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Central pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) decrease the baroreflex sensitivity in trout

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ABSTRACT

Although PACAP and VIP exert diverse actions on heart and blood vessels along the vertebrate phylum, no information is currently available concerning the potential role of these peptides on the regulation of the baroreflex response, a major mechanism for blood pressure homeostasis. Consequently, the goal of this study was to examine in our experimental model, the unanesthetized rainbow trout *Oncorhynchus mykiss*, whether PACAP and VIP are involved in the regulation of the cardiac baroreflex sensitivity (BRS). Cross-spectral analysis techniques using a fast Fourier transform algorithm were employed to calculate the coherence, phase and gain of the transfer function between spontaneous fluctuations of systolic arterial blood pressure and R–R intervals of the electrocardiogram. The BRS was estimated as the mean of the gain of the transfer function when the coherence between the two signals was high and the phase negative. Compared with vehicle, intracerebroventricular (ICV) injections of trout PACAP-27 and trout VIP (25–100 pmol) dose-dependently reduced the cardiac BRS to the same extent with a threshold dose of 50 pmol for a significant effect. When injected intra-arterially at the same doses as for ICV injections, only the highest dose of VIP (100 pmol) significantly attenuated the BRS. These results suggest that the endogenous peptides PACAP and VIP might be implicated in the central control of cardiac baroreflex functions in trout.

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) was originally isolated from ovine hypothalamus on the basis of its ability to stimulate adenylate cyclase activity in adenohypophysial cells [28]. PACAP is found in two biologically active forms, a 38 amino-acid peptide (PACAP-38) and a C-terminally truncated 27 amino-acid peptide (PACAP-27). In humans, the N-terminal portion of PACAP shows 68% sequence identity with vasoactive intestinal peptide (VIP), identifying PACAP as a member of the VIP/secretin/glucagon/GH-releasing hormone superfamily of peptides [39]. PACAP and VIP exert their biological activities through three G-protein coupled receptors termed PAC1, VPAC1 and VPAC2 [20]. Consistent with the wide distribution of PACAP and VIP, and also their receptors, throughout the central nervous system and periphery, PACAP and VIP exert multiple actions [44]. On the peripheral cardiovascular system, PACAP and VIP are considered to have potent and direct vasodilatory properties on a variety of arterial blood vessels and also to exert stimulatory effects on the heart [14,44]. Nonetheless, the role of these peptides in central cardiovascular regulation is not so clearly delineated [14]. In particular, nothing is known about the possible action of central PACAP and VIP on baroreflex, a key mechanism for blood pressure homeostasis.

The baroreflex has been evolutionary conserved from Agnatha (lamprey) to humans [40,19]. The baroreflex in fish, as in humans, is working spontaneously under baseline conditions [22] and responds to adverse blood pressure changes [40,38]. This baroreflex response is probably used as a mechanism to protect the delicate vasculature of the fish gills against high blood pressure [3,40]. Additionally, the biologically active region of PACAP, (the N-terminal 27 amino acids), the sequence of VIP, and the primary structure of PACAP/VIP receptors have been remarkably well conserved from fish to humans [45,39,32]. In teleost fish, the PACAP/VIP system is widely distributed in peripheral tissues [35,18,10] and in the

Abbreviations: BRS, baroreflex sensitivity; ECG, electrocardiogram; HF, high frequency; HRV, heart rate variability; IA, intra-arterial; ICV, intracerebroventricular; LF, low frequency; NPO, preoptic nucleus; PACAP, pituitary adenylate cyclase-activating polypeptide; PAC1, PACAP receptor; P_{DA}, dorsal aortic blood pressure; PSD, power spectral density; Pw_{O2}, partial oxygen pressure in water; SBP, systolic blood pressure; VIP, vasoactive intestinal peptide; VPAC1, VIP/PACAP receptor subtype 1; VPAC2, VIP/PACAP receptor subtype 2.

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central nervous system [29]. We recently described the cardiorespiratory actions of these peptides after peripheral and central administration in trout [23]. After intracerebroventricular (ICV) injection, PACAP and to lesser extend VIP provoked an hyperventilatory effect but only PACAP produced an increase in mean dorsal aortic blood pressure (P_{DA}) without changing heart rate. Intra-arterial (IA) injections of either PACAP or VIP were without effect on ventilation and only VIP significantly elevated P_{DA} without changing heart rate. The lack of heart rate response to elevation of blood pressure suggests that the cardiac baroreflex sensitivity (BRS) may be depressed following central PACAP and IA VIP. Therefore, the aim of this study was to examine in our experimental model, the unanesthetized rainbow trout *Oncorhynchus mykiss,* whether PACAP and VIP are involved in the regulation of the BRS. For this purpose, trout PACAP and VIP were injected within the third ventricle of the brain and intra-arterially and the modern transfer function analysis technique was used to study the cardiac BRS.

2. Materials and methods

2.1. Peptides and chemicals

Trout PACAP-27 (HSDGIFTDSYSRYRKQMAVKKYLAAVL.NH2) and trout VIP (HSDAIFTDNYSRFRKQMAVKKYLNSVLT.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 mM): 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.

2.2. Animals and surgical procedures

Some of the results reported in the present paper refer to recordings made during our own previous study on the action of PACAP and VIP in trout [23]. Recordings were excluded from the analysis if they contained excessive artefacts on electrocardiogram (ECG) signal or on pulsatile blood pressure. Additional new experiments were also carried out on rainbow trout *O. mykiss* using experimental procedures that have been described in detail in previous work [23]. Briefly, rainbow trout (body wt. 240–270 g) were equipped with two electrocardiographic electrodes, a dorsal cannula, and an ICV microguide with a buccal catheter that was used to record the buccal ventilatory pressure (not quantified in the present study). After surgery, the animals were transferred to a 6 l blackened chamber supplied with dechlorinated and aerated tap water (10–11 °C) that was both recirculating and through-flowing. Oxygen pressure within the water tank (Pw_{O2}) and pH were continuously recorded and maintained at constant levels (Pw_{O2} = 20 kPa; pH = 7.4–7.6). The trout were allowed to recover from surgery and to become accustomed to their new environment for 48–72 h. Experimental protocols were approved by the Regional Ethics Committee in Animal Experiments of Brittany, France.

2.3. Intracerebroventricular and intra-arterial administrations of PACAP and VIP

For all protocols, the recording session lasted 30 min and all injections were made at the fifth minute of the test. For the ICV protocol, the fish received an ICV injection of vehicle (0.5 μl) or an ICV injection of trout PACAP or VIP (25, 50 and 100 pmol in 0.5 μl). For the IA protocol, 50 μl of vehicle, trout PACAP or VIP at doses of 25, 50 and 100 pmol was injected through the dorsal aorta cannula and immediately flushed by 150 μl of vehicle. Peptides were administered in random order.

2.4. Data acquisition and transfer function analysis of the cardiovascular variables

The ECG and P_{DA} signals were recorded using standardized electronic devices [23]. The output signals were digitalized at 1000 Hz, visualized on the screen of a PC during the 30 min recording period and finally stored using PowerLab 4/30 data acquisition system (ADInstruments, Oxford, England) and LabChart Pro software (v.6.0; ADInstruments, Oxford, England). ECG and P_{DA} signals were processed off-line with custom-made programs written in LabView 6.1 (Laboratory Virtual Instrument Engineering Workbench, National Instruments, Austin, USA). For all protocols, 10 min segments of ECG and P_{DA} signals were selected 15 min after ICV or IA injections of vehicle, PACAP or VIP. R–R intervals were determined after detection of the R waves from the ECG recordings and systolic blood pressure (SBP) was identified from the pulsatile P_{DA}. Their mean values were calculated. R–R interval and SBP time series were resampled at 2.56 Hz to obtain equidistant data points. The linear trend was removed from this new time series and 11 segments of 256 data points (100 s) overlapping by half were subjected to a Hanning window. Spectral and cross spectral techniques developed in the present study were adapted from methods described previously in human [9,34], rat [16] and lizard [12]. The power spectral density (PSD) of each segment was calculated using standard fast Fourier transform and the PSD spectrum obtained were averaged. In order to investigate to what extent the input signal (the SBP) influences the output signal (the R–R interval) the coherence, phase and transfer function spectra of SBP against R–R interval were determined. The coherence spectrum, which has values between 0 and 1, is a measure of the correlation between the variations of the two signals. The transfer function provides a measure of the degree to which input signal content, at a given frequency, appears in output energy. A high coherence (>0.5) between the two signals and a negative phase shift indicates that the SBP mediates the changes in R–R intervals. Consequently, the cardiac BRS was estimated as the mean of the gain of the transfer function when the coherence was high and the phase negative.

All calculations for R–R interval (in msec), SBP (in kPa), PSD (in kPa^2/Hz), coherence, phase function (in sec) and transfer gain (in kPa/msec) were made for the post-injection period of 20–30 min and the results were averaged for trout subjected to the same protocol.

2.5. Statistical analysis

Data are expressed as means ± SEM or +SEM. In the figures and table, data refer to absolute values. For comparison between groups, Kruskal–Wallis non-parametric one-way analysis of variance followed by Dunn's multiple comparison test was used. A value of $P < 0.05$ was considered significant. The statistical tests were performed and the graphs constructed using GraphPad Prism 5.0 (GraphPad, San Diego, CA).

3. Results

3.1. Baroreflex response to central PACAP

Fig. 1 shows a representative example of 5 min R–R interval time-series, and SBP time series recorded during the 20–30 min period in a trout receiving firstly an ICV injection of vehicle