concert with other well-known vasoactive peptides, endogenous Gal may have a role in central and peripheral cardiovascular regulation in trout.

Perspectives

The present study was directed toward the understanding of the cardiovascular effects of centrally and peripherally administered native tGal in trout. Because of the opposite effects of tGal on blood pressure in trout according to the site at which the peptide acts, it is important to evaluate which of the observed actions of Gal may have physiological significance. In comparison with other centrally acting peptides such as arginine vasotocin (20) and angiotensin II (21), relatively large doses of Gal were used to evoke significant hypertension after intracerebroventricular injection. Consequently, there is the possibility that the effects are mediated, at least in part, by nonspecific activation of, for example, sympathetic neurons. It is conceivable, therefore, that the primary function of Gal in the brain of the trout is other than cardiovascular action. However, it should be remembered that Gal is a neuropeptide, and its endogenous concentration at the synaptic level is probably quite large. Central galaninergic pathways may be recruited in response to a perturbation requiring cardiovascular adjustment so that the hypertensive effects of central Gal might override hypotensive actions in the periphery. Whether the cardiovascular effects of centrally administered Gal are accompanied by effects of the peptide on ingestive behavior remains to be determined. At the periphery, the physiological significance of the vasodepressor effects of tGal observed in the present study is also difficult to assess because the circulating concentration of Gal in trout is unknown. With the assumption that the plasma volume of a 300-g trout is ~20 ml/kg (6), a bolus injection of the threshold dose of 0.5 nmol/kg of tGal would produce a circulating concentration of the peptide of ~25 pmol/ml. It is unclear whether this systemic threshold level would be reached in a physiological situation. However, the high concentration of tGal produced in the immediate vicinity of Gal-containing neurons may be functionally important in the local regulation of regional hemodynamic parameters. A similar paracrine role for trout bradykinin in the trout circulation has been proposed (24), and, although no role for circulating substance P in cardiovascular regulation in fish has ever been suggested, trout substance P released from neurons is believed to exercise a locally acting effect on gastrointestinal blood flow in the trout (15). Studies in mammals have demonstrated that Gal is involved in the control of gastrointestinal function. However, despite high concentration of Gal in the trout gastrointestinal tract (1), tGal had no effect on the tension of isolated longitudinal and circular muscle strips from trout stomach and intestine (J. M. Conlon and J. J. Jensen, unpublished data), so the peptide is unlikely to be a regulator of gastrointestinal motility. The full complexity of the physiological role of Gal in fish remains to be elucidated.

We are grateful to R. Creach for animal care and M. Simon for typing the manuscript.

Peptide synthesis was supported by a grant from the Nebraska Cancer and Smoking-Related Diseases Program.

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Table 3. Overall changes in heart rate and mean dorsal aortic blood pressure of conscious trout in response to intra-arterial injection of tGal in control trout or in trout pretreated with inhibitors of either cyclooxygenase products or nitric oxide synthase

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>HR, beats/min</th>
<th>P&lt;sub&gt;DA&lt;/sub&gt;, kPa</th>
<th>ΔHR, beats/min</th>
<th>ΔP&lt;sub&gt;DA&lt;/sub&gt;, kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (control)</td>
<td>66.27 ± 2.3</td>
<td>2.96 ± 0.09</td>
<td>−0.50 ± 0.48</td>
<td>−0.28 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>66.03 ± 3.33</td>
<td>3.17 ± 0.19</td>
<td>+2.76 ± 1.02</td>
<td>−0.35 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medofenamate</td>
<td>61.08 ± 2.2</td>
<td>3.00 ± 0.09</td>
<td>+1.26 ± 1.42</td>
<td>−0.23 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-NAME</td>
<td>59.97 ± 3.24</td>
<td>2.73 ± 0.09</td>
<td>+1.39 ± 0.74</td>
<td>−0.10 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of tests. Changes are in response to 1 nmol/kg tGal. <sup>a</sup>P < 0.01 vs. control trout (Dunnett’s test). <sup>b</sup>P < 0.01 vs. preinjection values (paired t-test). <sup>c</sup>P < 0.05 vs. control trout (Dunnett’s test).
15. Received 4 February 1998; accepted in final form 2 July 1998.

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