Cholecystokinin as a regulator of cardiac function and postprandial gastrointestinal blood flow in rainbow trout (Oncorhynchus mykiss)

Henrik Seth, Albin Gräns, and Michael Axelsson

Department of Zoology, University of Gothenburg, Gothenburg, Sweden

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Seth H, Gräns A, Axelsson M. Cholecystokinin as a regulator of cardiac function and postprandial gastrointestinal blood flow in rainbow trout (Oncorhynchus mykiss). Am J Physiol Regul Integr Comp Physiol 298: R1240–R1248, 2010. First published February 17, 2010; doi:10.1152/ajpregu.00781.2009.—We have studied the potential role of CCK as a regulator/modulator of the postprandial increase in gastrointestinal blood flow. Rainbow trout (Oncorhynchus mykiss) were instrumented with pulsed Doppler flow probes to measure the effects of CCK on cardiac output and gastrointestinal blood flow. Furthermore, vascular preparations were used to study the direct effects of CCK on the vessels. In addition, we used in situ perfused hearts to further study the effects of CCK on the cardiovascular system. When the sulfated form of CCK-8 was injected at a physiological concentration (0.19 pmol/kg) in vivo, there was a significant increase in the gastrointestinal blood flow (18 ± 4%). This increase in gastrointestinal blood flow was followed by a subsequent increase in cardiac output (30 ± 6%). When the dose was increased to 0.76 pmol/kg, there was only a 14 ± 6% increase in gastrointestinal blood flow; possibly due to a dose-dependent increase in the gill vascular resistance as previously reported or a direct effect on the heart. Nevertheless, CCK did not affect the isolated vessel preparations, and thus, it seems unlikely that CCK has a direct effect on the blood vessels of the second or third order. CCK did, however, have profound effects on the dynamics of the heart, and without a change in cardiac output, there was a significant increase in the amplitude (59 ± 4%) and rate (dQ/dt: 55 ± 4%; -dQ/dt: 208 ± 49%) of the phasic flow profile. If and how this might be coupled to a postprandial gastrointestinal hyperemia remains to be determined. We conclude that CCK has the potential as a regulator of the postprandial gastrointestinal blood flow in fish and most likely has its effect by inducing a gastrointestinal hyperemia. The mechanism by which CCK acts is at present unknown.

hormonal regulation; CCK-8s; circulating plasma levels; hyperemia; teleost

WHEN FOOD IS INGESTED, SEVERAL cardiovascular changes occur to enable the animal to efficiently digest, absorb, and redistribute the nutrients. The nature of these rearrangements, as well as the exact regulation governing these cardiovascular changes has only received limited attention in fish.

We have previously shown, in two separated teleost species, the rainbow trout (Oncorhynchus mykiss) (45, 46) and the short-horn sculpin (Myxocephalus scorpius) (44), that the postprandial cardiovascular changes are influenced by mechanical and chemical stimuli, as well as the nutrient composition in a manner that is similar to what has been reported previously in mammals (reviewed by Refs 34 and 36). As a meal enters the stomach, there is a α-adrenergic increase in the systemic vascular resistance. Subsequently, as food enters and is hydrolyzed in the proximal intestine, the free nutrient components induce a local decrease in the gastrointestinal vascular resistance, and blood is thus shunted from the systemic to the gastrointestinal circulation. The increase in gastrointestinal blood flow (Qcm), resulting from this hyperemia, is in most fish species studied, enhanced by an increase in cardiac output (CO) (5, 6).

The fact that the postprandial hyperemia is influenced by the nutrient composition of the diet is also in concordance with what has been seen in mammals (16, 24). The reason for this difference and the exact regulation governing the nutrient-induced hyperemia has not been determined. Several mechanisms have been proposed in mammals, such as direct effects of the absorbed nutrients, metabolic and nonmetabolic vasoactive factors, as well as endocrine and neural involvement. These mechanisms have been extensively reviewed (25, 36), and the local decrease in tissue oxygen content, or alternatively an increase in osmolarity, that occurs as a consequence of nutrient uptake and assimilation, is most likely the most important initial trigger (8). Also, other factors could contribute and modulate the response, since an increase in gastrointestinal blood flow is not necessarily linked to an increase in oxygen consumption (specific dynamic action) and vice versa. It is now believed that most of the specific dynamic action depend on factors outside the gastrointestinal tract (3), such as protein assimilation (10, 11).

In mammals, several hormonal components have also been proposed to be involved in this regulation. These include peptides like CCK and vasoactive intestinal polypeptide (23). CCK is also a possible modulator of the postprandial cardiovascular response to different diets in rainbow trout, since it has been shown that the circulating plasma levels of CCK change depending on the protein and lipid content of the ingested meal (32).

CCK is a diverse peptide found in several different isoforms, as well as in numerous tissues in both mammals (13) and teleosts (28, 40). In rainbow trout, three different forms have been discovered, differing in the amino acid in position 6 counting from the c-terminal (Fig. 1). Different posttranscriptional splice variants result in peptides of differing length (7, 8, or 21 amino acids), all sharing the same conserved region and a sulfated tyrosine in position 7 from the c-terminal. The physiological significance of the difference in length and amino acid composition in position 6 is unknown; the different forms are, nevertheless, expressed differently in different parts of the trout (29). CCK is involved in the secretion of pancreatic juices in dogs (38), stimulating gallbladder contractions (1, 2), as well as regulating gastric emptying in rainbow trout (39) and controlling functions of the central nervous system, such as satiety in rats (12).

Address for reprint requests and other correspondence: H. Seth, Dept. of Zoology, Univ. of Gothenburg, Box 463, S-405 30 Gothenburg, Sweden (e-mail: henrik.seth@zool.gu.se).
CCK has previously been shown to affect the gastrointestinal blood flow in mammalian species (15, 27, 42). This effect could be mediated through a neurogenic release of NO with CCK acting peripherally (43), although some suggest this effect is indirect via an increase in, for example, the pancreatic secretion and thus a local vasodilation in this tissue (38), and still others suggest that it is only vasoactive in high doses (41), doses that nevertheless could occur at discrete locations within the intestinal wall.

Overall, there seems to be many contradictory results concerning the importance of CCK in regulating or modulating the postprandial hyperemia in mammals. In fish, even less is known about how CCK might affect the gastrointestinal vasculature, as well as the cardiovascular system, in general. We, therefore, aimed to investigate whether CCK participates in the postprandial regulation of the gastrointestinal blood flow in rainbow trout. This was done in vivo with intra-arterial injections of the sulfated form of CCK-8. The effect of CCK on isolated vascular preparations, as well as in situ perfused heart preparations, was also assessed.

MATERIALS AND METHODS

Experimental Animals

Rainbow trout (O. mykiss), ranging in size between 380 and 750 g (mean: 487 ± 21 g, n = 27), were purchased from a local hatchery. The fish were held in 2 m³ fiberglass tanks supplied with aerated freshwater (10°C) from the departmental recirculating water system and fed dry trout pellets at regular intervals. The photoperiod was adjusted to 12:12-h light-dark conditions. Upon arrival, fish were left for at least 1 wk prior to any experimental procedures. Ethical permit 13/2007 from the Animal Ethics Committee of Gothenburg, covered all experiments reported here.

In Vivo Surgical Procedures

Fish were fasted for ~1 wk prior to surgery. Individual fish were anesthetized in water containing MS-222 (150 mg/l) buffered with sodium bicarbonate (300 mg/l) and transferred to an operating table covered with soft water-soaked rubber foam. The gills were continuously irrigated with aerated freshwater (10°C), and 2 ml of heparin (5000 IU/ml) was injected into the caudal artery. The hepatic veins were then visualized through a ventral midline incision, and all but one was tied using silk suture. A custom-made stainless-steel cannula (inflow) was introduced via the open hepatic vein, the cannula was secured using a silk suture and then checked for leakage. The inflow (inflow) was introduced via the open hepatic vein, the cannula was tied of using silk suture. A custom-made stainless-steel cannula (inflow) was introduced via the open hepatic vein, the cannula was secured using a silk suture and then checked for leakage. The inflow cannula was connected to a Marriot bottle filled with heparinized (1%) Ringer solution (NaCl, 140.0 mM; NaHCO3, 15.0 mM; KCl, 2.5 mM; CaCl2 × 2 H2O, 1.5 mM; KH2PO4, 1.0 mM; MgSO4 × 7 H2O, 0.8 mM; HEPES, 5.0 mM; glucose, 10 mM; and adrenaline, 5 mM), with pH set to 7.8. The unbranched ventral aorta was then visualized by removing the gills and another stainless-steel cannula (outflow) was then placed into the ventral aorta just before the gills (secured with silk suture as above). The fish was then removed from the operating table, and the entire fish was immersed into a cooled (10°C) bath containing saline (0.9%). The pericardium, as well as the surrounding nerves, was left intact for the entire experimental protocol.

The inflow cannula was then disconnected from the Marriot bottle and attached to a constant pressure device, connected to a cooled (10°C) reservoir containing 1,000 ml of well aerated (0.3% CO2) Ringer solution (+0.5 nM adrenaline). The outflow cannula was connected to a transit-time flow probe (2N688 Flow through probe; Transonic, Ithaca, NY). The inflow and outflow pressures were adjusted by means of a hoist and to measure the inflow and outflow pressures, the cannulas were connected to pressure transducers (MLD-type disposable pressure transducers).

Vascular Preparations

Fish were euthanized with a blow to the head, and the CMA was dissected free and put on ice. Small rings with a width of ~1 mm were cut from second- to third-order branches of the CMA and mounted on force transducers (ENSELab 10-700003; Somedic Sales AB, Höby, Sweden) for the measurement of vascular wall isometric tension. Preparations were submersed in cooled (10°C) and well-aerated (0.3% CO2) Ringer solution (NaCl, 140.0 mM; NaHCO3, 15.0 mM; KCl, 2.5 mM; CaCl2 × 2 H2O, 1.5 mM; KH2PO4, 1.0 mM; MgSO4 × 7 H2O, 0.8 mM; HEPES, 5.0 mM) kept under a normalised tension (~1.0–1.5 g), as described earlier (47), and allowed to recover for 1 h to normalize preparation. Preparations were checked for viability using potassium-rich (>50 mM) Ringer solution.
Experimental Protocols

In vivo recordings. Animals were allowed to recover for at least 24 h prior to the experiment. To avoid too much twisting of the wires, the fish was not connected to either the Doppler amplifier or the pressure transducer during the initial 6–12 h. Thereafter, the fish was connected to the devices to allow for an undisturbed recovery and the recording of baseline variables. After this initial recovery period, routine baseline cardiovascular variables were recorded for 1 h. Once a stable baseline was attained, the sulfated form of CCK-8 (see below) was injected through the dorsal aortic catheter. One group (n = 6) received an injection of 0.19 pmol/kg followed by a recovery period of 4 h before another injection of 0.76 pmol/kg. The control group (n = 6), received two saline injections of corresponding volume (0.3–0.4 ml) separated by a recovery period of 4 h. Cardiovascular parameters were recorded at 2–5 min postinjection. The CCK-8s concentrations were chosen as to give an estimated final plasma concentration of 50 pM [physiological level according to Jönsson et al. (32)] and 200 pM respectively, although the absolute concentration would depend on several factors, such as tissue uptake, degradation, and clearance.

In situ perfused heart preparations. The inflow and outflow pressures were adjusted to within physiological levels (inflow: 0 ± 0.20 kPa; outflow: 4.5 ± 0.50 kPa), and the heart preparations (n = 8) were then allowed to stabilize for a period. To confirm the viability of the preparation, a standard power test was performed on each preparation by a stepwise increase in the outflow pressure (data not shown). The outflow pressure was returned, and the preparation was allowed to stabilize again. After a 10-min baseline recording, CCK was added to the Ringer solution to give a final concentration ranging from 0.1 nM to 0.1 μM.

Vascular preparations. The vascular preparations (n = 8) were precontracted with 0.3 μM of adrenaline and allowed to reach a stable baseline before the protocol commenced (~30 min). CCK was then added to the bath to give a final concentration of 0.1 nM. The dosage was then stepwise increased to 0.1 μM. Finally, the ability of the preparation to relax was examined using atropine 0.1 mM, which, except for being a muscarinic receptor antagonist, is a very potent vasorelaxant, at least in adrenergically stimulated vessels (33, 35).

Drugs

CCK-8 in its sulfated form was obtained from Ansynth Service BY (Roosendaal, The Netherlands). CCK exists in several subforms, out of which CCK-7, CCK-8, and CCK-21 have been found in rainbow trout. It is related to gastrin, both functionally and structurally (Fig. 1). The biological active site is situated near the C-terminal just by a conserved sulfated tyrosine residue. We used a mammalian CCK 8s, which differ from the trout forms by having methionine in the sixth position from the fully amidated C-terminal, compared with leucine (CCK-I), asparagin (CCK-n), and threonine (CCK-t) in the trout forms (29). However, it shares the conserved region with all of the trout forms and has been characterized as reviewed by Johnsen (30). CCK was diluted in saline (0.9%).

Atropine (Atropine sulfate salt, ≥97%, A-0257) and adrenaline [(+)-epinephrine (E4375) were obtained from Sigma-Aldrich (St. Louis, MO) and diluted in Ringer solution.

Data Acquisition

The Doppler flow probes were connected to a directional pulsed Doppler flowmeter (model 54SC-4; The University of Iowa, Iowa City, IA). For the in situ perfused heart preparations, transit-time flow probes (2N688 flow through probe; Transonic) were connected to a small animal blood flow meter (T206; Transonic). Pressure transducers (MLD-type) were connected to a 4-channel Amplifier (SENSELab, 4CAMP, Somedic, Höry, Sweden). For the strip preparations, eight force transducers (SENSELab 10-700-0003; Somedic) were connected to two 4-channel amplifiers (SENSELab, 4CHAMP 700; Somedic). In all cases, a PowerLab system connected to a PC running Chart 6 (ADIInstruments, Castle Hill, Australia) was used for the analog/digital conversion and data acquisition.

Data Analysis and Statistics

Heart rate (HR) was obtained from the phasic pressure traces, and cardiac stroke volume (SV) was calculated as SV = CO/HR. In vivo blood flows (CO and Qcma) are presented as relative changes with the baseline set to 100%. The routine values were averaged and compared with the values after the CCK injection by a two-sample t-test, confirming equal variance. The wall tension of the vascular preparations was analyzed for treatment effects using a repeated-measures ANOVA followed by a Tukey’s post hoc test.

Systolic flow slope (+dQ/dt), diastolic flow slope (-dQ/dt), amplitude, as well as time to peak (X) and time to return (Y), were obtained from each flow pulse (Fig. 2) and averaged over an arbitrarily chosen period of time for each treatment. The in situ perfused heart preparations were used to analyze the effects on CO, HR, and SV, as well as the above described parameters, using a repeated-measures ANOVA followed by a Dunnet’s post hoc test. 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 expressed as means ± SE. * and ** denote a significant difference from routine as well as control values (P < 0.05 and P < 0.01, respectively). † denotes a significant (P < 0.05) change compared with previous treatment.

RESULTS

In Vivo Recordings

The injection of the gastrointestinal hormone CCK-8s (0.19 pmol/kg) produced a significant (P < 0.05) increase (20%; 18 ± 4% compared with routine) in gastrointestinal blood flow (Qcma) compared with a control injection of saline (Fig. 3A). This increase is most likely due to a decrease in the resistance of the gastrointestinal vasculature and a significant increase in CO (21% compared with control; 30 ± 6% compared with routine; Fig. 3B), mediated through an increase in SV (16%...
compared with control; 32 ± 11% compared with routine; Fig. 3C). The increase in stroke volume is, however, not significant compared with the control injection. Saline injections did not produce any significant changes in cardiac output or stroke volume; the small, yet not significant, increase in cardiac output and stroke volume when injecting saline could most likely be attributed to a small increase in the circulating blood volume (5– 6%), as well as a subsequent decrease in viscosity due to a dilution of the blood (Fig. 3, A–D).

When increasing the amount of injected CCK to 0.76 pmol/kg, the response is decreased compared with the lower dose. The increase in Qcma is only 15% compared with the control injection (14 ± 6% compared with routine). This is most likely due to a now insignificant increase in CO (5% compared with control; 12 ± 2% compared with routine) and SV (6%; 20 ± 3% compared with routine), thereby removing the CO-mediated part of the increase in Qcma. The reason for this could be a dose-dependent CCK-induced increase in the gill resistances (below).

No significant change in HR was seen with any of the treatments (Fig. 3D).

In Situ Perfused Heart

CCK had no significant effect on cardiac output in the in situ perfused heart preparations (Fig. 4A). Stroke volume did not change, but heart rate increased significantly (11 ± 2%) at a CCK concentration of 10 nM and remained at the same level when increasing the concentration to 100 nM (Fig. 4, B and C). Despite only a minor change in heart rate, there was a considerable change in the shape of the phasic flow profile, as can be seen in Fig. 5. The maximum amplitude of each pulse increased by 35 ± 12% at a CCK concentration of 10 nM compared with the baseline. The amplitude increased even further (50 ± 17%) at 100 nM (Fig. 6). This increase can be explained by a change in systolic/diastolic slope (±dQ/dt; Fig. 7, A and B) or the time to peak/baseline (X and Y, respectively; Fig. 7, C and D). The systolic slope increased with 39 ± 12% and 55 ± 15% at 10 nM and 100 nM, respectively. There was an even more profound change in the relaxation slope, which increased with 96 ± 26% and 208 ± 48%, respectively. The changes in the above variables resulted in a decreased time to peak (11 ± 6%) and time to return (40 ± 6%) at a CCK dosage of 100 nM.

The relative ventricular mass of the perfused hearts was 0.095 ± 0.004%.

Vascular Ring Preparations

There was no change in the wall tension of the precontracted (0.3 μM adrenaline) vascular ring preparations with either a low (0.1 nM) or a high (100 nM) CCK concentration. Adding atropine (0.1 mM) relaxed the vessels and decreased vessel wall tension by 12.6 ± 2% (Fig. 8).

Fig. 3. Means ± SE of gastrointestinal blood flow (Qcma) (A) at the coeliacomesenteric artery (CMA), cardiac output (CO) (B), stroke volume (SV) (C) and heart rate (HR) (D) measured in vivo in rainbow trout (Oncorhynchus mykiss). 0.19 pmol/kg and 0.76 pmol/kg sulfated CCK-8 were injected intra-arterially (black bars) at an interval of 4 h in animals weighing 490 ± 58 g (n = 6). Control animals (n = 6, 625 ± 48 g) received an intra-arterial injection of saline only (gray bars). Dashed line (black) indicates routine pretreatment values for treated animals, whereas dashed line (gray) indicates routine pretreatment values for the control group. A two-sample t-test, assuming equal variance, was used to test for a significant difference between treatments. To correct for multiple comparisons a Holm-Bonferroni test was used. *Significant difference from both routine and control values for CCK-treated group (P < 0.05).
DISCUSSION

Different nutrients are not equally important in inducing a postprandial hyperemia, and several theories have been proposed as to what cause this locally defined hyperemia in mammals (14, 24). One possible theory is that different gastrointestinal hormones might contribute. CCK is one of these potentially important peptides that possibly modulate the gastrointestinal blood flow and thus could be involved in the postprandial hyperemia, as well as other cardiovascular changes (43).

For fish, little is known about where and how the postprandial hyperemia is regulated and the importance of endocrine substances such as CCK. The observed, CCK-stimulated increase in gastrointestinal blood flow, is in concordance with mammalian studies, in which CCK have either direct (15, 27) or indirect neurogenic effects ([7, 43]; reviewed by Ruiz-Gayo et al. [42]) on the gastrointestinal vessels. The effect of CCK can also be secondary to increases in, for example, pancreatic secretions and thus pancreatic blood flow, leading to overall increase in gastrointestinal blood flow (38).

Furthermore, results from the isolated vascular preparations indicate that there are, at least in rainbow trout, no direct effects of physiological levels of CCK on isolated vessels from second- to third-order branches of the coeliacomesenteric artery. Nevertheless, disparity exists, and many studies indicate that the level needed to induce a gastrointestinal hyperemia is much higher than the circulating plasma levels recorded in the postprandial state (23, 41). It is, thus possible that the hormone work in a more paracrine mode, assuming that higher CCK levels can be produced locally in the intestine. Our results, however, challenge this, given that there was little effect of high CCK concentrations. It could also be that the direct vascular effect of CCK is limited to the smaller-diameter submucosal/mucosal vessels that are not possible to use in the present experimental setup. This is less likely considering that, at least in mammals, for the most part, the control of the gastrointestinal vascular resistance lies within the major vessels before the beginning of the regional intestinal vasculature (9).

The increase in gastrointestinal blood flow initiated by circulating CCK seen in this study is considerably lower than postfeeding values presented for rainbow trout in previous studies, showing an increase of 100% and above (17, 26). It is, however, unlikely that CCK would account for the entire increase in gastrointestinal blood flow seen after feeding, and it is probably more important in adapting the cardiovascular response to the nutrient composition of the diet. The dynamics of the CCK release and the subsequent circulating levels are also unknown, and it could very well be that there is a continuous release of CCK during postprandial conditions. Therefore, the levels achieved in this study could be transient, ultimately depending on the rate of turnover or breakdown. Furthermore, by injecting the dose into the systemic circulation, it could also be that we see a generalized vasodilation not limited to the gastrointestinal tract and, consequently, there would be no redistribution of blood from the systemic to the gastrointestinal circulation. During normal postfeeding conditions, it may well be that most of the CCK is confined to the intestine and thus only limited amounts reach the systemic circulation.

The observed increase in gastrointestinal blood flow in the present study is most likely due to a decrease in the vascular resistance of the gastrointestinal tract. However, we did not simultaneously record arterial and venous pressure; thus, any calculation of vascular resistances is not possible. The reasons for this are numerous. First of all, an additional venous catheter would have imposed further stress on the animal and thus could have hidden the observed cardiovascular changes. Second, it would have proven hard to get accurate measurements of dorsal aortic pressure so soon after the intra-arterial injection of CCK, and artifacts from the injection would have been a major concern.
To maintain the increase in gastrointestinal blood flow, there was a subsequent increase in cardiac output. The increase was of such a magnitude that it most likely could account for the entire increase in gastrointestinal blood flow, without an additional redistribution of blood. The increase in cardiac output was mediated via an increase in stroke volume since heart rate did not change. However, the in vivo increase in cardiac output was not seen in the isolated perfused heart preparations, and thus, it is probably not a direct effect of CCK on the heart itself. The fact that neither the isolated vessels nor the heart preparations showed any change strengthens the notion that CCK could work through a neurogenic mechanism. This, however, remains to be established. The increase in cardiac output could also have been a passive effect, resulting from the decrease in vascular resistance, although this is unlikely, considering the intrinsic property of the heart to maintain the stroke volume over a wide range of aortic pressures [homeometric regulation; (18)].

Even though cardiac output did not change, interesting changes were seen upon CCK exposure in the perfused heart. The most obvious effect was the increase in the amplitude of cardiac flow profile. Amplitude increased as a consequence of an increase in systolic and diastolic rate (dQ/dt) with only a minor increase in heart rate. This increase of the systolic and diastolic rate of the phasic flow profile could be caused by a change in the windkessel effect of the bulbus arteriosus (19). The bulbus probably enables the very large stroke volumes seen in many fishes (37), as well as having the capability to adjust to changes in blood pressure (31). The bulbus receives sympathetic innervation, and Farrell et al. (21) indicated that a bulbus-mediated regulation of the pulsatility and hence, the blood flow patterns through the gills could be important in governing its synchrony with the ventilatory movements in fish. A stiffening of the bulbus would explain the change in flow profile seen, and CCK could possibly act through a NO-mediated mechanism, given the wide repertoire of functions of NO in the heart (49). The dosage at which CCK has its effect is high compared with circulating levels, but it could, nevertheless, be attained at defined locations, such as at synapses or in the vicinity of neuroendocrine cells.

Nonetheless, when increasing the injected dose of CCK, there was still a significant increase in gastrointestinal blood flow, but the response was reduced compared with the lower physiological dose. There are several possible explanations for this. It could be that the maximal dose needed had already been reached. This is, however, unlikely considering that CCK in local areas of the gastrointestinal tract probably reach much higher levels as has been speculated in several mammalian studies (41). Another much more plausible explanation is that there is a dose-dependent increase in the vascular resistance of the gill circulation, as previously reported by Sundin and Nilsson (48). An increase in the resistance of the gill vessels would increase the workload of the heart and thus lead to a decrease in stroke volume (when outside of the homeometric regulatory scope) through a Starling mechanism (20), causing the observed decrease in cardiac output. This is also consistent with in vivo recordings of CO and ventral aortic pressure (Pva) in cod (48), where CO decreases with an increase in Pva during increasing levels of CCK exposure. Ultimately, this means that when cardiac output is decreased, the increase in gastrointestinal blood flow cannot be maintained and gastrointestinal blood flow decreases, as seen here. Whether or not this holds true remains to be determined.

Fig. 5. Representative trace illustrating the change in the cardiac output flow profile associated with an application of CCK at levels of 10 nM and above.

Fig. 6. Means ± SE in the amplitude of the cardiac output flow profile (Fig. 2) measured in the in situ perfused hearts (n = 8, 422 ± 14 g with a mean relative ventricular mass of 0.095 ± 0.004%) of rainbow trout (O. mykiss). Samples were tested for a significant difference from routine using a repeated-measure ANOVA followed by a Dunnet’s post hoc test. Dashed line indicates routine pretreatment values. **Significant difference from routine values (P < 0.01).
Conclusions and Future Perspectives

In conclusion, CCK seems to affect the gastrointestinal blood flow and the dynamics of the phasic flow profile in the ventral aorta. CCK could be important in regulating the cardiovascular postprandial response to different diets, but CCK does not have a direct effect on the vasculature but instead probably acts on peripheral nerves relaying the signal to its target. Furthermore, one rather peculiar effect on the heart was discovered. This CCK-induced change in flow pattern could be important in modulating the flow through the gills during changing conditions, such as, for example, after feeding or during stress, such as hypoxia. To undoubtedly establish the role of CCK in the postprandial regulation of gastrointestinal blood flow, more studies are, however, needed. First and most

Fig. 7. Means ± SE of systolic slope (+dQ/dt) (A), diastolic slope (-dQ/dT) (B), X (time to peak) (C) and Y (time to routine) (D) of the cardiac output flow profile (Fig. 2) measured in the in situ perfused hearts (n = 8; 422 ± 14 g with a mean relative ventricular mass of 0.095 ± 0.004%) of rainbow trout (O. mykiss). Samples were tested for a significant difference from routine values using a repeated-measure ANOVA followed by a Dunnet’s post hoc test. Dashed line indicates routine pretreatment values. * and ** denotes a Significant difference from routine values (P < 0.05) and (P < 0.01), respectively.

Fig. 8. Representative recording of a vascular preparation from the coeliacomesenteric artery and its branches, precontracted with 0.3 μM adrenaline and allowed to normalize before application of CCK in stepwise increments from 0.1 nM to 100 nM. To verify the capacity of the preparation to relax, atropine (0.1 mM) was added at the end of the experiment. The small graph in the upper right corner shows the means ± SE for the change in contractile force of n = 32 vascular preparations obtained from n = 8 (465 ± 14 g) rainbow trout (O. mykiss). Samples were tested for a significant difference from routine and precontracted values using a repeated-measures ANOVA followed by a Tukey’s post hoc test. Dashed line indicates routine pretreatment values and † denotes a significant difference from the precontracted values (P < 0.05) for the atropine treatment.
importantly, one would have to resolve the dynamics of the CCK in the plasma. Given the huge lack of knowledge concerning such aspects in fish, this holds true for most other peptides or pharmaceuticals as well. This could be achieved at high resolution using a microdialysis approach, enabling not only the exact determination of postprandial levels of CCK, but also the dynamics of the injected molecule. This would make a setup where CCK (and other peptides) can be infused, so as to give physiological plasma levels on a long-term basis possible. Furthermore, the modulatory capacity of CCK on the heart or the bulbous arterial warrants future investigations.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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