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The peptide hormone cholecystokinin modulates the tonus and compliance of the bulbus arteriosus and pre-branchial vessels of the rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The bulbus arteriosus is a compliant structure between the ventricle and ventral aorta of teleost fish. It serves as a "wind-kessel" that dampens pressure variations during the cardiac cycle allowing a continuous flow of blood into the gills. The bulbus arteriosus receives sympathetic innervation and is affected by several circulating substances, indicating neurohumoral control. We have previously shown that the peptide hormone, cholecystokinin (CCK), affects the hemodynamics of the cardiovascular system in rainbow trout (*Oncorhynchus mykiss*) by increasing flow pulse amplitude without affecting cardiac output. We hypothesized that this could be explained by an altered tonus or compliance/distensibility of the bulbus arteriosus. Our results show that there is a substantial effect of CCK on the bulbus arteriosus. Concentrations of CCK that altered the cardiac function of in situ perfused hearts also contracted the bulbus arteriosus in vitro. Pressure–volume curves revealed a change in both the tonus and the compliance/distensibility of this structure. Furthermore, the stimulatory (constricting) effect of CCK was also evident in the ventricle and vasculature leading to the gills, but absent in the atrium, efferent branchial arteries and dorsal aorta. In conclusion, CCK alters the mechanical properties of the ventricle, bulbus arteriosus, ventral aorta and afferent gill vasculature, thus maintaining adequate branchial and systemic blood flow and pressure when cardiorespiratory demands change, such as after feeding.

1. Introduction

The bulbus arteriosus is a chamber of the teleost heart located after the ventricle that functions as a wind-kessel (Farrell, 1979; Icardo, 2006). During systole, blood is pumped from the ventricle into the bulbus arteriosus, which then expands to accommodate a proportion of the full stroke volume. During diastole, the elastic properties of the expanded bulbus arteriosus eject the stored blood into the ventral aorta resulting in a steady, although pulsatile, blood flow during the cardiac cycle (Licht and Harris, 1973). Therefore, the bulbus arteriosus works much like the mammalian aorta (Belz, 1995), although there are several morphological differences, such as differences in tissue composition (Priede, 1976; Watson and Cobb, 1979). Subsequently, its origin, either cardiac or vascular is controversial (Parsons, 1930; Licht and Harris, 1973; Priede, 1976; Durán et al., 2008). The dampening capacity of the bulbus arteriosus is larger than that of the aorta, which is important given that the gills are serially connected to the systemic circulation. The pressure generated by the heart needs to overcome the combined vascular resistances of the branchial and systemic circulations, while ensuring that the pressure does not damage the delicate branchial microvasculature. The capacity of the bulbus arteriosus to store between 25 and 100% of cardiac output reduces the ventricular afterload (Priede, 1976; Bushnell and Jones, 1994).

The bulbus arteriosus is distinguished by 3 layers composed of both connective and elastic tissues; the adventitia, the media and the intima (Licht and Harris, 1973; Priede, 1976; Watson and Cobb, 1979; Leknes, 2009; Icardo, 2013). The thin outer adventitia is composed of well-vascularized connective tissue and innervated by sympathetic nerves (Watson and Cobb, 1979). The media consists of circumferentially and spirally arranged smooth muscle cells covered with elastic tissue (Priede, 1976). The innermost layer, the intima, which is extensively innervated, is composed of a layer of endothelial cells. The spirally arranged smooth muscle within the media may also enter this layer. The outer portion of the intima is primarily composed of connective tissue (Watson and Cobb, 1979; Farrell and Jones, 1992).

Cholecystokinin (CCK) is a gastrointestinal peptide hormone involved in the regulation of fat and protein digestion in mammals (Chandra and Liddle, 2007), as well as in rainbow trout (Jönsson et al., 2006). CCK is released during digestion and has vasoactive properties that regulate oxygen delivery to, and nutrient delivery from, the gastrointestinal tract (Guth and Smith, 1976; Chou et al., 1977; Sanchez-Fernandez et al., 2004; Ruiz-Gayo et al., 2006). In a previous

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study, we showed that physiological concentrations of CCK, injected into the blood stream of rainbow trout, in vivo, caused a significant increase in both gastrointestinal blood flow and cardiac output (Seth et al., 2010). Moreover, there was a profound increase in the amplitude of the phasic flow profile of in situ perfused hearts when exposed to CCK at doses resembling plasma levels observed after feeding (Seth et al., 2010). However, in the in situ perfused heart, there were no changes in either heart rate or stroke volume and subsequently no change in cardiac output. Therefore, we speculate that the profound change in phasic flow profile was due to an altered tonus and/or compliance of the bulbus arteriosus. Since adrenaline contracts the bulbus arteriosus (Farrell, 1979) and an extensive adrenergic innervation has been described, it is likely that this structure, in contrast to the mammalian aorta, is under neurohumoral control (Gannon and Brunstock, 1969; Watson and Cobb, 1979; Farrell and Jones, 1992). Furthermore, the bulbus arteriosus of the European eel (*Anguilla anguilla*) constricts when exposed to endothelin, whereas nitric oxide (NO), sodium nitroprusside (SNP), natriuretic peptides (NP), and prostaglandin E1 produced a significant dilation (Evans et al., 2003). This suggests that the function of the teleost bulbus arteriosus can be modulated by a variety of vasoactive substances.

Our aim was to examine the role of CCK in regulating the tonus and the compliance/distensibility of the bulbus arteriosus. Also, due to the vasoactive properties of CCK on distal arterioles, we tested additional structures proximal to the bulbus, including the atrium, ventricle, ventral aorta, dorsal aorta, as well as the afferent and efferent branchial arteries.

2. Materials and methods

2.1. Experimental animals

Rainbow trout (*Oncorhynchus mykiss*) ranging between 448 and 1120 g (mean 659 ± 76 g, N = 72), were kept in 2 m^3 tanks with well aerated freshwater (~10 °C). Fish were held under a 12:12 photoperiod and fed commercially available trout pellets. All experiments were done at the Department of Biological and Environmental Science at the University of Gothenburg and covered by permit 13/2007 from the Animal Ethics Committee.

2.2. Tissue preparation

Fish were killed by a sharp blow to the head and weighed. We made an incision on the ventral side of the fish, and then quickly removed the heart and surrounding vasculature. The heart was immediately weighed and placed in an ice-cold Ringer solution (NaCl, 140.0 mM; $NaHCO_3$, 15 mM; KCl, 2.5 mM; $CaCl_2 \times 2H_2O$, 1.5 mM; KH_2PO_4, 1.0 mM; $MgSO_4 \times 7H_2O$, 0.8 mM; HEPES, 5.0 mM: pH 7.8–7.9; 302–305 osm). Circular sections were then made from the bulbus arteriosus, ventricle, ventral aorta, as well as the efferent and afferent branchial arteries. Proximal and distal sections were taken from both the bulbus and ventral aorta. Due to its position and connection to the surrounding tissue, the dorsal aorta was prepared as elongated 2 mm strips. The atrium was excised and used as whole. The preparations were mounted on custom made stainless steel hooks and connected to force transducers (SENSELab 10-700-0003; Somedic Sales AB, Hörby, Sweden) for the measurement of isometric force. The preparations were submersed in a well aerated (0.3% CO_2 in air) Ringer solution at a temperature of 10 °C. Each preparation was initially stretched to 15 mN and left to stabilize for 2 h as previously described (Klaverkamp and Dyer, 1974; Shahbazi et al., 2001; Seth et al., 2010). The atrial preparations were stretched to a lower initial tension (10 mN). In another set of experiments, we removed the heart as described above and placed a custom made cannula into the bulbus arteriosus through the ventricle, while tying off the bulbar outflow as previously described (Forster and Farrell, 1994). The preparation was then submerged in a well aerated (0.3% CO_2 in air) Ringer solution at a temperature of 10 °C for at least 30 min prior to the experiment.

2.3. Experimental protocol

Two preparations per animal were made from each segment. After the 2-h normalization period, CCK was added in cumulative increments of 10, yielding a series of concentrations from 0.1 to 100 nM, similar to what was used by Seth et al. (2010). The preparations were allowed for 30 min to stabilize before each increase in concentration. Every experiment was terminated by the addition of 50 μl of saturated KCl Ringer solution (>50 mM) to elicit a maximal contraction. For the pressure–volume curves, the bulbus arteriosus was filled with Ringer solution at increments of 0.02 ml kg^{-1} until a final volume of 0.5 ml kg^{-1} or an intrabulbar pressure of approximately 10 kPa was reached. This procedure was also repeated in a second trial with the preparation submerged in a Ringer solution containing 100 nM CCK. We repeated the experimental protocol in an additional set of bulbus arteriosus preparations without the addition of CCK to control for handling effects.

2.4. Drugs

The sulphated form of CCK-8 was obtained from Ansynth Service BY (J. Vermeerin, 202 4703 Le Roosendaal, The Netherlands). Although CCK exists in several sub-forms, the sub-forms present in rainbow trout are CCK-7, -8 and -21. The mammalian CCK-8S used in this study differ from the trout forms by having methionine in the sixth position from the fully amidated C-terminal, compared to leucine (CCK-l), asparagine (CCK-n) and threonine (CCK-t) in the trout forms (Jensen et al., 2001). However, it shares the conserved, biologically active region with all the trout forms as reviewed by Johnsen (1998). Potassium chloride (KCl) was also obtained from Sigma–Aldrich. All drugs were diluted in cold Ringer solution.

2.5. Data analysis and statistics

Analog signals from the force and pressure transducers (force: SENSELab 10-700-0003; Somedic Sales AB, Hörby, Sweden; pressure: model DPT-6100, pvb Medizintechnik, Kirchseeon, Germany) were amplified by a 4ChAmp amplifier (Somedic AB, Hörby, Sweden) and digitalized via a PowerLab unit (ADInstruments Pty Ltd, Castle Hill, Australia) connected to a laptop running LabChart Pro (version 7.1; ADInstruments Pty Ltd, Castle Hill, Australia). The isometric force of the preparations was analyzed for treatment effects using a repeated measure ANOVA followed by Tukey's post-hoc tests. For the pressure–volume curves, a linear mixed model was used to compare between trials. Individuals were set as subjects and the ten volumes within the plateau phase of the curve (defined as the section with the lowest slope) were set as repeated measures. As proximate measurements are better correlated than distant measurements, an autoregressive structure was chosen as a type of covariance structure. Pressure was set as a dependent variable and the trials together with volume as factors. Compliance (ml kg^{-1} kPa^{-1} i.e., the change in volume (ΔV) for a given change in pressure (ΔP)) and distensibility (% kPa^{-1} i.e., the compliance normalized to the original volume) was calculated as the slope of the pressure–volume curve within physiological pressures (between 2 and 5 kPa). Paired Student's t-tests were used to test for a significant treatment effect on compliance and distensibility. All statistical comparisons were made on raw untransformed data and to correct for multiple comparisons the Holm–Bonferroni algorithm was used. All reported values are means ± S.E.M. * denotes a significant difference from baseline values ($p < 0.05$).