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Ventilatory and cardiovascular actions of centrally and peripherally administered trout pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) in the unanaesthetized trout

J.-C. Le Mével[1,*], F. Lancien[1], N. Mimassi[1] and J. M. Conlon[2]

[1]Université Européenne de Bretagne, Université de Brest, INSERM U650, Laboratoire de Traitement de l'Information Médicale, Laboratoire de Neurophysiologie, IFR 148 ScInBioS, Faculté de Médecine et des Sciences de la Santé, 22 Avenue Camille Desmoulins, CS 93837, 29238 Brest Cedex 3, CHU de Brest, France and [2]Department of Biochemistry, Faculty of Medicine and Health Sciences, United Arab Emirates University, 17666 Al Ain, United Arab Emirates

*Author for correspondence (jean-claude.lemevel@univ-brest.fr)

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SUMMARY

In mammals, pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are involved in cardiovascular and respiratory regulation. Several studies have demonstrated the presence of PACAP, VIP and their receptors in various tissues of teleost fish, including the brain, but little is known about their respiratory and cardiovascular effects. The present study was undertaken to compare the central and peripheral actions of graded doses (25–100 pmol) of trout PACAP and trout VIP on ventilatory and cardiovascular variables in the unanaesthetized rainbow trout. Compared with vehicle, only intracerebroventricular injection of PACAP significantly (P<0.05) elevated the ventilation frequency and the ventilation amplitude, but both peptides significantly increased the total ventilation ($\dot{V}_{TOT}$). However, the maximum hyperventilatory effect of PACAP was approximately 2.5-fold higher than the effect of VIP at the 100 pmol dose (PACAP, $\dot{V}_{TOT}$=+5407±921 arbitrary units, a.u.; VIP, $\dot{V}_{TOT}$=+2056±874 a.u.; means ± s.e.m.). When injected centrally, only PACAP produced a significant increase in mean dorsal aortic blood pressure (P_{DA}) (100 pmol: +21%) but neither peptide affected heart rate (f_H). Intra-arterial injections of either PACAP or VIP were without effect on the ventilatory variables. PACAP was without significant action on P_{DA} and f_H while VIP significantly elevated P_{DA} (100 pmol: +36%) without changing f_H. In conclusion, the selective central hyperventilatory actions of exogenously administered trout PACAP, and to a lesser extent VIP, suggest that the endogenous peptides may be implicated in important neuroregulatory functions related to the central control of ventilation in trout.

Key words: PACAP, VIP, ventilatory control, heart rate, blood pressure, intracerebroventricular injection, teleost.

INTRODUCTION

Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) belong to the secretin–glucagon superfamily of peptides (Sherwood et al., 2000). PACAP is found in two forms, a 38 amino acid peptide (PACAP-38) and the C-terminally truncated 27 amino acid peptide (PACAP-27). PACAP and VIP share sequence similarity and, in mammals, these peptides exert their actions by binding to three receptors, PAC1, VPAC1 and VPAC2 (Laburthe et al., 2007). The peptides and their receptors have a widespread distribution in mammalian tissues and are involved in numerous physiological functions. In the brain, PACAP and VIP are known to act as neuroendocrine peptides, but also as neurotransmitters or neuromodulators controlling multiple physiological processes (Vaudry et al., 2000). In particular, PACAP-signalling pathways are vital for neuronal control of breathing (Cummings et al., 2004; Li et al., 2006; Wilson and Cumming, 2008) and play a role in the control of the cardiovascular system (Farham et al., 2008). In addition, PACAP and VIP have neuroprotective and neurotrophic actions in brain tissues (Vaudry et al., 2000). In the periphery, PACAP and VIP function as local hormones or neurotransmitters acting on various tissues. Acting in an autocrine manner, PACAP and VIP are implicated in the pathogenesis of breast and lung cancer (Moody and Jensen, 2006).

PACAP and VIP appeared very early during evolution and the primary structure of these peptides and their receptors has been remarkably well conserved from fish to mammals (Wong et al., 1998; Sherwood et al., 2000; Montpetit et al., 2003). Within the central nervous system of teleosts, PACAP- and VIP-like immunoreactivity are localized mainly in neuronal perikarya of the diencephalon at the level of the preoptic nucleus (NPO) with their fibers projecting both into the adenohypophysis (Matsuda et al., 1997; Montero et al., 1998; Wong et al., 1998; Mathieu et al., 2001) and towards many other hypothalamo-hypophysial areas (Montero et al., 1998; Mathieu et al., 2001; Matsuda et al., 2005). These observations suggest that PACAP and VIP act not only as hypophysiotropic hormones (Parker et al., 1997; Wong et al., 1998; Chang et al., 2001; Kelley et al., 1988; Montero et al., 1998; Matsuda et al., 2008) but also as neurotransmitters and/or neuromodulators (Matsuda et al., 2006).

PACAP and VIP are also present in peripheral tissues of teleosts. VIP is thought to be an endogenous vasodilating neuropeptide in trout (Kagstrom and Holmgren, 1997), and in cod VIP increased blood flow in the celiac artery (Jensen et al., 1991). PACAP and VIP co-exist in neuronal elements of the gill arch of the goldfish *Carassius auratus* (de Girolamo et al., 1998). Neuronal nerve fibres immunoreactive to PACAP and VIP are also present in the vicinity of the chromaffin cells of various teleosts including the rainbow trout (Reid et al., 1995) and PACAP and VIP stimulate the release of adrenaline from the *in situ* saline-perfused trout posterior cardinal vein (Montpetit and Perry, 1999; Montpetit and Perry, 2000).

However, to our knowledge, the peripheral effect of PACAP on any biological function *in vivo* has never been explored in fish, except for the suppressive effect of peripheral PACAP on feeding behaviour in the goldfish (Matsuda et al., 2006).

Given that PACAP and VIP (1) have been highly conserved during vertebrate evolution, (2) are widely distributed in all mammalian and fish tissues, and (3) in mammals are crucial for breathing and cardiovascular regulation (see above), we proposed the hypothesis that these peptides could exert a pivotal role in the control of these systems in fish, an area that has been poorly explored. Consequently, the aim of the present study was to investigate the effects of intracerebroventricular (i.c.v.) administration of synthetic replicates of trout PACAP (Krueckl and Sherwood, 2001) and trout VIP (Wang and Conlon, 1995) on ventilation rate (f_V), ventilation amplitude ($\dot{V}_{AMP}$), dorsal aortic blood pressure (P_{DA}) and heart rate (f_H) in the unanaesthetized rainbow trout, *Oncorhynchus mykiss*. The central actions of the peptides on these variables were compared with their effects after intra-arterial (i.a.) administration.

MATERIALS AND METHODS

Peptides and chemicals

Trout PACAP-27 (HSDGIFTDSYSRYRKQMAVKKYLAA-VL.NH2) and trout VIP (HSDAIFTDNYSRFRKQMAVK-KYLNSVLT.NH2) were synthesized by GL Biochem (Shanghai, China) and purified to near homogeneity (>98% purity) by reversed-phase HPLC. The identities of the peptides were confirmed by electrospray mass spectrometry. Peptides were stored in stock solution (0.01% HCl) at –25°C. For injections, the peptides were diluted to the desired concentration with Ringer's solution (vehicle) immediately prior to use. The composition of the Ringer's solution was (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). All solutions were sterilized by filtration through 0.22 μm filters (Millipore, Molsheim, France) before injection.

Animals

Adult rainbow trout (*Oncorhynchus mykiss* Walbaum 1792; body mass 261±3 g mean ± s.e.m., N=59) 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 l tank containing circulating dechlorinated, aerated tap water (11–12°C), under a standard photoperiod (lights on 09:00–20:00 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 of Brittany, France.

Experimental procedures

All surgical procedures were made under tricaine methane sulfonate (3-amino-benzoic acid ethyl ester; 60 mg l^{-1} in tap water buffered with $NaHCO_3$ to pH 7.3–7.5) anaesthesia. The techniques used for placement of the electrocardiographic (ECG) electrodes, placement of the buccal catheter, cannulation of the dorsal aorta and insertion of the i.c.v. 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, Clay Adams) 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 i.c.v. 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 (Le Mével et al., 2009). An obturator was placed at the end of the PE-10 tubing and the cranial surface was covered with haemostatic tissue followed by light quick-curing resin. After surgery, the animals were force-ventilated with dechlorinated tap water and, following recovery of opercular movements, were transferred to a 6 l blackened chamber supplied with dechlorinated and aerated tap water (10–11°C) that was both re-circulating and through-flowing. Oxygen pressure within the water tank ($P_{W_{O_2}}$) and pH were continuously recorded and maintained at constant levels ($P_{W_{O_2}}$=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 i.a. or i.c.v. 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 behaviour, checking the ventilatory and the cardiovascular variables, and measuring their haematocrit. Animals that did not appear healthy, according to the range of values detailed in our previous studies, were discarded. After stable f_V, $\dot{V}_{AMP}$, P_{DA} and f_H were maintained for at least 90 min, parameters were recorded for 30 min without any manipulation, i.a. or i.c.v. injection in control experiments.

Intracerebroventricular administration of PACAP and VIP

The injector was introduced within the i.c.v. guide prior to the beginning of a recording session which lasted 30 min. All injections were made at the 5th minute 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 first received an i.c.v. injection of vehicle (0.5 μl), and 30 min later an i.c.v. injection of PACAP or VIP (25, 50 and 100 pmol in 0.5 μl). Previous control experiments using two i.c.v. injections 30 min apart have shown the absence of time-dependent changes in the measured variables using this protocol (Le Mével et al., 2009). The animals received no more than two i.c.v. injections of peptide per day with a delay of at least 5 h between the injections. The peptides were given in random order within and between fish. 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.

Intra-arterial administration of PACAP and VIP

Five minutes after the beginning of the recording session, 50 μl of vehicle, or trout PACAP or VIP at the appropriate concentration was injected through the dorsal aorta and immediately flushed with 150 μl of vehicle. PACAP and VIP were tested at doses of 25, 50 and 100 pmol. Peptides were administered in random order.

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

tank water 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 1000 Hz and visualized on the screen of a PC using PowerLab 4/30 data acquisition system (ADInstruments, Oxford, UK) and LabChart Pro software (v.6.0; ADInstruments) during the 30 min recording period and the data were stored on a disk. The time series related to the ventilatory, the pulsatile P_{DA} and the ECG signals were then processed off-line with custom-made programs written in LabView 6.1 (Laboratory Virtual Instrument Engineering Workbench, National Instruments, Austin, TX, USA). The ventilatory and cardiovascular variables were calculated as previously described (Lancien et al., 2004; Le Mével et al., 2007). Segments free from any movement artifacts on the ventilatory signal were selected and f_V (breaths min^{-1}) and $\dot{V}_{AMP}$ (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. $\dot{V}_{AMP}$ 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 $\dot{V}_{AMP}$ on ventilation was estimated according to the formula $\dot{V}_{TOT}=f_V\times\dot{V}_{AMP}$ where $\dot{V}_{TOT}$ (a.u.) is total ventilation. The mean P_{DA} (kPa) was calculated from the pulsatile P_{DA} as the arithmetic mean between 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, $\dot{V}_{AMP}$, $\dot{V}_{TOT}$, 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 table, data refer to absolute values or maximal changes from baseline (pre-injection) values, and in the text as percentage changes. For the time course studies, the data were analysed initially using two-way ANOVA (treatments and time) followed by the Bonferroni *post hoc* test for comparisons between groups. Within each group of trout receiving peptide, when the overall preceding two-way ANOVA analysis demonstrated statistically significant differences compared with vehicle-injected trout, Dunnett's test was used for comparison of post-injection values with pre-injection values. Student's *t*-test was used to compare the maximum difference between the pre-injection and the post-injection ventilatory variables between PACAP- and VIP-injected trout. The criterion for statistical difference between groups was $P<0.05$. The statistical tests were performed using GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA).

RESULTS

Ventilatory and cardiovascular responses to central PACAP

Fig. 1 illustrates 30 s recordings in a single trout of the ventilatory, blood pressure and ECG signals taken during the pre-injection period (Fig. 1A) and during the 25–30 min post-injection period (Fig. 1B)

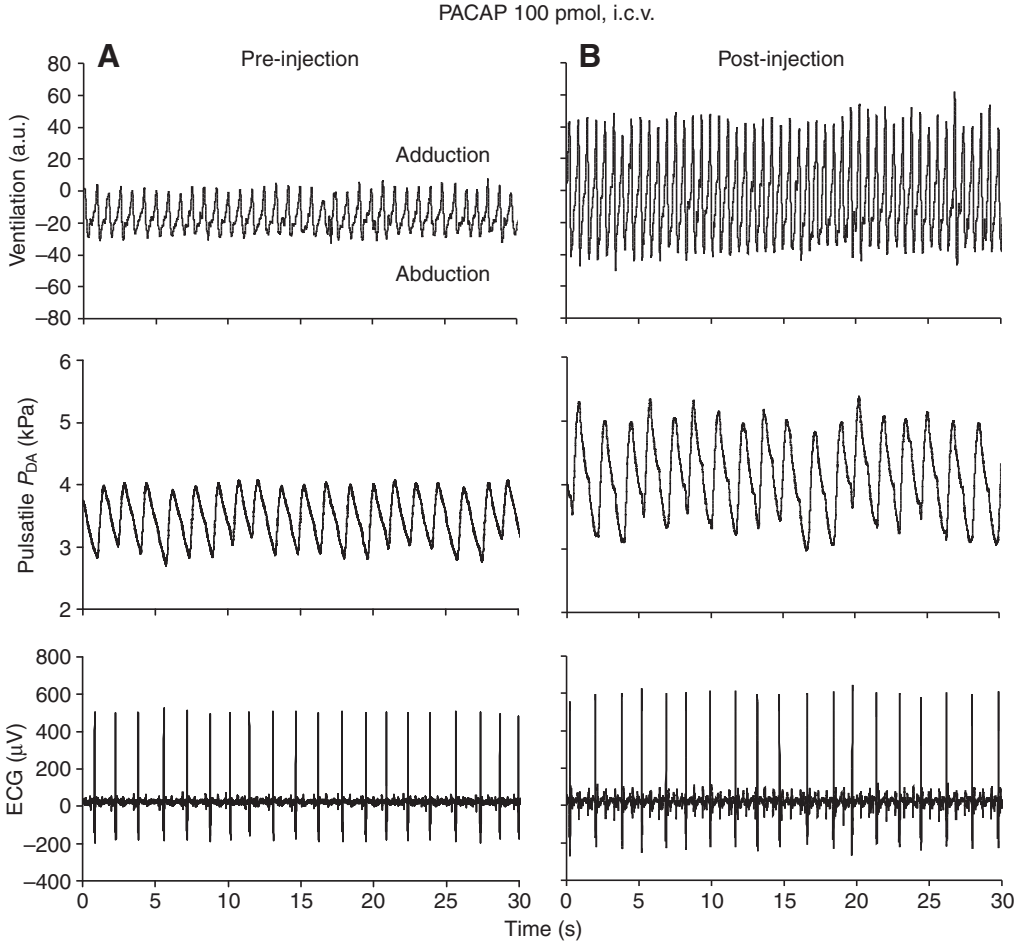

Fig. 1. Recording traces of 30 s duration in the same unanaesthetized trout illustrating the changes observed in ventilatory movements (ventilation), dorsal aortic blood pressure (P_{DA}) and electrocardiographic (ECG) signals between the pre-injection period (0–5 min) and the post-injection period (20–25 min) after intracerebroventricular (i.c.v.) injection of 100 pmol pituitary adenylate cyclase-activating polypeptide (PACAP). Note that, compared with the pre-injection period, i.c.v. injection of PACAP produces an increase in the ventilation rate and amplitude, an elevation of blood pressure but no change in heart rate.

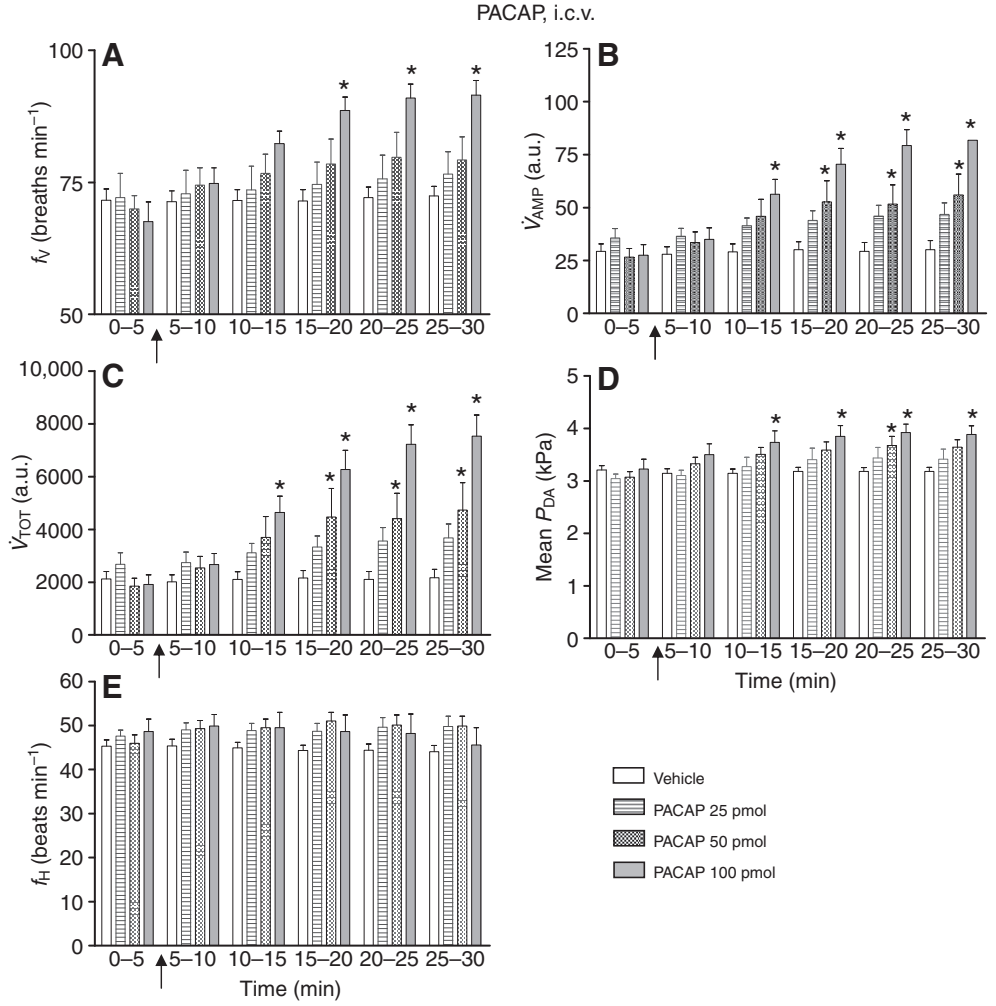

Fig. 2. Time course of the effects of i.c.v. injection of (1) 0.5 µl of vehicle (N=16), (2) 25 pmol PACAP (N=8), (3) 50 pmol PACAP (N=9) and (4) 100 pmol PACAP (N=11) on (A) ventilation rate (f_V), (B) ventilation amplitude ($\dot{V}_{AMP}$), (C) total ventilation ($\dot{V}_{TOT}$), (D) mean P_{DA} and (E) heart rate (f_H) in unanaesthetized trout. The arrow indicates when the injection was given. *P<0.05 *vs* vehicle at corresponding post-injection period and *vs* pre-injection value.

after i.c.v. injection of 100 pmol PACAP. Comparing the post-injection and the pre-injection signals, PACAP caused a marked elevation in f_V and $\dot{V}_{AMP}$. Concurrently, i.c.v. injection of PACAP also produced an increase in mean P_{DA} without any change in f_H.

Fig. 2 summarizes the time course of changes observed in the ventilatory and cardiovascular variables following i.c.v. injection of vehicle or a range of doses (25–100 pmol) of PACAP. The i.c.v. injection of vehicle produced no significant change in the ventilatory and cardiovascular variables compared with pre-injection values. Compared with i.c.v. injection of vehicle, PACAP evoked a dose- and time-dependent elevation of f_V (Fig. 2A) and $\dot{V}_{AMP}$ (Fig. 2B). Consequently, the net effect of the peptide was a hyperventilatory response involving a gradual and significant dose-dependent increase in $\dot{V}_{TOT}$ (Fig. 2C). The threshold dose for an effect of PACAP on f_V was 100 pmol, but a significant effect on $\dot{V}_{AMP}$ and $\dot{V}_{TOT}$ occurred at 50 pmol and this was observed 15 min after injection of the peptide. The actions of PACAP on the ventilatory variables were long lasting as values had not returned to baseline levels by the end of the post-injection period of 25 min. The most pronounced action of PACAP was in evoking hyperventilation through an increase in $\dot{V}_{AMP}$ instead of f_V. For instance, after 50 and 100 pmol PACAP, the maximal change in $\dot{V}_{AMP}$ expressed as a percentage from the pre-injection value reached about 100% (55.9±9.9 *vs* 26.5±4.0 a.u., P<0.05) and 200% (81.8±7.8 *vs* 27.4±5.0 a.u., P<0.05), respectively, while the elevation of f_V was only about 10% (79.2±4.3 *vs* 69.9±2.5 breaths min^{-1}, not significant) and 35% (91.4±2.7 *vs* 67.5±3.7 breaths min^{-1}, P<0.05) (Fig. 2A,B and Table 1). After i.c.v. injection, only the highest dose of PACAP produced a weak, but significant, sustained increase in P_{DA} (Fig. 2D). However, there was no change in f_H after i.c.v. injection of PACAP at any dose (Fig. 2E).

Ventilatory and cardiovascular responses to central VIP

Fig. 3 depicts the results obtained following i.c.v. injection of vehicle or graded doses (25–100 pmol) of VIP. After i.c.v. injection, the effects of VIP on the ventilatory variables were quite different from those following i.c.v. injection of PACAP (Fig. 2A–C). VIP did not produce a significant increase in f_V (Fig. 3A) or $\dot{V}_{AMP}$ (Fig. 3B) but nonetheless the resultant action of this peptide was a small, transient but significant elevation of $\dot{V}_{TOT}$ (Fig. 3C) at the highest dose tested. Moreover, statistical analysis of the results obtained following i.c.v. injection indicated that the maximum increase in f_V, $\dot{V}_{AMP}$ and $\dot{V}_{TOT}$ after i.c.v. injection of 100 pmol PACAP relative to the pre-injection values was about 2.5-fold higher than the maximum ventilatory effects of the same dose of VIP (Table 1). VIP did not produce any change in either P_{DA} (Fig. 3D) or f_H (Fig. 3E).

Ventilatory and cardiovascular responses to peripheral PACAP and VIP

In contrast to its i.c.v. effects, i.a. injection of PACAP at doses of 25–100 pmol produced no change in f_V, $\dot{V}_{AMP}$ or $\dot{V}_{TOT}$ (Fig. 4A–C). Peripherally injected PACAP did not cause any significant change

Table 1. Maximal effect of intracerebroventricular (i.c.v.) administration of 100 pmol PACAP and VIP on ventilation frequency (f_V), ventilation amplitude ($\dot{V}_{AMP}$) and total ventilation ($\dot{V}_{TOT}$) during the post-injection period

		Pre-injection (0–5 min)			Post-injection		
Protocol	*N*	f_V (breaths min^{-1})	$\dot{V}_{AMP}$ (a.u.)	$\dot{V}_{TOT}$ (a.u.)	Δf_V (breaths min^{-1})	$\Delta\dot{V}_{AMP}$ (a.u.)	$\Delta\dot{V}_{TOT}$ (a.u.)
PACAP	11	67.5±3.7	27.4±5.0	1922±357	+22.2±4.6*,†	+52.2±8.5*,†	+5407±921*,†
VIP	11	64.3±4.1	28.2±4.5	1909±377	+9.2±2.7	+22.2±10	+2056±874*

Results for the post-injection period are shown as changes from the baseline (pre-injection, 0–5 min) level.
PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal peptide.
All data are presented as means ± s.e.m. a.u., arbitrary units. Data from trout receiving PACAP and VIP are from Fig. 4 and Fig. 5, respectively. *P<0.05 *vs* pre-injection values (Dunnett's test); †P<0.05 *vs* VIP (Student's *t*-test).

in either P_{DA} or f_H (Fig. 4D,E). As with i.a. PACAP, i.a. injection of VIP caused no change in the ventilatory variables (Fig. 5A–C). In contrast to PACAP, bolus peripheral injection of VIP produced a robust dose-dependent and sustained hypertensive response (maximum increase; 50 pmol: +30%; 100 pmol: +36 %; Fig. 5D) without any change in f_H (Fig. 5E).

DISCUSSION

Ventilatory and cardiovascular actions of centrally administered PACAP and VIP

The most important outcome of this study was the clear demonstration for the first time in a non-mammalian species that i.c.v. administration of native PACAP, and to a much lesser extent VIP, increased ventilation in trout. In addition to this hyperventilatory action, centrally administered PACAP produced a modest but sustained hypertensive effect at the highest dose of peptide tested (100 pmol). It is possible that after i.c.v. injection, PACAP and VIP were removed from the ventricular system to the vascular space by crossing the blood–brain barrier so that their peripheral action on the ventilatory and cardiovascular apparatus may be responsible for the effects observed. However, the response to centrally injected PACAP and VIP is more probably mediated by direct central mechanisms because the i.a. injection of equimolar doses of these two peptides did not produce any effect on the ventilatory variables and because the cardiovascular actions of PACAP and VIP were not similar to their central actions.

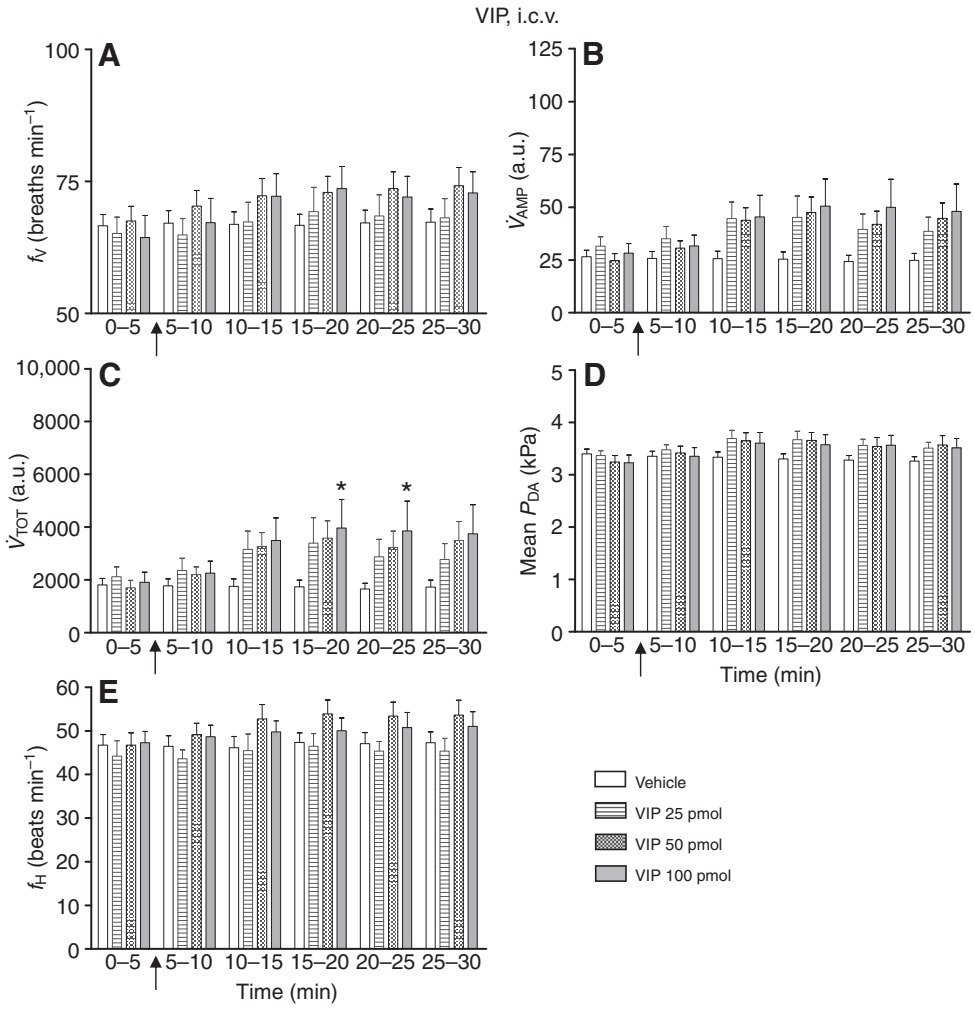

Fig. 3. Time course of the effects of i.c.v. injection of (1) 0.5 µl of vehicle (*N*=16), (2) 25 pmol vasoactive intestinal peptide (VIP, *N*=8), (3) 50 pmol VIP (*N*=9) and (4) 100 pmol VIP (*N*=11) on (A) f_V, (B) $\dot{V}_{AMP}$, (C) $\dot{V}_{TOT}$, (D) mean P_{DA} and (E) f_H in unanaesthetized trout. The arrow indicates when the injection was given. *P<0.05 *vs* vehicle at corresponding post-injection period and *vs* pre-injection value.

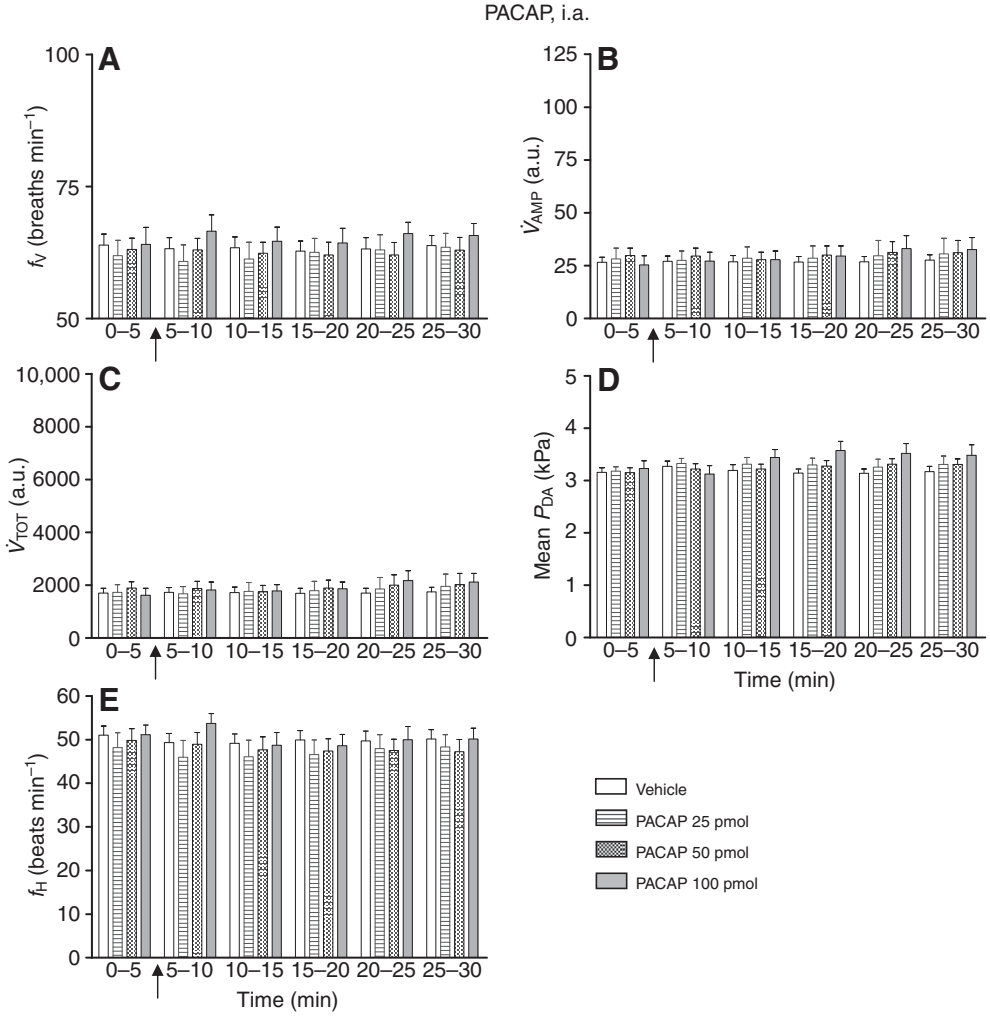

Fig. 4. Time course of the effects of intra-arterial (i.a.) injection of (1) 50 µl of vehicle (N=16), (2) 25 pmol PACAP (N=8), (3) 50 pmol PACAP (N=9) and (4) 100 pmol PACAP (N=8) on (A) f_V, (B) $\dot{V}_{AMP}$, (C) $\dot{V}_{TOT}$, (D) mean P_{DA} and (E) f_H in unanaesthetized trout. The arrow indicates when the injection was given. No significant change in the ventilatory and cardiovascular variables occurred after the injection of PACAP.

Rhythmic ventilatory movements in fish are generated by a diffuse central pattern generator (Taylor et al., 1999) whose activity is modulated by inputs originating from the peripheral chemoreceptors (see Burleson et al., 1992) and also from higher brain centres (Taylor et al., 1999). However, little is known about the specific pathways, the neurotransmitters and/or neuropeptides, and the receptors that permit integration of the various inputs at the level of the central pattern generator to control the final output motor impulses that govern the frequency and amplitude of the ventilatory movements (Gilmour and Perry, 2007). In the brainstem of the dogfish, *Squalus acanthias*, catecholamines regulate the electrical activity of respiratory neurons (Randall and Taylor, 1991). In the channel catfish, *Ictalurus punctatus*, glutamatergic pathways within the caudal part of the nucleus tractus solitarius (NTS) are essential for the control of ventilation, and NMDA (*N*-methyl-D-aspartate) receptors mediate f_V, while AMPA (α-amino-3-hydroxy-5-methyl-4-isoazole propionic acid) receptor or another ionotropic receptor mediates $\dot{V}_{AMP}$ (Turesson and Sundin, 2003; Sundin et al., 2003). The present findings provide important information relevant to the understanding how the central PACAP/VIPergic system could affect ventilatory drive in a teleost fish. Using a similar experimental protocol we recently demonstrated that other biologically active neuropeptides, corticotropin-releasing factor (Le Mével et al., 2009), neuropeptide gamma (Le Mével et al., 2007) and urotensin II (Lancien et al., 2004), can act on the trout brain to provoke important changes in the ventilatory movements. Collectively, these results support the idea that, in the trout brain, multiple neuropeptides might be involved in the fine control of ventilation, with each peptide having a selective action on f_V or $\dot{V}_{AMP}$, or both ventilatory variables.

PACAP-38 is also present in the brain of many vertebrate species including fish (Arimura et al., 1991; Chartrel et al., 1991; Montero et al., 2000). It is known, however, that the C-terminally truncated 27 amino acid alpha-amidated peptide, PACAP-27, exhibits the full biological activity of the peptide (Vaudry et al., 2000). We cannot exclude the possibility, however, that trout PACAP-38 may have different potencies from trout PACAP-27 in our model. In mammals, PACAP and VIP generally act on one of three different G-protein-coupled receptors – the PACAP-preferring PAC1 receptor or the VPAC1 and VPAC2 receptors that exhibit a similar high affinity for PACAP and VIP (Laburthe et al., 2007). PAC1 and VPAC receptors were cloned from brain and peripheral tissues of goldfish (Wong et al., 1998) and rainbow trout (Montpetit et al., 2003). In these two species, the receptors share relatively high sequence similarity with their mammalian counterparts. The present study has shown that only PACAP at doses of 50 and 100 pmol produced a significant increase in f_V and $\dot{V}_{AMP}$, and at a dose of 100 pmol, PACAP produced about a 2.5-fold higher increase in $\dot{V}_{TOT}$ than did VIP. In addition, central PACAP but not VIP produced hypertensive effects. These data suggest that within the brain of the trout, PACAP

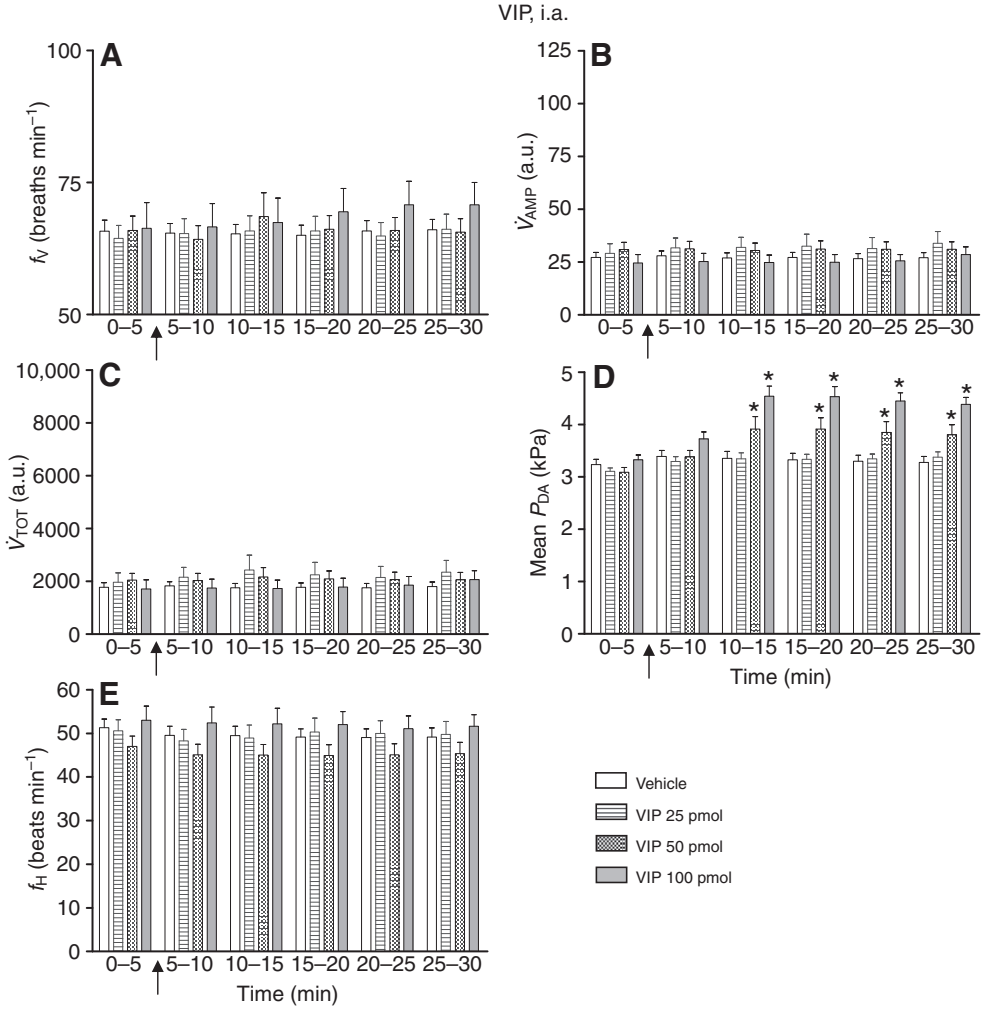

Fig. 5. Time course of the effects of i.a. injection of (1) 50 µl of vehicle (*N*=16), (2) 25 pmol VIP (*N*=8), (3) 50 pmol VIP (*N*=9) and (4) 100 pmol VIP (*N*=8) on (A) f_V, (B) $\dot{V}_{AMP}$, (C) $\dot{V}_{TOT}$, (D) mean P_{DA} and (E) f_H in unanaesthetized trout. The arrow indicates when the injection was given. **P*<0.05 *vs* vehicle at corresponding post-injection period and *vs* pre-injection value.

may bind preferentially to PAC1 receptor rather than to VPAC receptors.

To produce hyperventilation, PACAP and VIP, injected i.c.v., must access receptors critical for the control of respiratory motor neurons. However, the site(s) of action of PACAP and VIP cannot be determined from the present study. Since PACAP and VIP were injected in close proximity to the NPO they might mimic the action of the endogenous peptides that are present within neuronal perikarya of this diencephalic nucleus (Matsuda et al., 1997; Montero et al., 1998; Wong et al., 1998; Mathieu et al., 2001). PACAP- and VIP-containing, and also arginine vasotocin- and isotocin-containing, preoptic neurons project not only to the hypophysis but also towards the mesencephalon (Montero et al., 1998; Mathieu et al., 2001), and the medulla oblongata (Montero et al., 1998; Mathieu et al., 2001) where these neuropeptides may affect the activity of brainstem respiratory and cardiovascular nuclei including the NTS and the dorsal vagal motor nucleus (Batten et al., 1990; Saito et al., 2004). It is worth mentioning that in mammals, PACAP and PACAP receptors are present within the paraventricular nucleus (PVN), a nucleus homologous to the teleostean NPO (Arimura et al., 1991; Vaudry et al., 2000), and that PACAP excites PVN neurons (Vaudry et al., 2000). Vasopressin and oxytocin PVN neurons project to important respiratory-related regions of the medulla and spinal cord, including the pre-Bötzinger complex and the phrenic motor nuclei (Mack et al., 2002; Kc et al., 2002; Wilson et al., 2008). Finally in our model, the exogenously injected peptides may affect breathing though diffusion within the cerebrospinal fluid towards critical respiratory brainstem nuclei.

Ventilatory and cardiovascular actions of peripherally administered PACAP and VIP

PACAP and VIP injected peripherally produced no change in the ventilatory parameters. No change in P_{DA} and f_H was produced by PACAP while VIP evoked a significant increase in P_{DA} without changing f_H. These cardiovascular actions of VIP are consistent with previous studies using another teleost fish, the cod *Gadus morhua* (Jensen et al., 1991). In this species VIP (100 pmol kg^{-1}) also caused an increase in visceral blood flow which was due in part to an increase in cardiac output and a decrease in resistance in the celiac vascular bed (Jensen et al., 1991). To the best of our knowledge, the *in vivo* peripheral cardiovascular actions of PACAP have never been reported in fish. In mammals, the vascular actions of PACAP are variable as the peptide has both vasodilator and vasoconstrictor actions and these effects appear to be dose and species dependent (Runcie et al., 1995). Previous studies have shown that, in the anaesthetized dog, intravenous administration of PACAP-27 caused potentiation of cardiac slowing evoked by stimulation of the vagus nerve and a marked increase in ventilation through a probable stimulation of peripheral chemoreceptors (Runcie et al., 1995). VIP- and PACAP-immunoreactive nerve cell bodies and fibres exist in

the sino-atrial tissue (Zaccone et al., 2009) and a high level of mRNA for type I PACAP receptors is found in the goldfish heart (Wong et al., 1998) suggesting that PACAP may also play some role in the control of cardiac functions in fish. In our study, we did not observe any significant change in f_H. Neuronal nerve fibres immunoreactive to PACAP and VIP are also present at the vicinity of the chromaffin cells of various teleosts, including the rainbow trout (Reid et al., 1995). PACAP and VIP cause the release of adrenaline from *in situ* saline-perfused trout posterior cardinal vein (Montpetit and Perry, 1999; Montpetit and Perry, 2000). This action of VIP and PACAP is mediated by VIP-binding sites that exhibit the properties of VPAC receptors. The fact that in the present study only peripheral VIP caused a hypertensive action strongly suggests that VPAC receptors mediate vasoregulatory mechanisms in trout. It remains to be determined whether the slow increase in P_{DA} observed after the systemic administration of VIP might be due either to the release of adrenaline from chromaffin cells to compensate for a possible vasodilatory action of VIP or to another mechanism.

The possible physiological significance of the observed effects of PACAP and VIP is unknown and we can only propose a hypothesis. As we demonstrated that central PACAP mainly promotes hyperventilation, this response may be crucial for trout facing environmental hypoxia to maintain energy homeostasis through oxidative processes (Perry et al., 2009). In the periphery, VIP has more pronounced effects than PACAP and is more likely to be involved in the fine control of blood pressure.

In conclusion, the potent and selective central hyperventilatory action of exogenously administered trout PACAP suggests that the endogenous peptide within the trout brain might be implicated as a neurotransmitter and/or a neuromodulator in the regulation of ventilation. In addition, central PACAP might be involved in the control of blood pressure. In contrast, the selective systemic hypertensive action of exogenously injected VIP suggests that the endogenous peptide may act as a local and/or circulating hormone preferentially involved in vasoregulatory mechanisms.

LIST OF SYMBOLS AND ABBREVIATIONS

a.u.	arbitrary unit
ECG	electrocardiographic
f_H	heart rate
f_V	ventilation rate
i.a.	intra-arterial
i.c.v.	intracerebroventricular
NPO	preoptic nucleus
NTS	nucleus tractus solitarius
PACAP	pituitary adenylate cyclase-activating polypeptide
PAC1	PACAP receptor
P_{DA}	dorsal aortic blood pressure
PVN	paraventricular nucleus
$P_{W_{O_2}}$	partial oxygen pressure in water
$\dot{V}_{AMP}$	ventilation amplitude
VIP	vasoactive intestinal peptide
VPAC1	VIP/PACAP receptor subtype 1
VPAC2	VIP/PACAP receptor subtype 2
$\dot{V}_{TOT}$	total ventilation

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