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Effects of serotonin and fluoxetine on blood glucose regulation in two decapod species

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Abstract

One of the best known crustacean hormones is the crustacean hyperglycemic hormone (CHH). However, the mechanisms involved in hormone release in these animals are poorly understood, and thus constitute the central objective of the present study. Different groups of crustaceans belonging to diverse taxa (*Chasmagnathus granulata*, a grapsid crab and *Orconectes limosus*, an astacid) were injected with serotonin, fluoxetine, or a mixture of both, and glycemic values (*C. granulata* and *O. limosus*) and CHH levels (*O. limosus*) were determined after 2 h in either submerged animals or animals exposed to atmospheric air. Both serotonin and fluoxetine caused significant hyperglycemia ($P<0.05$) after injection into the blood sinus of the two species, an effect enhanced after exposure to atmospheric air. In *C. granulata* blood glucose increased from 6.1 to 43.3 and 11.4 mg/100 ml in submerged animals and from 5.7 to 55.2 and 22.5 mg/100 ml in air-exposed animals after treatment with serotonin and fluoxetine, respectively. In *O. limosus* the increases were from 1.2 to 59.7 and 135.2 mg/100 ml in submerged animals and from 2.5 to 200.3 and 193.6 mg/100 ml in air-exposed animals after treatment with serotonin and fluoxetine, respectively. Serotonin and fluoxetine also caused a significant increase in the circulating levels of CHH in *O. limosus*, from 11.9 to 43 and 45.7 fmol/ml in submerged animals and from 13.2 to 32.6 and 45.7 fmol/ml in air-exposed animals, respectively, thus confirming their action as neuroregulators in these invertebrates.

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- Crustacean
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Introduction

In crustaceans the X-organ/sinus gland complex, typically located in the eyestalk of decapods, plays a central role in physiological regulation of biological activities (1,2). Hormones produced and released by this system are known to regulate reproduction, nutrient metabolism, chromatic adaptation and growth, among several others. One of the best known crustacean hormones, with a relatively fast response time (between 1 and 2 h for maximum response), is the crustacean hyperglycemic hormone (CHH) (3-5).

Neuroregulators are compounds that function either as neurotransmitters by acting on the transfer of information between a neuron and an adjacent target cell or as neuromodulators by amplifying or dampening neurotransmitter activity (6).

Some of the most successful neuroregulators experimentally used so far in crustaceans are serotonin (5-hydroxytryptamine, 5-HT) and some of its related drugs or modulators, like fenfluramine (a serotonin releaser) and fluoxetine (a serotonin potentiator) (7).

As part of our research directed at the understanding of hormone release regulation in crustaceans, in the present investigation we studied the effect of serotonin and fluoxetine on blood glucose levels in the decapods *Chasmagnathus granulata* and *Orconectes limosus*.

Material and Methods

Adult *Chasmagnathus granulata* (Grapsidae) males were collected at Samborombon Bay (Buenos Aires Province, Argentina), and transported to the laboratory at the University of Buenos Aires, where they remained for at least 15 days. During this period they were regularly fed (three times a week) beef liver and commercial rabbit food pellets *ad libitum*. Temperature, salinity and photoperiod were set at 20°C, 12‰ and 12:12-h light-dark cycle, respectively. Animals were fasted 24 to 48 h before the experiments.

Adult *Orconectes limosus* (Astacidae) males were obtained through commercial sources from the Havel River (Berlin, Germany). In the laboratory, at the University of Bonn, they were kept under running tap water at a temperature of about 10°C and on a 12:12-h light-dark cycle. They were regularly fed commercial cat chow *ad libitum*. Feeding was discontinued at least 48 h before the experiments. Animals at intermolt, or at most early pre-molt, were employed in all experiments, which were performed during afternoon hours.

Experiments were divided into two main sets, the first to determine if either of the drugs known to stimulate ovarian development (serotonin and fluoxetine) was able to cause hyperglycemia in submerged animals, and the second, to establish if either drug could inhibit or potentiate a hyperglycemic response.

For both sets, animals were divided into 5 groups: 1) intact submerged animals prior to any treatment, 2) saline-injected animals, 3) serotonin-injected animals (0.5 µmol/animal in 50 µl saline), 4) fluoxetine-injected animals (1.5 nmol/animal in 50 µl of saline), and 5) serotonin- and fluoxetine-injected animals (0.5 µmol and 1.5 nmol/animal in 50 µl of saline, respectively). All injections were performed at the base of the fourth pair of pereiopods. Saline consisted of either 12‰ seawater for *C. granulata*, or "van Harreveld" solution (8) for *O. limosus*.

In the first set of experiments, hemolymph was withdrawn from submerged animals from the blood sinus at the base of the fourth or third pair of pereiopods (100 µl) 2 h after injection. In the second group, animals were injected and then exposed to a known hyperglycemia-inducing stress, i.e., exposure to atmospheric air for 2 h. After this period, hemolymph was similarly obtained and analyzed.

Fluoxetine was used as the commercial formulation of Prozac (Eli Lilly do Brasil Ltda., São Paulo, SP, Brazil), and serotonin as hydrochloride (Sigma Chemical Co., St. Louis, MO, USA).

Hemolymph glucose content of both species was determined by the glucose-oxidase method using available commercial diagnostic kits (Wiener Laboratories, Rosario, Argentina, and Boehringer-Mannheim Corp., Mannheim, Germany, for *C. granulata* and *O. limosus*, respectively).

Hemolymph CHH content of *O. limosus* was determined using a "sandwich-type" ELISA (5,9,10).

Means were compared by one-way analysis of variance (ANOVA), followed by Tukey's multiple range test (HSD). Paired means were compared by the standard Student *t*-test. Data were also tested for normality and homogeneity of variances, and transformed to log when necessary. All analyses were performed using the statistical package "Statistics for Windows" version 4.2 (Statsoft Inc., 1993). The level of significance was set at $P<0.05$.

Results and Discussion

Serotonin has long been known to have a potent hyperglycemic effect (11), and the present results confirm such findings ($P<0.05$) (Table 1). In submerged *C. granulata* fluoxetine alone had a comparatively mild hyperglycemic effect ($P<0.05$), while a combination of both drugs had an effect similar to that of serotonin alone. As expected, saline injection caused no changes in hemolymph glucose in these animals ($P>0.05$).

C. granulata exposed to atmospheric air presented hyperglycemia in all experimental situations (Table 1). Nevertheless, it should be pointed out that after injection of either saline or fluoxetine, hemolymph glucose was significantly higher in the air-exposed than in the submerged animals ($P<0.05$). Similar results were obtained for *O. limosus* (Table 1).

The observed hemolymph CHH levels were in full accordance with the glycemic changes, i.e., the injection of either fluoxe-

Table 1 - Effects of serotonin (5-HT), fluoxetine (Flx) and a mixture of both drugs (5-HT + Flx) on glucose levels (mg/100 ml hemolymph) in *Chasmagnathus granulata* and *Orconectes limosus* hemolymph.

Animals were either kept submerged or exposed to atmospheric air after drug administration. Note that control animals were always kept submerged. Data are reported as mean ± SD. N = 5-8 animals in all cases. Same letters along a line indicate means not significantly different ($P>0.05$, ANOVA followed by Tukey multiple range test).

	Control	Saline	5-HT	Flx	5-HT + Flx
			C. granulata		
Submerged	6.1 (1.9)^{A}	5.0 (1.6)^{A}	43.3 (13.0)^{C}	11.4 (2.6)^{B}	34.0 (8.9)^{C}
Air	5.7 (2.5)^{A}	12.1 (6.4)^{B}	55.2 (7.9)^{C}	22.5 (3.9)^{B}	43.9 (19.8)^{C}
			O. limosus		
Submerged	1.2 (0.7)^{A}	3.4(0.7)^{A}	59.7 (31.1)^{B}	135.2 (44.7)^{C}	149.7 (61.8)^{C}
Air	2.5 (1.0)^{A}	79.4 (27.7)^{B}	200.3 (73.2)^{C}	193.6 (95.6)^{C}	161.3 (128.7)^{BC}

Table 2 - Effects of serotonin (5-HT), fluoxetine (Flx) and a mixture of both drugs (5-HT + Flx) on CHH levels (fmol/ml) in the hemolymph of *Orconectes limosus*.

Animals were either kept submerged or exposed to atmospheric air after drug administration. Note that control animals were always kept submerged. Data are reported as mean ± SD. N = 6-10 animals in all cases. Same letters along a line indicate means not significantly different ($P>0.05$, ANOVA followed by Tukey multiple range test).

	Control	Saline	5-HT	Flx	5-HT + Flx
Submerged	11.9 (3.6)^{A}	13.9 (10.2)^{A}	43.0 (26.0)^{B}	45.7 (27.8)^{B}	29.2 (13.4)^{B}
Air	13.2 (11.6)^{A}	23.4 (6.1)^{AB}	32.6 (9.7)^{BC}	45.7 (29.6)^{C}	44.0 (21.8)^{C}

tine or serotonin, or the exposure to atmospheric air, which caused a marked and significant hyperglycemia ($P<0.05$), also led to increases in the circulating levels of CHH (Table 2).

One thing to be noted from the above results is that hemolymph glucose responds to substances quite well known as stimulators of ovarian development in crustaceans, i.e., serotonin and fluoxetine (7,12).

Fluoxetine did not potentiate this serotonin effect in *C. granulata*, either submerged or air exposed, suggesting that a maximum response had already been reached with serotonin alone. Nevertheless, following exposure to atmospheric air, which naturally causes hyperglycemia in both species (3,13), fluoxetine alone caused a larger increase in hemolymph glucose than when injected into submerged crabs ($P<0.05$), although not in crayfish ($P>0.05$). These results suggest that serotonin may be released under this circumstances and that its effect is then amplified by fluoxetine. Also, serotonin levels must have been relatively low in non-stressed submerged *C. granulata*, although this does not seem to be the case for *O. limosus*, and for this reason fluoxetine was able to cause only a discrete, although significant ($P<0.05$), hyperglycemia under such conditions.

These differences in the results obtained for *C. granulata* and *O. limosus* may be the consequence of a species-specific time course for the glycemic responses and/or a result of distinct adaptations of physiological mechanisms. *C. granulata*, an intertidal animal, seems to be less affected by air exposure than *O. limosus*. On the other hand, both fluoxetine and serotonin were more effective in promoting hyperglycemia in the latter species.

An increase in CHH release due to exposure to atmospheric air has been recently reported for *Cancer pagurus* (14), even though it may be absent in some species, as seems to be the case for *Carcinus maenas* (Santos EA and Keller R, unpublished results).

That serotonin is involved in the regulation of hemolymph glucose concentration, possibly controlling the release of neurohormones from the X-organ/sinus gland complex, has been demonstrated several times (15-18). Nevertheless, the exact nature of this effect is not well established. Our data are the first direct evidence that 5-HT is in fact related to the release of CHH, since fluoxetine injection had a direct effect on the circulating levels of this hormone (Table 2). However, some of the published data are conflicting. *In vivo* administration of serotonin, as shown in the present paper as well as by several investigators (11,15,19,20), leads to a significant hyperglycemia. On the other hand, it has been suggested that serotonin should decrease CHH release, since it had an inhibitory effect on the spontaneous electrical activity of CHH neurosecretory cells, previously isolated from the X-organ (21). Such effect, as also reported, is quite similar to that evoked by glucose. This carbohydrate has also been demonstrated to have an inhibitory effect on CHH release both *in vivo* (5) and *in vitro* (21). Nevertheless, in spite of a similarity in neuronal depression caused by glucose and serotonin, as reported in the cited experiments (21), our present results leave no doubt that serotonin has a hyperglycemic effect, which seems to be mediated by the release of CHH. Increased circulating levels of CHH after serotonin injection have been demonstrated in this study for the first time. This CHH release is in accordance with the previously reported increase in firing rate and induction of action potential by serotonin in neurosecretory cells of the X-organ (22), even though the CHH-producing cells were not specifically identified.

Another conflicting issue is the effect of CHH on ovarian development. This hormone has been recently shown to inhibit the synthesis of methyl farnesoate (23), as well as of proteins and mRNA in ovarian fragments (24). Thus, even though fluoxetine,

which we have demonstrated to increase circulating levels of CHH, would not be expected to stimulate ovarian development, it actually does (12). On the other hand, during purification of sinus gland extracts from *Homarus americanus*, a gonad-stimulating activity in the same HPLC fraction containing CHH has been reported (25), raising the possibility of a stimulatory action on reproduction.

Clearly, the above results suggest that much is still to be learned about the endocrine control of metabolism and especially of reproduction in crustaceans, and that hemolymph glucose may be used as a faster screening system for drugs which modulate the release of neurohormones, especially those of eyestalk origin. Alternative administration routes for such drugs, which are able to promote ovarian development and cause hyperglycemia, are presently under investigation in this model.

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