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Ventilatory and cardiovascular actions of centrally administered trout tachykinins in the unanesthetized trout

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Summary

The brains of teleost fish contain members of the tachykinin family that are the products of orthologous genes expressed in mammalian nervous tissues, but little is known regarding the physiological effects of these peptides in their species of origin. The present study compares the central actions of trout neuropeptide gamma (NPγ), substance P (SP) and neurokinin A (NKA) (5–250 pmol) on ventilatory and cardiovascular parameters in the unanesthetized rainbow trout *Oncorhynchus mykiss*. Intracerebroventricular (ICV) injection of NPγ evoked a dose-dependent elevation of the ventilation rate (f_V) but a reduction of the ventilation amplitude (V_{AMP}) that was caused by a reduction of the magnitude of the adduction phase of the ventilatory signal. The net effect of NPγ was to produce an hypoventilatory response since the total ventilation (V_{TOT}) was significantly reduced. The minimum effective dose for a significant effect of NPγ on f_V and V_{AMP} was 50 pmol. SP evoked a significant elevation of f_V, a concomitant depression of V_{AMP}, and a resultant decrease in V_{TOT} but only at the highest dose (250 pmol). NKA was without action on f_V but significantly decreased V_{AMP} at only the highest dose tested. In this case also, the net effect of NKA was to reduce V_{TOT}. When injected centrally, none of the three peptides, at any dose tested, produced changes in heart rate or mean dorsal aortic blood pressure (P_{DA}). Intra-arterial injection of the three tachykinins (250 pmol) produced a significant ($P<0.05$) increase in P_{DA}, but only SP and NKA induced concomitant bradycardia. None of the three peptides produced any change in f_V or V_{AMP}. In conclusion, our results demonstrate that centrally injected tachykinins, particularly NPγ, produce a strong hypoventilatory response in a teleost fish and so suggest that endogenous tachykinins may be differentially implicated in neuroregulatory control of ventilation.

Key words: neuropeptide γ, substance P, neurokinin A, ventilatory control, intracerebroventricular injection, teleost.

Introduction

The tachykinins are a family of biologically active peptides that are characterized structurally by the common carboxy-terminal pentapeptide sequence Phe-Xaa-Gly-Leu-Met-NH_2. In mammals, substance P (SP), neurokinin A (NKA), neuropeptide gamma (NPγ) and neuropeptide K (NPK) are encoded by the single copy *preprotachykinin A* gene. Neurokinin B is derived from the *preprotachykinin B* gene while the *preprotachykinin C* gene encodes three peptides (hemokinin 1, endokinin C and endokinin D), with limited structural similarity with SP (see Conlon, 2004). The tachykinins exert their actions by binding to G-protein coupled receptors that are widely distributed within vascular, endocrine and nervous tissues. SP is the preferential agonist of the NK-1 receptor; NKA along with NPγ and NPK are regarded as endogenous ligands of the NK-2 receptor; and NKB is the preferred agonists of the NK-3 receptor (Patacchini and Maggi, 2004). In mammals, there is strong evidence for the importance of central nervous system (CNS) tachykinins in the control of respiration (Kumar and Prabhakar, 2003). Micro-injection or bath application of SP into the pre-Bötzinger complex, the primary source of rhythmogenesis situated in the reticular formation (Smith et al., 1991), increased respiratory frequency through an action on the NK-1 receptor (Monteau et al., 1996; Gray et al., 1999; Gray et al., 2001). Furthermore, NKA, NKB and agonists selective for NK-2 and NK-3 receptors reduced respiratory frequency but increased tidal volume after injection within the nucleus tractus solitarius (NTS) (Mazzone and Geraghty, 2000). In addition, central tachykinins are involved in cardiovascular regulation, neuroendocrine secretion, pain transmission, and in certain behavioral responses (see Satake and Kawada, 2006). In the periphery, the presence of tachykinins and their receptors in lung indicate an important physiological role in local regulation of the pulmonary system (see Meini and Lecci, 2006), and tachykinins acting as neurotransmitters and/or neuromodulators are implicated in regulation of cardiovascular and gastrointestinal functions and

inflammatory and immune processes (see Holzer, 2006; Page, 2006; Walsh and McWilliams, 2006).

Orthologs of the mammalian tachykinins have been isolated and structurally characterized in a wide range of tetrapod and non-tetrapod species (see Conlon, 2004). In particular, SP (Jensen and Conlon, 1992), NKA (Jensen and Conlon, 1992) and NPγ (Jensen et al., 1993) have been purified from tissues of the rainbow trout *Oncorhynchus mykiss*. In the unanesthetized trout, trout SP and trout NKA given intra-arterially were equally effective in increasing both systemic and coeliac resistance, leading to hypertension, bradycardia and a decrease in cardiac output (Kagstrom et al., 1996). These peptides were also approximately equipotent in increasing the trout dorsal aortic vascular resistance in an *in vitro* perfusion system (Kagstrom et al., 1996). In contrast, trout SP was more potent than NKA in stimulating the motility of the isolated trout intestinal smooth muscle and the vascularly perfused trout stomach (Jensen et al., 1993). Neuroanatomical studies have revealed the presence of tachykinin-like immunoreactivity in neuronal cell bodies and fibers throughout the brain of several teleost fish, including the trout (Vecino et al., 1989; Batten et al., 1990; Holmqvist and Ekstrom, 1991; Moons et al., 1992), together with high density of tachykinin binding sites (Moons et al., 1992) but central actions of tachykinins in fish are unknown. Rhythmic ventilatory movements in fish are generated by a diffuse central pattern generator (CPG), probably located within the reticular formation (see Taylor et al., 1999), whose activity is modulated by inputs originating from higher brain centers (Shelton, 1959). Because of the crucial role of central tachykinins in the control of respiratory rhythmogenesis in mammals and the presence of a central tachykinergic system in teleost fish, we propose the hypothesis that tachykinins in fish might be involved in the central control of ventilation. Consequently, the present study was carried out to investigate the effects of intracerebroventricular (ICV) administration of synthetic replicates of trout NPγ, SP and NKA on ventilation rate (f_V), ventilation amplitude (V_{AMP}), dorsal aortic blood pressure (P_{DA}) and heart rate (f_H) in the unanesthetized rainbow trout. The central actions of the peptides on these parameters were compared with their effects after intra-arterial (IA) administration.

Materials and methods

Peptides and chemicals

Trout SP (KPRPHQFFGLM.NH_2), NKA (HKINSFVG-LM.NH_2) and NPγ (SSANPQITHKRHKINSFVGLM.NH_2) were supplied in crude form by GL Biochem Ltd (Shanghai, China) and purified to near homogeneity by reversed-phase high-pressure liquid chromatography (HPLC) on a (2.2×25 cm) Vydac 218TP1022 (C-18) column (Separations Group, Hesperia, CA, USA). The purity of all peptides tested was >98% and their identities were confirmed by electrospray mass spectrometry. Peptides were firstly dissolved in 0.1% v/v acetic acid and aliquots were stored at –25°C. For injections, the peptides were freshly diluted to the desired concentration with Ringer's solution (composition in mmol l^{-1}: NaCl 124, KCl 3, $CaCl_2$ 0.75, $MgSO_4$ 1.30, KH_2PO_4 1.24, $NaHCO_3$ 12, glucose 10 (pH 7.8) immediately prior to use. Vehicle was made from Ringer solution containing an appropriate concentration of acetic acid. All solutions were sterilized by filtration through 0.22 μm filters (Millipore, Molsheim, France) before injection.

Animals

Adult rainbow trout (body mass 276±2 g; mean ± s.e.m., *N*=70) of both sexes were purchased locally and transferred in a well-oxygenated and thermostatically controlled water tank to the laboratory. All the fish were kept in a 1000-liter tank containing circulating dechlorinated, aerated tapwater (11–12°C), under a standard photoperiod (lights on 09:00 h–20:00 h). The fish were allowed at least 3 weeks to acclimate under these conditions before the experiments were started. Experimental protocols were approved by the Regional Ethics Committee in Animal Experiments, Brittany, France.

Experimental procedures

All surgical procedures were made under tricaine methane sulfonate (3-amino-benzoic acid ethyl ester; 60 mg l^{-1} in tapwater buffered with $NaHCO_3$ to pH 7.3–7.5) anesthesia. The techniques used for placement of the electrocardiographic (ECG) electrodes, placement of the buccal catheter, cannulation of the dorsal aorta and insertion of the ICV microguide have previously been described in detail (Le Mével et al., 1993; Lancien et al., 2004). Briefly, two ECG AgCl electrodes (Comepa, 93541 Bagnolet, France) were subcutaneously implanted ventrally and longitudinally at the level of the pectoral fins. The incision was sutured across the electrodes and the leads were sutured to the skin. The dorsal aorta was cannulated with a PE-50 catheter (Clay Adams, Le Pont De Claix, France). A flared cannula (PE-160) was inserted into a hole drilled between the nares such that its flared end was resting against the roof of the mouth. This cannula was used to record any changes in buccal ventilatory pressure (Holeton and Randall, 1967). The absence of a neocortex in fish allowed the accurate placement of the ICV microguide under stereomicroscopic guidance. A 25-gauge needle fitted with a PE-10 polyethylene catheter was inserted between the two habenular ganglia and descended into the third ventricle until its tip lay between the two preoptic nuclei. An obturator was placed at the end of the PE-10 tubing and the cranial surface was covered with hemostatic tissue followed by light quick-curing resin. After surgery, the animals were force-ventilated with dechlorinated tapwater and, following recovery of opercular movements, were transferred to a 6-liter blackened chamber supplied with dechlorinated and aerated tapwater (10–11°C) that was both re-circulating and through-flowing. Oxygen pressure within the water tank (Pw_{O_2}) and pH were continuously recorded and maintained at constant levels (Pw_{O_2}: 20 kPa; pH 7.4–7.6). A small horizontal aperture was made along the upper edge of the chamber in order to connect the ECG leads to an amplifier and to connect the dorsal aorta and the buccal cannula to pressure transducers. This aperture permitted ICV injections of peptides without disturbing the trout.

The trout were allowed to recover from surgery and to become accustomed to their new environment for 48–72 h. Each day, the general condition of the animals was assessed by observing their behavior, checking the ventilatory and the

cardiovascular variables, and measuring their hematocrit. Animals that did not appear healthy, according to the range of values detailed in our previous studies, were discarded. After f_V, V_{AMP}, P_{DA} and f_H were maintained stable for at least 90 min, parameters were recorded for 30 min without any manipulation or ICV injection in control experiments.

Intracerebroventricular administration of tachykinins

The injector was introduced within the ICV guide prior to the beginning of a recording session, which lasted 30 min. All injections were made at the fifth min of the test but the injector was left in place for a further 5 min to allow for complete diffusion of the agent and to minimize the spread of substances upwards in the cannula tract. The fish received an ICV injection of vehicle (0.5 μl) and 30 min later, an ICV injection of trout NPγ (5, 25, 50 and 100 pmol in 0.5 μl), SP or NKA (50, 100 and 250 pmol in 0.5 μl). The animals received a single ICV injection of one dose of peptide per day. No single fish was studied for more than 2 days and control experiments revealed that there was no significant change in performance over this period. Pilot experiments showed that the initial ICV injection of vehicle had no effect on the subsequent ICV injection of peptide. Furthermore, the possibility of time-dependent changes in the measured variables was evaluated by performing two sequential ICV injections of vehicle 30 min apart. There were no significant changes in the recorded variables following the second injection of vehicle compared to the changes observed after the first one.

Intraarterial administration of tachykinins

5 min after the beginning of the recording session, 50 μl of vehicle, or trout tachykinins at an appropriate concentration, were injected through the dorsal aorta and immediately flushed by 150 μl of vehicle. NPγ, SP and NKA were tested at doses of 50, 100 and 250 pmol.

Data acquisition and analysis of the ventilatory and the cardiovascular variables

The ECG electrodes were connected to a differential amplifier (band pass: 5–50 Hz; Bioelectric amplifier, Gould & Nicolet, 91942 Courtaboeuf, France) and a stainless steel bar was immersed in the water of the tank to act as a reference electrode. The aortic cannula and the buccal catheter were connected to P23XL pressure transducers (band-pass: 0–15 Hz; Gould & Nicolet). These pressure transducers were calibrated each day using a static water column. At the beginning of the experiments, the zero-buccal pressure level was set electronically. The output signals from the devices were digitalized at 500 Hz (PCI-1200 board, National Instruments, Austin, TX, USA) during the 30 min recording period and the data were stored on a disc. The time-series related to the ventilatory, the pulsatile P_{DA} and the ECG signals were processed off-line with custom-made programs written in LabView 6.1 (Laboratory Virtual Instrument Engineering Workbench, National Instruments). The ventilatory parameters were calculated as previously described (Lancien et al., 2004). Segments free of any movement artifacts on the ventilatory signal were selected and f_V (breaths min^{-1}) and the V_{AMP} (arbitrary units, a.u.) were determined. The f_V was calculated from the first harmonic of the power spectrum of the ventilatory signal using the fast Fourier transformation. V_{AMP} was calculated from the difference between the maximal abduction phase and the maximal adduction phase for each of the ventilatory movements. The net effect of the changes in f_V and V_{AMP} on ventilation was determined according to the formula $V_{TOT}=f_V \times V_{AMP}$, where V_{TOT} is total ventilation. The overall ventilatory response is determined by the combined output of the f_V and ventilatory stroke volume. By using only buccal pressure, we used an indirect technique to estimate ventilatory water flow since V_{AMP} is not necessarily proportional to the ventilatory stroke volume. However, this technique has the advantage of necessitating only minimal surgery and is widely used in cardiorespiratory studies on fish (Fritsche and Nilsson, 1993). The mean P_{DA} (kPa) was calculated from the pulsatile P_{DA} as the arithmetic mean of the systolic blood pressure and the diastolic blood pressure, and the mean f_H (beats min^{-1}) was determined from the ECG signal. All calculations for f_V, V_{AMP}, V_{TOT}, P_{DA} and f_H were made for the pre-injection period (0–5 min) and for five post-injection periods of 5 min for each trout and the results were averaged for trout subjected to the same protocol.

Statistical analysis

Data are expressed as means ± s.e.m. or + s.e.m. for each 5 min period. In the figures and text, data refer to absolute values (f_V in breaths min^{-1}; V_{AMP} in a.u.; V_{TOT} in a.u.; P_{DA} in kPa; f_H in beats min^{-1}) or maximal changes from baseline (pre-injection) values. The data were analyzed using two-way ANOVA followed by the Bonferroni *post hoc* test for comparisons between groups. Within each group, when the overall preceding ANOVA analyses demonstrated statistically significant differences, Dunnett's test was used for comparisons of post-injection values with pre-injection values. The criterion for statistical difference between groups was $P<0.05$. The statistical tests were performed using GraphPad Prism 3.0 (GraphPad, San Diego, CA, USA).

Results

Ventilatory and cardiovascular responses to central and peripheral NPγ

Fig. 1 illustrates recordings for 30 s in a single trout of the ventilatory, blood pressure and ECG signals taken during the 15–20 min post-injection period after ICV injection of vehicle (Fig. 1A) or 50 pmol NPγ (Fig. 1B). Comparing the vehicle-treated and NPγ-injected trout, NPγ caused an impressive reduction in V_{AMP} by decreasing in the magnitude of the adduction phase of the lower jaw, i.e. by reducing the mouth closing phase of the ventilatory cycle. Concurrently, NPγ caused a potent elevation of f_V. However, NPγ was without effect on either P_{DA} or f_H.

The time course of effects observed in the ventilatory and cardiovascular variables following ICV injections of vehicle or a range of doses (25–100 pmol) of NPγ are summarized in Fig. 2. Since 5 pmol NPγ was without action on any parameters, these data are omitted from Fig. 2. Table 1 shows the maximal changes in the ventilatory variables. There was no statistically significant difference between the baseline values of the

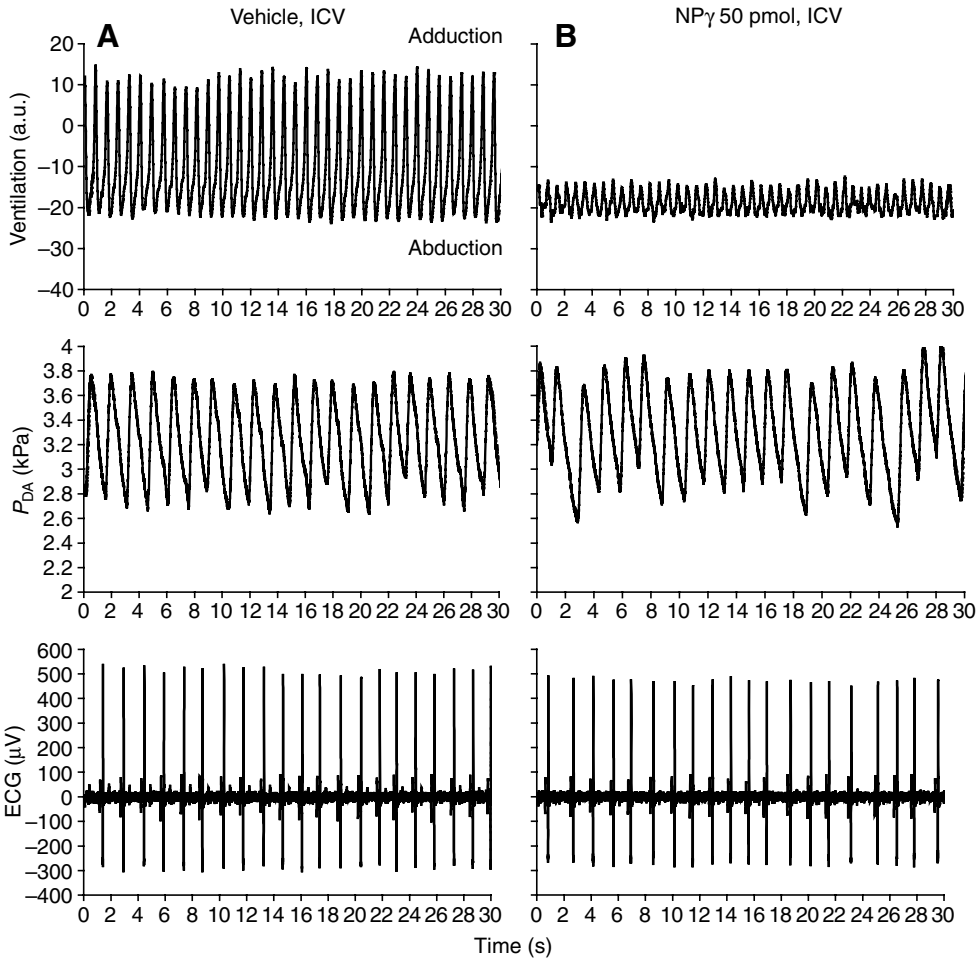

Fig. 1. Recording traces in a single unanesthetized trout, illustrating the changes observed in ventilatory movements (ventilation), dorsal aortic blood pressure (P_{DA}) and electrocardiographic (ECG) signals 15 min after intracerebroventricular (ICV) injection of (A) 0.5 μl vehicle and (B) 50 pmol NPγ. Note that, compared with ICV injection of vehicle, the injection of NPγ produces an increase in ventilation rate but a potent reduction in ventilation amplitude.

ventilatory and the cardiovascular variables recorded during the control period and during the pre-injection period prior to ICV injection of vehicle (data not shown). The ICV injection of vehicle produced no significant change in the ventilatory and in the cardiovascular parameters compared to pre-injection values (Fig. 2, Table 1). Compared with ICV injection of vehicle, NPγ evoked a gradual dose-dependent elevation of f_V (Fig. 2A, Table 1) but a progressive dose-dependent reduction of V_{AMP} (Fig. 2B and Table 1). The net effect of the peptide was a hypoventilatory response involving a significant dose-

Table 1. *Maximal changes in baseline level of ventilation rate, ventilation amplitude and total ventilation in the unanesthetized trout in response to intracerebroventricular injection of NPγ, SP and NKA*

			Pre-injection (0–5 min)			Post-injection (5–30 min)		
Peptides	Doses (pmol)	*N*	f_V (breaths min^{-1})	V_{AMP} (a.u.)	V_{TOT} (a.u.)	Δf_V (breaths min^{-1})	ΔV_{AMP} (a.u.)	ΔV_{TOT} (a.u.)
NPγ	0 (vehicle)	16	71.2 ± 2.5	29.4±2.5	2210±189	–1.4±1.1	–1.1±0.6	–140.0±66
	25	8	71.6±2.6	31.6±5.5	2203±372	15.7±4.2*	–8.4±4.1	–343±227
	50	9	74.2±3.2	26.9±2.6	2013 + 240	18.4±4.1*	–14.6±2.6*	– 945±236*
	100	8	70.2±5.1	32.6±4.4	2340±424	26.9±2.3*	–23.9±3.6*	–14851±369*
SP	0 (vehicle)	16	66.2±1.8	27.9±2.9	2179±339	–1.6±0.7	–3.2±1.4	–178±91
	250	10	69.6±3.1	28.4±3.7	1992±273	18.2±4.2*	–16.7±3.5*	–1071±246*
NKA	0 (vehicle)	16	68.2±2.6	33.9±4.0	2353±312	1.5±0.6	1.8±1.3	153±98
	250	9	67.8±3.0	32.0±3.0	2196±244	10.6±2.6	–14.7±3.0*	–898±218*

f_V, ventilation rate; V_{AMP}, ventilation amplitude; V_{TOT}, total ventilation; NPγ, neuropeptide γ; SP, substance P; NKA, neurokinin A; a.u., arbitrary units.

All data are presented as means ± s.e.m.; *N*=number of trout. Values and statistics are from Figs 2, 4 and 5. Changes in ventilatory variables following the action of 5 pmol NPγ and 50 and 100 pmol SP and NKA were omitted since they were not significant (see text and Figs 2, 4 and 5). *P<0.05 *vs* pre-injection values.

dependent decrease in V_{TOT} (Fig. 2C, Table 1). NPγ at a dose of 25 pmol evoked a progressive elevation of f_V, reaching significance 25 min after injection, without significant change in V_{AMP} and V_{TOT}. The threshold dose for an effect of NPγ on both f_V, V_{AMP} and V_{TOT} was 50 pmol and this was observed 15 min after the injection of the peptide (Fig. 1A–C, Table 1). At the maximum dose of NPγ tested (100 pmol), a significant increase in f_V but reduction in V_{AMP} and V_{TOT} occurred 10 min after ICV injection. In two trout out of ten, the ICV injection of 100 pmol NPγ was followed by a dramatic reduction in V_{AMP} to near the noise level of the recording system for periods of 10 to 20 s, giving the appearance of an apneic response. During this phase, the inferior jaw of the trout remained largely abducted. No f_V could be accurately determined and results from these two trout were not included within the data set. All actions of NPγ on the ventilatory variables were of long duration since, after reaching their peak value, parameters did not return to baseline values by the end of the recording period. During the period in which NPγ produced marked changes in f_V and V_{AMP}, there was no significant change either in mean P_{DA} (Fig. 2D) or in f_H (Fig. 2E).

As shown in Fig. 3, IA injections of NPγ at doses of

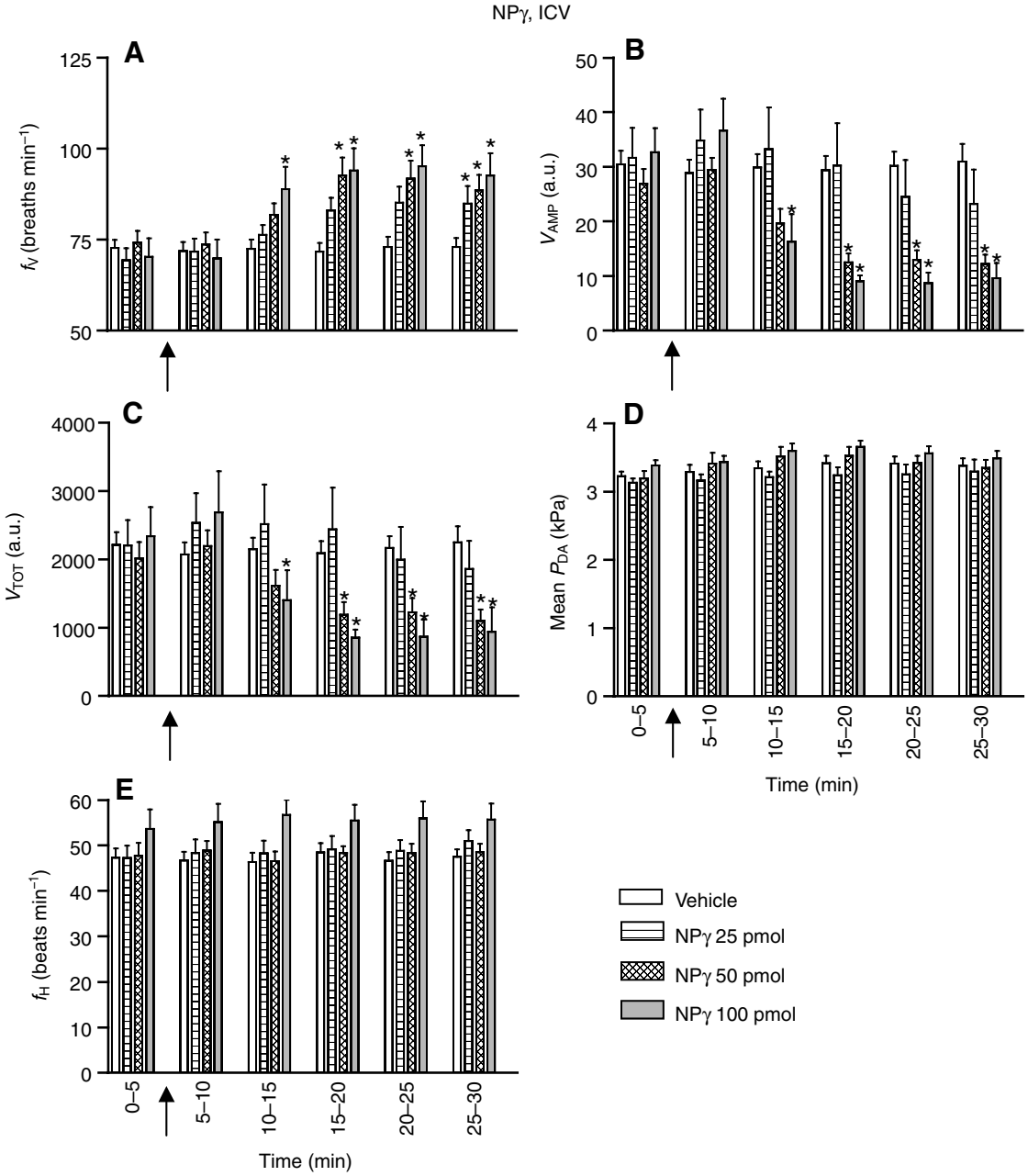

Fig. 2. Time course of the effects of intracerebroventricular injection of (1) 0.5 μl vehicle (open bars, *N*=16), (2) 25 pmol NPγ (horizontal-hatched bars, *N*=8), (3) 50 pmol NPγ (cross-hatched bars, *N*=9) and (4) 100 pmol NPγ (gray bars, *N*=8) on (A) f_V, (B) V_{AMP}, (C) V_{TOT}, (D) mean P_{DA}, and (E) f_H in unanesthetized trout. The arrow indicates when the injection was given. **P*<0.05 *vs* vehicle at corresponding post-injection period and *vs* pre-injection value.

50–250 pmol produced no change in either f_V, V_{AMP} or V_{TOT} (Fig. 3A–C). However, NPγ (250 pmol) caused a significant increase in mean P_{DA} (Fig. 3D). During this hypertensive response there was no change in f_H (Fig. 3E).

Ventilatory and cardiovascular responses to central and peripheral SP

The results obtained following ICV injections of graded doses (50–250 pmol) of SP are shown in Fig. 4. In contrast to the action of NPγ (Fig. 2), the effects of SP were not dose dependent and only the highest dose of SP (250 pmol) produced a significant elevation of f_V (Fig. 4A, Table 1), a significant reduction of V_{AMP} (Fig. 4B, Table 1) and a resultant significant decrease of V_{TOT} (Fig. 4C and Table 1). The changes in these parameters reached significance 10–15 min after ICV injection. No significant change occurred in either P_{DA} or f_H following the ICV injection of SP (Fig. 4D,E).

IA injection of SP caused no change in the ventilatory variables (not shown) and only the injection of 250 pmol SP produced a significant increase in mean P_{DA} together with a fall in f_H (P_{DA}: +0.63±0.14 kPa; f_H:–5.38±1.15 beats min^{-1}). These changes were transient, reaching their maximal value 5 min after injection and returning to basal level 15 min after injection (not shown).

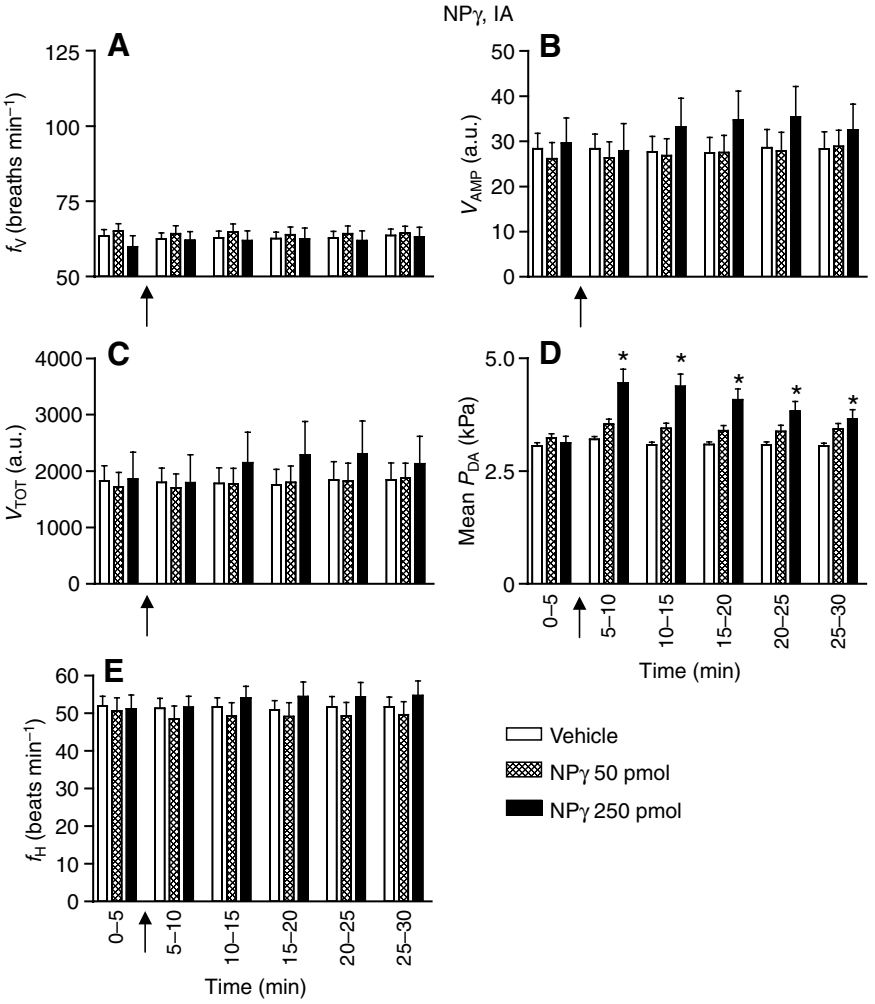

Fig. 3. Time course of the effects of intra-arterial injection of (1) 50 μl vehicle (open bars, *N*=16), (2) 50 pmol NPγ (cross-hatched bars, *N*=8), and (3) 250 pmol NPγ (filled bars, *N*=8) on (A) f_V, (B) V_{AMP}, (C) V_{TOT}, (D) mean P_{DA} and (E) f_H, in unanesthetized trout. The arrow indicates when the injection was given. *P<0.05 *vs* vehicle at corresponding post-injection period and *vs* pre-injection value.

Ventilatory and cardiovascular responses to central and peripheral NKA

The results obtained following ICV injections of graded doses (50–250 pmol) of NKA are shown in Fig. 5. As with SP, the effect of NKA on the ventilatory variables (Fig. 5A–C) was relatively minor, with only the highest dose (250 pmol) producing a significant decrease in V_{AMP} (Fig. 5B and Table 1) and an overall significant fall in V_{TOT} (Fig. 5C). This action of NKA was of short duration with V_{AMP} returning rapidly to baseline values. No significant changes in mean P_{DA} (Fig. 5D) or f_H (Fig. 5E) were observed following ICV injection of NKA.

No change in f_V and V_{AMP} or in the cardiovascular variables was observed following the IA injections of 50 pmol NKA (not shown). The highest dose of NKA (250 pmol) was also without effect on the ventilatory variables but this dose NKA produced a slight but significant increase in mean P_{DA} (+0.29±0.04 kPa) and a concomitant significant fall in f_H (–3.16±0.50 beats min^{-1}) 5 min after injection (not shown).

Discussion

Ventilatory and cardiovascular actions of centrally administered trout tachykinins

This study has investigated for the first time in any species the central action of NPγ on ventilation and demonstrates that ICV administration of the native tachykinins NPγ, SP and NKA differentially affect ventilatory movements in the unanesthetized trout. We provide evidence that the three tachykinins tested have a differential action on the neuronal substrate involved in respiratory rhythmogenesis. Since none of the peptides, at any dose tested, produced substantial changes in either P_{DA} or f_H, a strong argument is presented that observed changes in the ventilatory pattern were not secondary to cardiovascular changes. Furthermore, since the IA administration of the tachykinins did not affect f_V or V_{AMP} at any dose tested, the response observed following ICV injection clearly originated from the CNS rather than from diffusion of the peptides to the periphery. The data indicate that NPγ was about fivefold more potent than SP and NKA in producing an elevation of f_V, a reduction of V_{AMP}, and an overall hypoventilatory response. The N-terminal extension to the NKA sequence in NPγ is not considered to be involved in receptor interaction (see Conlon, 2004) so that the increased potency of NPγ is probably a consequence of its

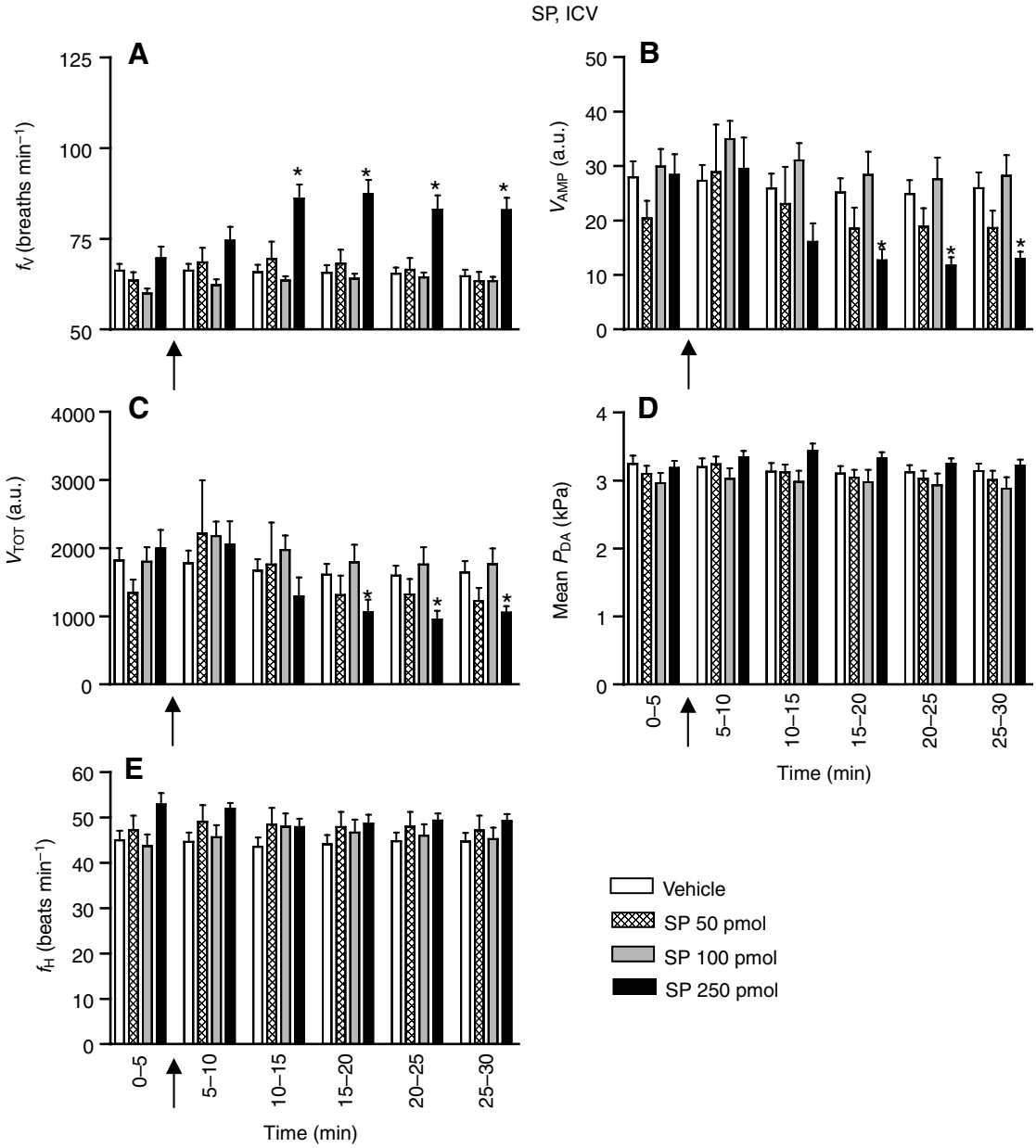

Fig. 4. Time course of the effects of intracerebroventricular injection of (1) 0.5 μl vehicle (open bars, *N*=16), (2) 50 pmol SP (cross-hatched bars, *N*=8), (3) 100 pmol SP (gray bars, *N*=10) and (4) 250 pmol SP (filled bars, *N*=10) on (A) f_V, (B) V_{AMP}, (C) V_{TOT}, (D) mean P_{DA} and (E) f_H, in unanesthetized trout. The arrow indicates when the injection was given. **P*<0.05 *vs* vehicle at corresponding post-injection period and *vs* pre-injection value.

increased stability within the third ventricle relative to NKA and SP.

The distribution of tachykinin receptors in the trout brain has not been reported but in another teleost species, the sea bass *Dicentrarchus labra*, tachykinin-binding sites are located in various brain regions including the entire hypothalamus and the medulla oblongata (Moons et al., 1992). However, the pharmacological properties of tachykinin receptors in fish have been studied much less extensively than in mammals. In the unanesthetized rat, NPγ evoked dose-dependent increases in mean arterial blood pressure and f_H, and produced behavioral responses that were attenuated by the NK2-receptor antagonist SR48968. The NK1-receptor antagonist RP67580 was without effect, indicating that central actions of NPγ are mediated, at least in part, through interaction with NK2-receptors (Picard and Couture, 1996). In mammals, the pre-Bötzinger complex is considered to be the primary source of respiratory rhythmogenesis (Smith et al., 1991) and micro-injection or bath application of SP into the pre-Bötzinger complex increased respiratory frequency (Monteau et al., 1996; Gray et al., 1999). The effect of SP was mediated by the NK-1 receptor (Gray et al., 2001). The central action of other mammalian tachykinins have not been studied so extensively but in the anesthetized rat, NKA, NKB and agonists selective for NK-2 and NK-3 receptors reduced respiratory frequency but increased tidal volume after injection within the NTS (Mazzone and Geraghty, 2000).

Further studies are required to determine whether the central action of NPγ on ventilatory variables in trout involves interaction with a receptor that resembles the mammalian NK-2 receptor more closely than the NK1-receptor.

The respiratory rhythm in fish is generated by a diffuse CPG located within the brainstem (Shelton, 1970). This CPG controls the activity of trigeminal Vth, facial VIIth, glossopharyngeal IXth and vagal Xth motor nuclei, all of which drive the breathing muscles (see Taylor et al., 1999). The CPG receives modulatory inputs from various sources, including peripheral mechano- and chemoreceptors, and also from the higher brain centers, including the mesencephalon and the forebrain (Shelton, 1959; Randall and Taylor, 1991; Taylor et al., 1999). There have been few studies in fish describing the afferent pathways from peripheral receptors and their general central projections to the brainstem (see Burleson et al., 1992). Immunohistochemical and physiological investigations have demonstrated that in the channel catfish *Ictalurus punctatus*, the primary general visceral nuclei situated at the caudal part of the NTS are crucial for maintaining basal ventilation and utilizes glutamate as a neurotransmitter for oxygen chemoreflexes (Sundin et al., 2003a). Studies conducted in a

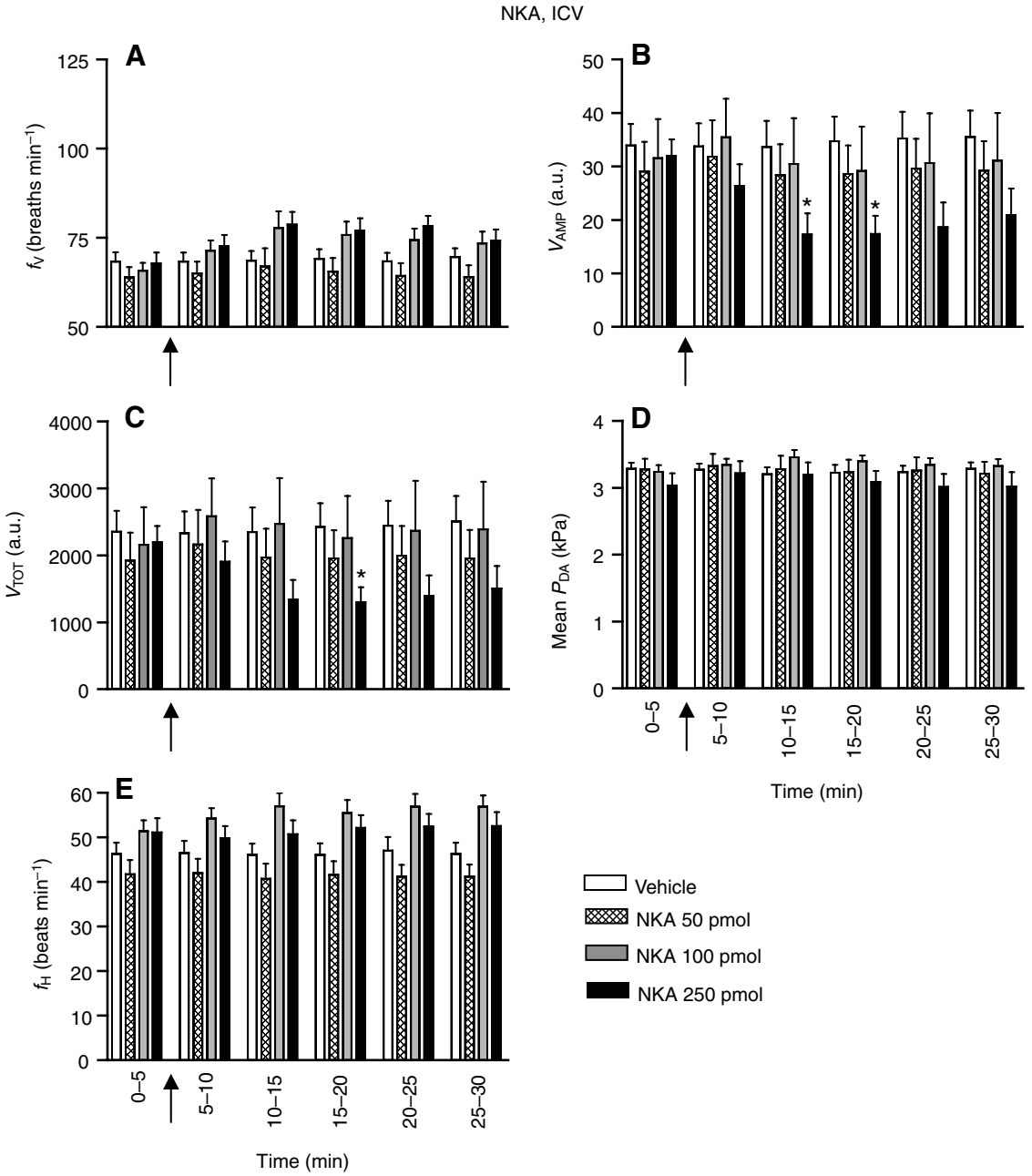

Fig. 5. Time course of the effects of intracerebroventricular injection of (1) 0.5 μl of vehicle (open bars, *N*=16), (2) 50 pmol NKA (cross-hatched bars, *N*=7), (3) 100 pmol NKA (gray bars, *N*=8), and (4) 250 pmol NKA (filled bars, *N*=7) on (a) f_V, (B) V_{AMP}, (C) V_{TOT}, (D) mean P_{DA} and (E) f_H, in unanesthetized trout. The arrow indicates when the injection was given. **P*<0.05 *vs* vehicle at corresponding post-injection period and *vs* pre-injection value.

teleost fish, the shorthorn sculpin *Myoxocephalus scorpius*, have also revealed that within the sensory vagal area of the brainstem, glutamate may be a key excitatory neurotransmitter released by the afferents of baro- and chemoreceptors (Sundin et al., 2003b). In the brainstem of the dogfish *Squalus acanthias*, catecholamines may also be involved in the control of the electrical activity of respiratory neurons (Randall and Taylor, 1991). However, nothing is known regarding either the origin and the actions of higher brain centers neurons that project towards the CPG or the respiratory motoneurons or the neurotransmitters and the receptors involved. The central pathways mediating the effects of NPγ and other tachykinins were not investigated in the present study. It is reasonable, however, to speculate that ICV injection of tachykinins activated receptor sites located mainly in the diencephalon, including the hypothalamus, where they can modulate the activity of various nuclei including the preoptic nuclei. Arginine vasotocin and isotocin neurons from the preoptic nuclei are known to project their axons not only to the neurohypophysis but also towards brainstem nuclei, including the NTS and the dorsal vagal motor nucleus (Batten et al., 1990; Saito et al., 2004). In this context it should be emphasized that immunocytochemical studies have shown that, in the brain of the rainbow trout, the diencephalon contains the largest number of SP-immunoreactive fibers and nuclei (Vecino et al., 1989). In addition, we can speculate about a possible diffusion of the injected tachykinins within the cerebrospinal fluid (CSF) towards the brainstem nuclei. Thus, exogenous tachykinins may produce effects on various nuclei including those previously described to be crucial for maintaining basal ventilation by both direct and indirect actions.

Ventilatory and cardiovascular actions of intraarterially administered trout tachykinins

It has previously been shown in the trout that trout SP and NKA increase both systemic vascular resistance and P_{DA} but decrease f_H and cardiac output (Kagstrom et al., 1996). Our present data are consistent with these results and indicate that SP was more potent than NKA in inducing the increase in P_{DA} and decrease in f_H. In this context, it should also be mentioned that trout SP was more effective than trout NKA in stimulating gastric motility in the rainbow trout (Jensen et al., 1993). In addition, we have demonstrated for the first time in a fish that intra-arterial injection of NPγ in trout causes a hypertensive response but without change in f_H. The lack of bradycardia following the IA injection of NPγ is intriguing since the hypertensive response is relatively robust. Absence of bradycardia suggests that, after IA injection of NPγ, the cardio-inhibitory baroreflex response is blunted or that the reflexogenic decrease in f_H is counteracted by a positive chronotropic effect on the heart. Further studies are needed to clarify this issue. The effects of the tachykinins on the peripheral cardiovascular system in trout stand in sharp contrast with the effects of NPγ, SP and NKA in mammals, where the three peptides are potent vasodilators of several vascular beds (see Conlon, 2004; Walsh and McWilliams, 2006). In the anesthetized guinea pig, intravenous injection of NPγ produced a fall in blood pressure that was mediated through interaction with NK-1 receptors, together with an increase in total pulmonary resistance and decrease in dynamic lung compliance that were mediated through NK-2 receptors (Yuan et al., 1994).

Possible physiological significance

The study provides insight into a possible role of endogenous tachykinins in the regulation of ventilatory and cardiovascular processes in the trout. The potent and selective central ventilatory actions of these peptides, particularly of NPγ, in increasing f_V and decreasing V_{AMP} thereby resulting in a potent hypoventilatory action, together with lack of effect on these variables after peripheral administration, suggest that receptors mediating ventilatory changes exist in the brain but not in the periphery. Conversely, the cardiovascular actions of the tachykinins after peripheral administration, particularly the hypertensive effect of NPγ, but the absence of cardiovascular actions after central administration, are consistent with peripheral rather than central localization of receptor(s) mediating cardiovascular changes.

The relevance of the present data to trout respiratory physiology is emphasized by the demonstration that numerous tachykinin neurons of a CSF-contacting type are present within the paraventricular organ of the hypothalamus in the Atlantic salmon *Salmo salar* (Holmqvist and Ekstrom, 1991), giving strong neuroanatomical support for a possible secretion of the endogenous products within the CSF compartment. Further studies are required to determine whether the observed central effects of the exogenously injected tachykinins, particularly NPγ, within the third ventricle mimic those of the endogenous peptides and to reveal under what circumstances tachykininergic systems of the brain are activated to control the respiratory system. As a working hypothesis, it is tempting to speculate that the central tachykininergic system might be recruited under conditions of environmental hyperoxia, a situation that is known to promote a hypoventilatory response in trout and other teleost species (Kinkead and Perry, 1990).

List of abbreviations and symbols

a.u.	arbitrary unit
CNS	central nervous system
CPG	central pattern generator
CSF	cerebrospinal fluid
ECG	electrocardiographic
f_H	heart rate
f_V	ventilation rate
IA	intra-arterial
ICV	intracerebroventricular
NK	neurokinin
NPγ	neuropeptide gamma
NPK	neuropeptide K
NTS	nucleus tractus solitarius
P_{DA}	dorsal aortic blood pressure
$P_{W_{O_2}}$	partial oxygen pressure in water
SP	substance P
V_{AMP}	ventilation amplitude
V_{TOT}	total ventilation

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