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RESEARCH ARTICLE

Central ventilatory and cardiovascular actions of calcitonin gene-related peptide in unanesthetized trout

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SUMMARY

Calcitonin gene-related peptide (CGRP) and its receptors are widely distributed in the tissues of teleost fish, including the brain, but little is known about the ventilatory and cardiovascular effects of the peptide in these vertebrates. The present study was undertaken to compare the central and peripheral actions of graded doses (5–50 pmol) of trout CGRP on ventilatory and cardiovascular variables in unanesthetized rainbow trout. Compared with vehicle, intracerebroventricular injection of CGRP significantly elevated the ventilation frequency (f_V) and the ventilation amplitude (V_{AMP}) and, consequently, the total ventilation (V_{TOT}). The maximum hyperventilatory effect of CGRP (V_{TOT}: +300%), observed at a dose of 50 pmol, was mostly due to its stimulatory action on V_{AMP} (+200%) rather than f_V (+30%). In addition, CGRP produced a significant and dose-dependent increase in mean dorsal aortic blood pressure (P_{DA}) (50 pmol: +40%) but the increase in heart rate (f_H) was not significant. Intra-arterial injections of CGRP were without effect on the ventilatory variables but significantly and dose-dependently elevated P_{DA} (50 pmol: +36%) without changing f_H. At the highest dose tested, this hypertensive phase was preceded by a rapid and transient hypotensive response. In conclusion, our study suggests that endogenous CGRP within the brain of the trout may act as a potent neurotransmitter and/or neuromodulator in the regulation of cardio-ventilatory functions. In the periphery, endogenous CGRP may act as a local and/or circulating hormone preferentially involved in vasoregulatory mechanisms.

Key words: central CGRP, hyperventilation, hypertension, teleost.

INTRODUCTION

The 37-amino-acid peptide calcitonin gene-related peptide (CGRP) is derived from the tissue-specific splicing of the primary transcript of the calcitonin gene (Amara et al., 1982). CGRP is thus a member of the calcitonin/CGRP peptide family, which includes adrenomedullin (AM), adrenomedullin-2 (or intermedin), amylin and calcitonin receptor-stimulating peptide (Ogoshi et al., 2006; Sawada et al., 2006). CGRP binds to a seven transmembrane G-protein-coupled calcitonin receptor-like receptor that is complexed with one of three receptor-activity-modifying proteins (Tam and Brain, 2006). In mammals, CGRP and its receptors are widely distributed throughout the peripheral and central nervous systems (CNS). In the periphery, CGRP is a vasodilator, a hypotensive peptide and produces positive chronotropic and inotropic effects in the heart (Ando et al., 1990; Brain and Grant, 2004; Smillie and Brain, 2011). CGRP also affects gastrointestinal functions (Martinez and Tache, 2006). In the CNS, CGRP acts as a neurotransmitter and/or neuromodulator involved in multiple physiological and behavioural processes, including the hypothalamic regulation of feeding (Krahn et al., 1984). In addition, CGRP regulates the local vasodilation of cerebral vessels contributing to the pathophysiology of migraine headaches, and the peptide modulates pain responses at the level of the spinal cord (Tam and Brain, 2006). Central CGRP also plays a role in the autonomic regulation of the cardiovascular system. In contrast to its hypotensive effect in the periphery, intracerebroventricular (ICV) injection of CGRP produces a hypertensive response by activating the sympathetic nerves in rats (Fisher et al., 1983) and CGRP augments the baroreflex controls of renal sympathetic nerve activity and heart rate (f_H) in the unanesthetized rabbit (Matsumura et al., 1999).

CGRP has an ancient evolutionary history. In fish, cDNAs encoding for CGRP have been isolated from a number of species (Jansz and Zandberg, 1992; Clark et al., 2002; Ogoshi et al., 2006; Martinez-Alvarez et al., 2008) and CGRP mRNA is expressed in peripheral and central tissues (Clark et al., 2002; Martinez-Alvarez et al., 2008). Moreover, the primary sequence of the peptide has been highly conserved from fish to humans (Shahbazi et al., 1998). The presence of CGRP-immunoreactive nerve fibers in the vascular wall of the coeliac arteries and gastrointestinal tract has also been described in rainbow trout, *Oncorhynchus mykiss* (Kagstrom and Holmgren, 1998), and Atlantic cod, *Gadus morhua* (Shahbazi et al., 1998; Shahbazi et al., 2009), together with the presence of immunologically and biologically active CGRP in the heart of the eel *Anguilla anguilla* (Lafont et al., 2004). *In vitro*, CGRP induces vasodilation in precontracted vessels of trout (Kagström and Holmgren, 1998) and cod (Shahbazi et al., 2009) and is involved in regulation of gut motility in cod (Shahbazi et al., 1998). As in many cerebral regions, the hypothalamus expresses CGRP mRNA (Martinez-Alvarez et al., 2008) and some CGRP-like immunoreactive fibers represent ascending projections from brainstem areas involved in autonomic

functions (Batten et al., 1989; Batten et al., 1990). Interestingly, in the goldfish *Carassius auratus* and in the pufferfish *Fugu rubripes* (Clark et al., 2002), the strongest expression of calcitonin/CGRP transcripts was observed in the posterior brain at the level of autonomic nuclei and spinal cord. In addition, CGRP receptors are present within the brain and heart of the flounder *Paralichthys olivaceus* (Suzuki and Kurokawa, 2000). Collectively, these neuroanatomical data support a role for CGRP in neuroendocrine function and behaviour, and in autonomic and cardiovascular regulation in fish. However, to date, there is little information regarding the central role that CGRP may play in teleosts and only the anorexigenic action of centrally administered CGRP in the goldfish *C. auratus* has been described (Martinez-Alvarez et al., 2009). We have demonstrated previously that a number of brain neuropeptides are involved in the central control of the cardiovascular and ventilatory systems of trout (Lancien et al., 2004; Le Mével et al., 2007; Le Mével et al., 2009a; Le Mével et al., 2009b). Consequently, the aim of the present study was to investigate whether CGRP is another component acting centrally to control cardio-respiratory functions. To this end, we analyzed the effects of ICV administration of synthetic replicate of trout/salmon CGRP on ventilation rate (f_V), ventilation amplitude (V_{AMP}), dorsal aortic blood pressure (P_{DA}) and f_H in unanesthetized rainbow trout, *O. mykiss* Walbaum 1792. Additionally, the central actions of the peptide were compared with its effects after intra-arterial (IA) injections.

MATERIALS AND METHODS

Peptides and chemicals

Trout/salmon CGRP (ACNTATCVTHRLADFLNRSGGMGN-SNFVPTNVGAKAF.NH2) (Jansz and Zandberg, 1992) was synthesized by GL Biochem (Shanghai, China) and purified to near homogeneity (>98% purity) by reverse-phase HPLC. The identity of the peptide was confirmed by electrospray mass spectrometry. The peptide was stored in stock solution (0.01% HCl) at –25°C. For injections, CGRP was 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 (mean ± s.e.m. body mass=259±3.3 g, N=46) 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) 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, 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) (Soivio et al., 1972). 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 allows 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 (Le Mével et al., 2009a). 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 tap water and, following recovery of opercular movements, were transferred to a 6 l blackened flow-through chamber supplied with re-circulating dechlorinated and aerated tap water (10–11°C). Oxygen partial pressure within the water tank (P_{wO_2}) and pH were continuously recorded and maintained at constant levels (P_{wO_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 and IA 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 hematocrit. Animals that did not appear healthy, according to the range of values detailed in our previous studies, were discarded. After stable f_V, V_{AMP}, P_{DA} and f_H were maintained for at least 90 min, parameters were recorded for 30 min without any manipulation, and without ICV or IA injection in control experiments.

ICV administration of CGRP

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 first an ICV injection of vehicle (0.5 μl) and 30 min later, an ICV injection of CGRP (1, 5 and 50 pmol in 0.5 μl). Previous control experiments using two ICV injections 30 min apart have shown the absence of time-dependent changes in the measured variables using this protocol (Le Mével et al., 2009a). The animals received no more than two ICV injections of peptide per day with a delay of at least 5 h between the injections. 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.

IA administration of CGRP

Five minutes after the beginning of the recording session, 50 μl of vehicle, or trout CGRP at doses of 5, 20 and 50 pmol, was injected through the dorsal aorta and immediately flushed by 150 μl of vehicle.

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,

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 1000 Hz and visualized on the screen of a PC using the 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 disc. The time series related to the ventilatory, pulsatile P_{DA} and 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 of any movement artifacts on the ventilatory signal were selected and f_V (breaths min^{-1}) and V_{AMP} [arbitrary units (a.u.)] were determined. 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 ventilatory cycle. The net effect of the changes in f_V and V_{AMP} on ventilation was estimated according to the formula $V_{TOT}=f_V V_{AMP}$, where 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 mean 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. A high-resolution time course curve (sampling time: 1 s) for mean P_{DA} was also constructed and averaged among trout receiving an IA injection of 50 pmol CGRP.

Statistical analysis

Data are expressed as means ± s.e.m. or +s.e.m. for each 5-min period on time course histograms or at selected times for the time course curve. Data refer to absolute values, maximal changes from baseline (pre-injection) values or percentage changes. The data were analyzed 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 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 5.0 (GraphPad, La Jolla, CA, USA).

RESULTS

Ventilatory and cardiovascular responses to central CGRP

Fig. 1 illustrates recordings for 30 s in a single trout of the ventilatory, blood pressure and ECG signals taken during the pre-

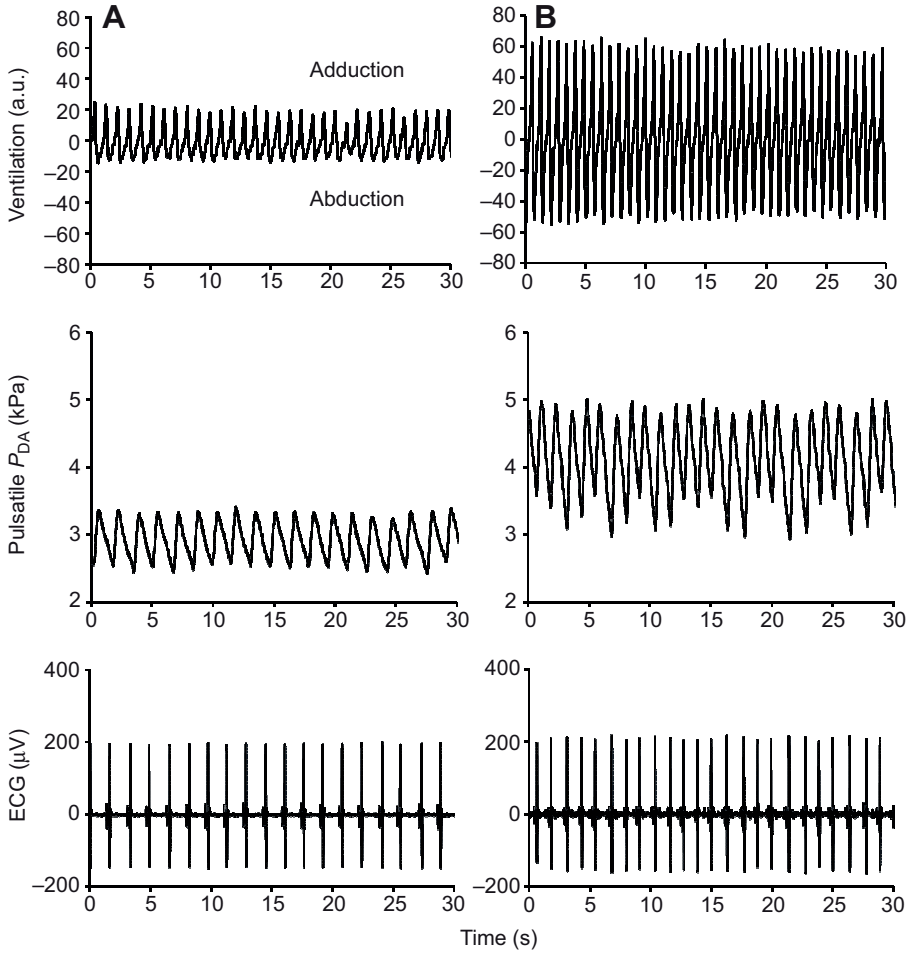

Fig. 1. Recording traces of 30 s duration in the same unanesthetized trout illustrating the changes observed in ventilatory movements (ventilation), pulsatile dorsal aortic blood pressure (P_{DA}) and electrocardiographic (ECG) signals between (A) the pre-injection period (0–5 min) and (B) the post-injection period (15–20 min) after intracerebroventricular (ICV) injection of 50 pmol calcitonin gene-related peptide (CGRP). Note that, compared with the pre-injection period, the ICV injection of CGRP produces an increase in the ventilation rate and amplitude, and an elevation of blood pressure and heart rate.

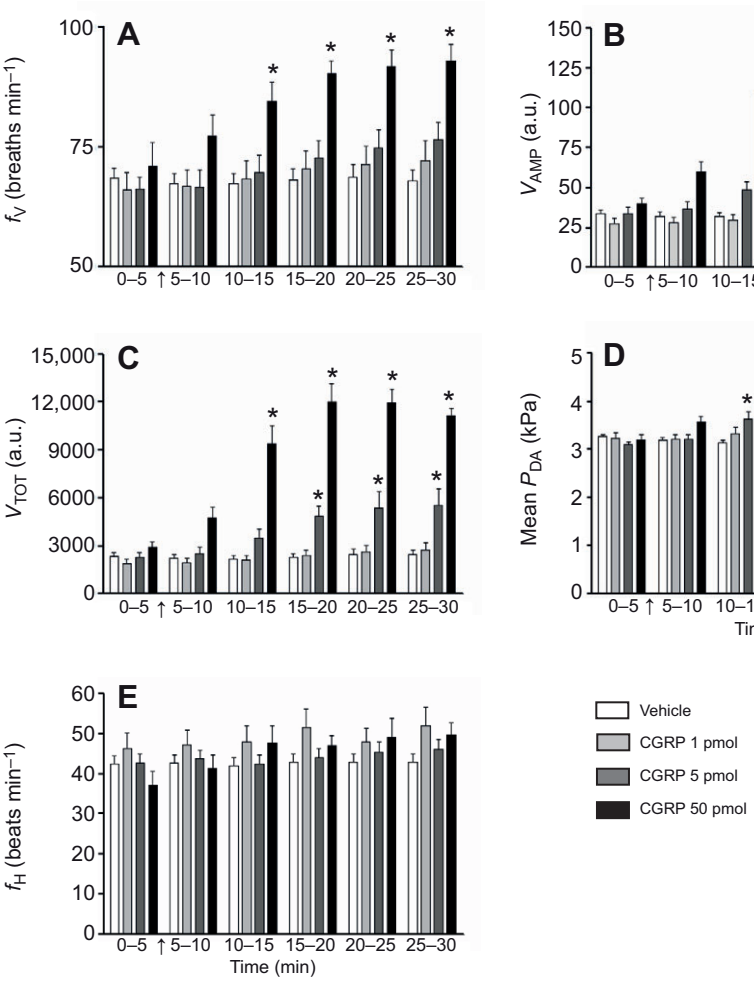

Fig. 2. Histograms showing the time course of the effects of intracerebroventricular injection of (1) 0.5 µl of vehicle (*N*=25), (2) 1 pmol CGRP (*N*=9), (3) 5 pmol CGRP (*N*=11) and (4) 50 pmol CGRP (*N*=7) on (A) ventilation rate (f_V), (B) ventilation amplitude (V_{AMP}), (C) total ventilation (V_{TOT}), (D) mean dorsal aortic blood pressure (P_{DA}) and (E) heart rate (f_H) in unanesthetized trout. Arrows indicate when the injection was given. *N*, number of trout. Asterisks indicate significant differences *vs* vehicle at the corresponding post-injection period and *vs* the pre-injection value (*P<0.05).

injection period (Fig. 1A) and during the 15–20 min post-injection period (Fig. 1B) after ICV injection of 50 pmol CGRP. Comparing the post-injection and the pre-injection signals, CGRP caused a marked elevation in f_V and V_{AMP}. Concurrently, ICV injection of CGRP produced an increase in systolic and diastolic P_{DA} and, consequently, CGRP caused an increase in mean P_{DA}. CGRP also caused tachycardia.

Fig. 2 summarizes the time course of effects observed in the ventilatory and cardiovascular variables following ICV injections of vehicle or a range of doses (1–50 pmol) of CGRP. ICV injection of vehicle produced no significant change in the ventilatory and cardiovascular variables compared with pre-injection values. Compared with ICV injection of vehicle, CGRP evoked a dose- and time-dependent elevation of f_V (Fig. 2A) and V_{AMP} (Fig. 2B). Consequently, the net effect of the peptide was a hyperventilatory response involving a gradual and significant dose-dependent increase in V_{TOT} (Fig. 2C). The threshold dose for an effect of CGRP on f_V was 50 pmol (Fig. 2A) but only 5 pmol for V_{AMP} (Fig. 2B). The actions of CGRP on these ventilatory variables were long-lasting because values had not returned to baseline levels by the end of the post-injection period of 25 min. The most pronounced action of CGRP was evoking hyperventilation through an increase in V_{AMP} instead of f_V. For instance, at a dose of 50 pmol, during the 15–20 min post-injection period when V_{TOT} was maximal and increased by 300% from the baseline value (11,997±1157 *vs* 2907±362 a.u., P<0.05; Fig. 2C), the change in V_{AMP} expressed as a percentage from pre-injection value was more than 200% (133±12.5 *vs* 40.4±3.0 a.u., P<0.05) whereas the elevation of f_V was only approximately 30% (90.2±2.5 *vs* 71.1±4.9 breaths min^{-1}, P<0.05; Fig. 2A,B). After ICV injection, CGRP produced a significant dose-dependent and sustained increase in P_{DA} (maximum increase; 5 pmol: +30%; 50 pmol: +40%; Fig. 2D). Although there was a clear trend for central CGRP to increase f_H (Fig. 1, Fig. 2E), two-way ANOVA applied to all the data shown in Fig. 2E did not indicate a significant action of CGRP on f_H.

Ventilatory and cardiovascular responses to peripheral CGRP

In contrast to its ICV effects, IA injections of CGRP at doses of 5–50 pmol produced no change in f_V, V_{AMP} or V_{TOT} (Fig. 3A–C). Nonetheless, peripherally injected CGRP caused an overall robust dose-dependent and sustained hypertensive response (maximum increase; 50 pmol: +50%; Fig. 3D) without any change in f_H (Fig. 3E). However, precise data analysis indicated that IA injection of the highest dose of CGRP first caused a rapid but transient decrease in P_{DA}, reaching its nadir approximately 90 s after injection (Fig. 4) before returning to pre-injection level 120 s later. Thereafter, the hypertensive phase developed and P_{DA} did not return to its pre-injection level until 60 min after IA injection (not shown).

DISCUSSION

A major finding of this study is that ICV injection of picomolar doses of trout CGRP in trout produces a robust hyperventilation through a stimulatory action of V_{AMP}. To the best of our knowledge,

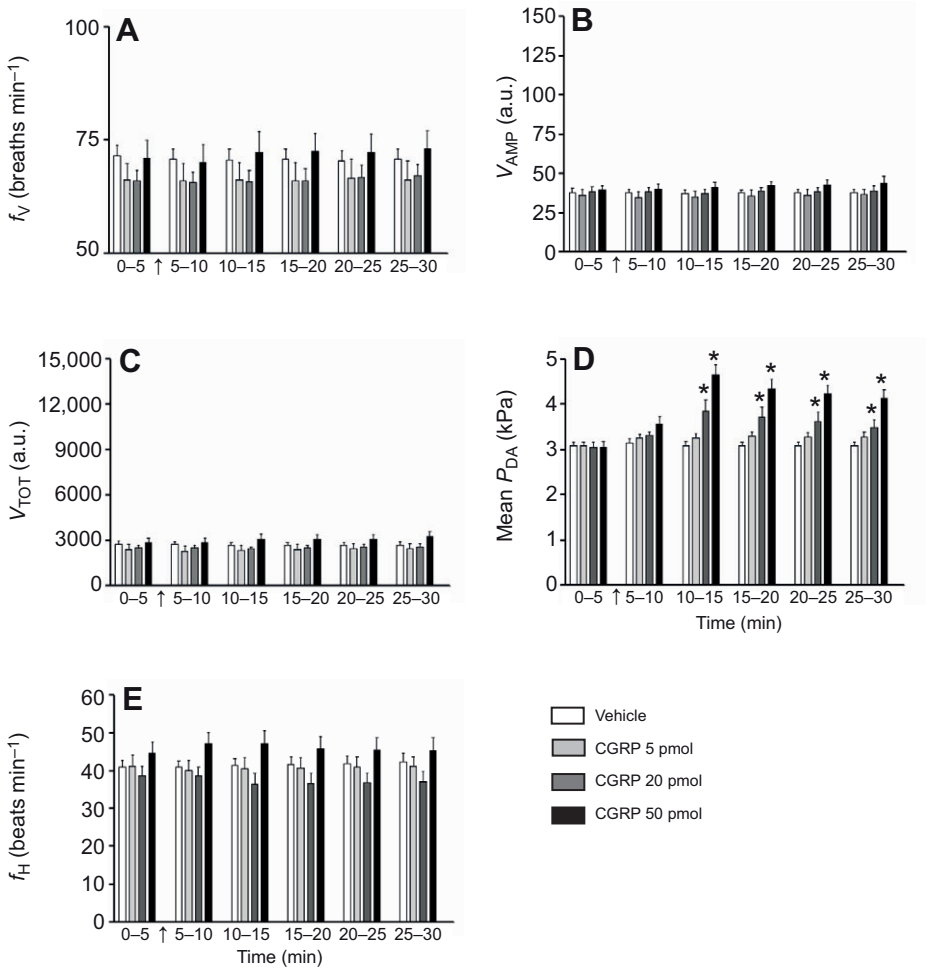

Fig. 3. Histograms showing the time course of the effects of intra-arterial injection of (1) 50 µl of vehicle (N=24), (2) 5 pmol CGRP (N=10), (3) 20 pmol CGRP (N=8), and (4) 50 pmol CGRP (N=12) on (A) ventilation rate (f_V), (B) ventilation amplitude (V_{AMP}), (C) total ventilation (V_{TOT}), (D) mean dorsal aortic blood pressure (P_{DA}) and (E) heart rate (f_H) in unanesthetized trout. The arrow indicates when the injection was given. N, number of trout. Asterisks indicate significant differences *vs* vehicle at the corresponding post-injection period and *vs* the pre-injection value (*P<0.05).

this is the first demonstration in any vertebrate species that exogenously administered native CGRP within the brain causes a potent hyperventilation. In addition, CGRP potently and dose-dependently elevates P_{DA} without significantly affecting f_H. The demonstration that ICV injections of CGRP, but not peripheral administration of equimolar doses of the peptide, cause hyperventilation suggests that the CNS is probably the only site where CGRP can access receptors critical for its hyperventilatory action. Because both central and systemic injections of CGRP provoke a hypertensive response without significant change in f_H, our findings suggest that exogenous CGRP can act through neurogenic mechanisms and also through direct or indirect peripheral mechanisms to control blood pressure and f_H.

Ventilatory and cardiovascular actions of centrally administered CGRP

Our results demonstrating that third ventricle injection of CGRP in trout causes hypertension without change in f_H may be compared with previous studies conducted with CGRP and the AMs in other vertebrate species. In teleost fish, five AM peptides have been identified (Ogoshi et al., 2003; Ogoshi et al., 2006). In the eel, the central actions of native AM1, AM2 and AM5 on cardiovascular functions are complex, depending on both the nature of the AM used and the route of administration. Third ventricle administration of AM2, but not AM1, increases P_{DA} and decreases f_H, but the injection of AM5 depresses blood pressure and elevates f_H. In contrast, the three AMs decrease blood pressure when injected within the fourth ventricle (Ogoshi et al., 2008), with only AM1 elevating f_H. Hypertensive actions of AM were also observed in unanesthetized rats as the ICV injection of AM results in elevated blood pressure and f_H (Samson et al., 1998; Saita et al., 1998). In unanesthetized, freely moving rats, ICV injection of CGRP (2 nmol) elicits an increase in blood pressure and f_H that is probably due to noradrenaline release after sympathetic stimulation (Fisher et al., 1983). However, in contrast to the hypertensive action of centrally administered CGRP, intravenous injection of the peptide in rats rapidly decreases blood pressure. This hypotension is accompanied by tachycardia (Fisher et al., 1983). In unanesthetized rabbits, ICV infusion of human CGRP (1 nmol h^{-1}) does not produce any effects on blood pressure or f_H but CGRP promotes the baroreflex control of f_H and renal sympathetic nerve activity (Matsumura et al., 1999). It should be pointed out that the mammalian ICV studies have used high doses of CGRP to determine the cardiovascular action of the peptide. Although the concentration of CGRP producing effects under physiological conditions is unknown, our study in the trout model used low picomolar doses of exogenous peptide that might be more relevant to possible physiological action of the endogenous peptide.

To produce hyperventilation and cardiovascular actions, ICV injections of CGRP must access receptors critical for the control of cardio-ventilatory motor neurons. However, the receptor site(s) initiating cellular transduction mechanisms and the multisynaptic chain of events that ultimately translate into alteration in neuronal

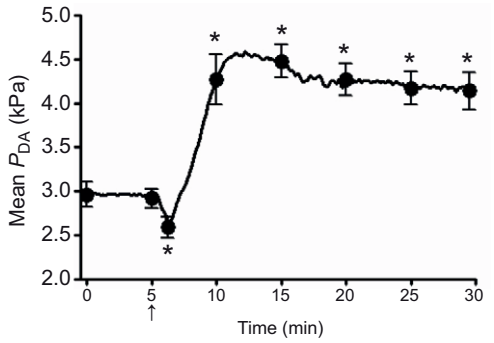

Fig. 4. Time course curve for the effects of intra-arterial injection of 50 pmol CGRP (N=12) on mean dorsal aortic blood pressure (P_{DA}) in unanesthetized trout. The arrow indicates the time of intra-arterial injection. The curve represents mean (±s.e.m. at selected times) for 12 trout. Asterisks indicate significant differences *vs* the 0 and 5 min time points (Dunnett's test, *P<0.05). The injection artifacts have been removed during signal processing.

control of motor ventilatory and cardiovascular neurons were not determined in the present study. CGRP and its receptor(s) are present within the brains of teleost fish, notably at the level of the hypothalamus and posterior brain (Batten et al., 1989; Batten et al., 1990; Fouchereau-Peron et al., 1990; Suzuki and Kurokawa, 2000; Clark et al., 2002; Martinez-Alvarez et al., 2008). CGRP was injected in close proximity to a major neuroendocrine hypothalamic nucleus, the preoptic nucleus, and so may mimic the action of the endogenous peptide in triggering, for example, the activity of peptidergic or non-peptidergic preoptic neurons that project their axons towards brainstem respiratory and cardiovascular nuclei (Batten et al., 1989; Batten et al., 1990; Saito et al., 2004). Alternatively, the site of action of centrally administered CGRP may be caudal to the third ventricle because the ventilatory and cardiovascular actions of this peptide are delayed in onset by approximately 5–10 min after ICV injection. The exogenously injected peptide may thus diffuse within the cerebrospinal fluid towards critical cardiovascular and respiratory brainstem nuclei.

Ventilatory and cardiovascular actions of peripherally administered CGRP

The vasodilator action of CGRP in mammals including humans is well documented (for reviews, see Brain and Grant, 2004; Smillie and Brain, 2011). CGRP-induced vasodilation is mediated in part through a nitric oxide (NO)-dependent mechanism or by production of prostaglandins. *In vivo*, IA or intravenous administration of CGRP is associated with hypotension and inotropic and chronotropic actions. This general vasodilator action of CGRP has been maintained throughout the vertebrate phylum, as chicken CGRP has been shown to relax precontracted rainbow trout, *O. mykiss*, and Atlantic cod, *G. morhua*, coeliac arteries (Kagstrom and Holmgren, 1998; Shahbazi et al., 2009) and various arteries in the bullfrog *Rana catesbiana* (Kline et al., 1988). In a study examining isolated peripheral arteries and veins, Olson and Villa (Olson and Villa, 1991) were unable to find NO-dependent mechanisms in trout vasoregulation. It is probable, therefore, that the vasorelaxant effect of CGRP in trout and in cod is not mediated by the release of NO or even by prostaglandins (Kagström and Holmgren, 1998; Shahbazi et al., 2009). In trout, the receptor involved in CGRP-induced vasorelaxation is a mammalian-like CGRP-1 receptor (Kagström and Holmgren, 1998). *In vivo* studies are crucial to determine the action of peptides in cardiovascular regulation and, to our knowledge, this approach has not yet been performed for CGRP in a non-mammalian vertebrate. In the eel model, the structurally related peptides AM2 and AM5 have potent hypotensive actions when injected peripherally, but no change occurred in f_H (Nobata et al., 2008; Ogoshi et al., 2008). In addition, a high dose of AM1 (5 nmol kg^{-1} body mass) increases P_{DA} after an initial and transient hypotension. We demonstrate in the present study that IA administration of trout CGRP in trout produces a sustained hypertensive phase with no change in f_H. This hypertensive response is quite surprising, taking into account both the *in vitro* vasodilator action of CGRP in trout and the hypotensive action of AMs in the eel. This hypertensive phase might be a compensatory mechanism activated when the blood pressure falls. In fact, precise analysis of the P_{DA} recordings revealed that a high dose of CGRP (50 pmol, ~0.2 nmol kg^{-1} body mass) elicits a rapid, transient and significant hypotensive phase within seconds of IA injection (Fig. 4) followed by a sustained hypertensive period. This rapid and transient hypotension was never observed after ICV injection of an equimolar dose of CGRP. We suggest that this first hypotensive phase is due to a direct vasodilatory action of CGRP on blood vessels whereas the second hypertensive phase is indirectly mediated by vasopressive agents. The renin–angiotensin system and catecholamines act separately or in conjunction to counteract a hypotensive phase (Olson, 1992; Bernier et al., 1999a). Moreover, it has been proposed that the renin–angiotensin system in trout is a catecholamine secretagogue during periods of hypotension (Bernier et al., 1999b). These mechanisms could well be the means by which the CGRP-evoked hypotensive phase is reversed to a hypertensive period. An alternative explanation is that the potent hypertensive phase is unrelated to the hypotensive stimulus and arises from a direct stimulatory action of CGRP on the release of catecholamines and/or angiotensin II. In addition, because P_{DA} is globally increased post-IA injection of CGRP, this would provide a baroreflex stimulus for the f_H to decrease. Instead, f_H remained unchanged or slightly elevated, suggesting that the baroreflex response was impaired or that either CGRP itself or catecholamines act directly on the heart to blunt the cardio-inhibitory baroreflex response. It should be also kept in mind that peripherally administered CGRP could not only have direct actions on the vasculature and heart but could gain access to critical target sites in the brain that lack the blood–brain barrier to modulate cardiovascular regulation including the baroreflex response. We have demonstrated that some neuropeptides are able to modulate the cardiac baroreflex sensitivity in trout (Lancien and Le Mével, 2007; Lancien et al., 2011). In mammals, rather conflicting results have been obtained regarding the change in f_H during CGRP-induced hypotension after systemic injection so that the effect of the peptide on modulation of baroreflex functions is unclear (Okamoto et al., 1992; Fukuhara et al., 1995; Kunz et al., 2007). Further studies are needed to determine the precise mechanisms involved in central and peripheral cardiovascular and ventilatory actions of CGRP in trout.

Possible physiological significance

The present results add CGRP to the list of peptides [pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide (Le Mével et al., 2009b), corticotropin-releasing factor (Le Mével et al., 2009a), neuropeptide gamma (Le Mével et al., 2007) and urotensin II (Lancien et al., 2004)] that act centrally to modulate the neuronal outputs that ultimately affect the ventilatory and cardiovascular effectors in the trout model. The hyperventilatory and hypertensive actions of central CGRP may

be viewed as cardio-ventilatory mechanisms to protect against adverse hypoventilation and hypotension. However, it remains to be determined whether the observed actions of exogenously administered CGRP (and other neuropeptides) can be translated into evidence for endogenous regulation of physiological functions. Similarly, the circumstances leading to the release of endogenous CGRP into the synaptic cleft to control the autonomic nuclei also remain to be delineated.

In conclusion, we demonstrate for the first time in any animal species that exogenously administered picomolar doses of CGRP in the brain exert a potent stimulatory effect on ventilation. In addition, trout CGRP increases blood pressure in unanesthetized trout without changing f_H. These results suggest that the endogenous CGRP within the brain of the trout may be implicated as a neurotransmitter and/or a neuromodulator in the regulation of cardio-ventilatory functions. The systemic administration of CGRP evokes a hypertensive response that may be due to either an indirect mechanism to counterbalance the rapid and transient vasodilatory and hypotensive direct action of the peptide or a direct hypertensive mechanism unrelated to the hypotensive stimulus, suggesting that endogenous CGRP may act as a local and/or circulating hormone involved in vasoregulatory mechanisms.

LIST OF SYMBOLS AND ABBREVIATIONS

AM	adrenomedullin
CGRP	calcitonin gene-related peptide
CNS	central nervous system
ECG	electrocardiographic
f_H	heart rate
f_V	ventilation rate
IA	intra-arterial
ICV	intracerebroventricular
P_{DA}	dorsal aortic blood pressure
P_{wO2}	partial oxygen pressure in water
V_{AMP}	ventilation amplitude
V_{TOT}	total ventilation

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