Comparative Biochemistry and Physiology, Part A 158 (2011) 126–131

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

In vitro effects of both dopaminergic and enkephalinergic antagonists on the ovarian growth of *Cherax quadricarinatus* (Decapoda, Parastacidae), at different periods of the reproductive cycle

Alejandra Valeria Cahansky, Daniel Alberto Medesani, Anouk Chaulet, Enrique Marcelo Rodríguez *

Dept. of Biodiversity and Experimental Biology, FCEyN, University of Buenos Aires, Ciudad Universitaria, Pab. II. C1428EHA, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 3 August 2010
Received in revised form 20 September 2010
Accepted 21 September 2010
Available online 29 September 2010

Keywords:
Crayfish
Reproduction
Ovary
Spiperone
Naloxone
Thoracic ganglion
Dopamine
Enkephalin

ABSTRACT

The *in vitro* effect of both spiperone (dopaminergic antagonist) and naloxone (enkephalinergic antagonist), was assayed on small pieces of ovary dissected from *C. quadricarinatus* females, with the eventual addition of some neuroendocrine organs, such as thoracic ganglion or eyestalk tissue. The incorporation of tritiated leucine by the ovary was measured in order to estimate the ovarian growth. During the post-reproductive period, both mentioned antagonists were able to significantly stimulate the ovary in the presence of thoracic ganglion, but did not produce any significant effect in the preparation containing ovary and eyestalk tissue, or only ovary. No significant effects of the assayed antagonists were noted during the pre-reproductive period. These results were in accordance with previous models describing the neuroendocrine control of crustacean reproduction, and represent new findings about the hormonal context in different periods of the reproductive cycle of crayfish. Besides, by means of the experimental combination of the tested antagonists with dopamine or met-enkephalin, a new model dealing with the interaction of these two neurotransmitters on the hormonal secretion of thoracic ganglion has been proposed.

1. Introduction

Among the freshwater crayfish species used in aquaculture, *Cherax quadricarinatus* (Parastacidae, Astacidea, Decapoda) is one of the most valuables from an economic point of view. Commonly named as "red claw" (because of the conspicuous red patch in the external margin of male chelae), this species native from northern Australia has a high potential for culturing, due to its rapid growth, multiple spawning throughout the reproductive season and its high resistance to suboptimum environmental conditions (Lawrence and Jones, 2002; Villarreal Colmenares and Naranjo Páramo, 2008).

The reproductive period of *C. quadricarinatus* is the spring and summer months, when most of spawning takes place in natural conditions. A single female can be impregnated by males several times during the reproductive cycle (Jones, 1997). This seasonal reproductive pattern is clearly observed in farms from temperate climates, and even under constant laboratory conditions throughout the year (Barki et al., 1997). During the winter (pre-reproductive period), the ovary grows significantly, to fully ripen at the beginning of spring. During the autumn (post-reproductive period), the ovaries remain in a quiescent stage (Karplus et al., 2003). According to Cahansky et al. (2008b), ovaries dissected from *C. quadricarinatus* females in August (pre-reproductive period) are in a mid-vitellogenic stage (yolk stage, according to the scale proposed by Abdu et al., 2000), while ovaries corresponding to the post-reproductive period are expected to be the primary-vitellogenic phase described by Abdu et al. (2000).

Ovarian growth occurs in two stages; the previtellogenic stage is characterized by mitotic proliferation of gonia and their differentiation into primary oocytes, followed by the vitellogenic stage, i.e., vitellogenin synthesis, initially produced in oocytes (primary or endogenous vitellogenesis) and subsequently by extra-oocyte cells and then taken up by the oocytes (secondary or exogenous vitellogenesis) (Abdu et al., 2000; Tsukimura, 2001; Kulkarni et al., 1991; Charniaux-Cotton, 1985). During exogenous vitellogenesis the follicular cells that surround the oocytes are involved in the uptake of this vitellogenin by the oocytes (Wainwright and Rees, 2001; Abdu et al., 2000). In *C. quadricarinatus*, exogenous vitellogenin synthesis takes place in the hepatopancreas (Abdu et al., 2002; Serrano-Pinto et al., 2004); as in other species, vitellogenin is then exported to the ovary through the hemolymph (Meusy and Charniaux-Cotton, 1984).

The so-called gonad-inhibiting hormone (GIH), produced and secreted in the eyestalks, would probably block, among other effects, the uptake of vitellogenin by the oocytes during the exogenous vitellogenesis stage (Charniaux-Cotton, 1985). There is also strong evidence that a gonad-stimulating hormone (GSH), secreted by the brain and thoracic ganglia, stimulates ovarian growth by acting

* Corresponding author. Tel.: +54 11 45763300x210; fax: +54 11 45763384.
E-mail address: enrique@bg.fcen.uba.ar (E.M. Rodríguez).

1095-6433/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2010.09.016

directly on the ovary and also probably on other tissues such as the hepatopancreas (Charniaux-Cotton, 1985; Fingerman, 1995, 1997). An isoform of the crustacean hyperglycemic hormone (CHH) present in the ventral nerve chain of lobsters, has been suggested to function there as GSH (De Kleijn and Van Herp, 1998).

Dopamine is a classical neurotransmitter found in crustaceans, and its role in modulating the secretion of neurohormones is well documented (Fingerman 1995, 1997). The dopaminergic antagonist spiperone (D2 receptors blocker) has been shown to suppress the inhibitory effect of dopamine on secretion of the crustacean hyperglycemic hormone (CHH) by the crustacean eyestalks (Sarojini et al., 1995a). At the reproductive level, spiperone was able to induce ovarian maturation of the crayfish *Procambarus clarkii* (Rodríguez et al., 2002a), as well as to produce an increment of both oocyte diameter and gonadosomatic index in the crustaceans *Chasmagnathus granulata* (Zapata et al., 2003), *C. quadricarinatus* (Cahansky et al., 2002, 2003) and *Aegla platensis* (Cahansky et al., 2008a). These results, taken together, suggest that dopamine could be arresting ovarian growth by inhibiting the secretion of GSH in the brain/thoracic ganglia, by stimulating the secretion of GIH from the eyestalks, or by both mechanisms (Fingerman, 1997; Wainwright and Rees, 2001).

Among the neuropeptides involved in the regulation of crustacean hormone secretion, some opioid compounds such as met-enkephalin and leu-enkephalin have been reported (Nagabhushanam et al., 1995; Fingerman and Kulkarni, 1991; Fingerman et al., 1985). Several studies have established the inhibitory effects of these opioids on ovarian maturity of decapod crustaceans, such as the fiddler crab *Uca pugilator* (Sarojini et al., 1995b) and the crayfish *P. clarkii* (Sarojini et al., 1996, 1997). As mentioned for dopamine, a possible inhibition of GSH secretion and/or stimulation of GIH secretion have been proposed for crustacean endogenous opioids (Fingerman, 1997). Naloxone, an enkephalinergic antagonist, has been able to induce the ovarian growth in several crustacean species, likely by antagonizing the effect of the enkephalins above mentioned (Nagabhushanam et al., 1995; Sarojini et al., 1995b, 1996; Cahansky et al., 2008a,b).

This study was aimed at determining, by means of an *in vitro* procedure, the role of both endogenous dopamine and enkephalins on the regulation of any endocrine factor secreted by the thoracic ganglia and eyestalks, at different periods of the reproductive cycle of the crayfish *C. quadricarinatus*.

2. Materials and methods

2.1. In vitro incubation

Adult females of *C. quadricarinatus* were obtained form a local aquaculture facility (Pinzas Rojas S.R.L.). Once in the laboratory, the animals were acclimated for 15 days to constant temperature (20–22 °C) and photoperiod (14:10 L:D), being daily fed with pellets for fish (Tetra Diskus®), provided *ad libitum*, and supplemented with leaves of *Elodea* sp.

For the *in vitro* incubation, the following procedure was followed, adapted from those used in previous studies (Eastman-Reks and Fingerman, 1985; Khalaila et al., 2002; Rodríguez et al., 2002a). Briefly, each ovarian piece (approximately 10 mm long) was put in a sterile vial filled with 2 mL of M199 culture medium (Sigma Chemical) dissolved in Van Harreveld's crustacean saline. Osmolarity was adjusted to 428 mOsm and pH to 7.4. The culture medium was also supplemented with antibiotics (penicillin G and streptomycin, each one at 100 UI/mL), 10% bovine serum, antiproteases (0.1 mM of phenylmethanesulfonyl fluoride) and EDTA 1 mM. Each vial received 3 μCi of tritiated leucine (Perkin Elmer Life Sciences, Net-460) in order to measure the subsequent incorporation of this amino acid into the acid-precipitable protein fraction of the ovary. For this purpose, all vials were incubated for 24 h in CO_2 chambers at 28 °C in constant darkness. After that, each piece of ovary was surface dried, weighed (at a precision of ± 0.0001 g), homogenized and centrifuged at 3000 *g* for 10 min at 4 °C. The resulting pellet was resuspended in 2 mL of 10% trichloroacetic acid and filtered in a Millipore suction filtration funnel. The filters (nitrocellulose discs; 0.22 μm) were then air dried and transferred to vials with fluor solution (Optiphase Hi Safe 2) for 24 h at 4 °C, and finally the counts per minute (CPM) were determined in a Beckman scintillation counter.

2.2. Experimental design

The following experimental groups were run, for each of the following experiments (OV: ovary, M: muscle, a small piece serving as control tissue, SP: spiperone, NX: naloxone, DA: dopamine, ENK: Met-enkephalin, TG: thoracic ganglion, ET: eyestalk tissue).

Experiment 1, evaluation of the spiperone effect: 1) OV + M, 2) OV + M + SP, 3) OV + TG, 4) OV + TG + SP, 5) OV + ET, 6) OV + ET + SP. This experiment was conducted both during the pre-reproductive period (July, mean body mass of females: 36.39 g ± 2.19, N = 10) and the post-reproductive period (March, mean body mass of females: 45.20 g ± 2.78; N = 6).

Experiment 2, evaluation of the naloxone effect: 1) OV + M, 2) OV + M + NX, 3) OV + TG, 4) OV + TG + NX, 5) OV + ET, 6) OV + ET + NX. This experiment was conducted both during the pre-reproductive period (August, mean body mass of females: 69.98 g ± 1.38, N = 6) and the post-reproductive period (March, mean body mass of females: 40.65 g ± 4.44, N = 6).

Experiment 3, evaluation of the crossed interaction between antagonist and endogenous ligands: This experiment was only conducted during the post-reproductive period, and consisted of two different trials:

First trial: 1) OV + M, 2) OV + M + DA + NX, 3) OV + TG, 4) OV + TG + DA + NX. Females having a mean body mass of 39.26 g ± 2.86 (N = 9) were used.

Second trial: 1) OV + M, 2) OV + M + ENK + SP, 3) OV + TG, 4) OV + TG + ENK + SP. Females having a mean body mass of 46.58 g ± 2.91 (N = 9) were used.

In all the experiments, a piece of ovary from each female was put into to one vial in every experimental group (repeated measure design). When required, the tissue from one eyestalk tissue was put in one vial containing a piece of ovary taken from the same female, while a thoracic ganglion was cut in two halves, and put into one vial that also contained an ovarian piece from the same female.

The dose used for all assayed compounds was 125 μM, as in previous assays carried out with other decapod crustacean species (Rodríguez et al., 2002b; Zapata et al., 2003). All drugs were purchased form Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Both dopamine and naloxone were used as hydrochloride, while methionine enkephalin (met-enkephalin) was used as acetate salt and spiperone as powder (spiroperidol). All of them were dissolved in dimethyl sulfoxide as vehicle, but spiperone was previously dissolved with some drops of acetic acid. An aliquot of 15 μl of any drug was added to each vial; control vials received the same volume of vehicle.

2.3. Data analysis

A repeated measure one-way ANOVA, followed by the multiple comparison LSD tests (Sokal and Rohlf, 1981) were used to compare mean values (CPM/g of ovary) among experimental groups. A 5% significant level was always considered.

3. Results

3.1. Effect of spiperone

Fig. 1 shows a significant ($p<0.05$) ovarian growth by the addition of thoracic ganglion to the *in vitro* preparation of ovary, either with or without spiperone, during the pre-reproductive period. On the other hand, although the spiperone added to the preparation of ovary and eyestalk tissue produced some increment in the ovarian growth, this was not statistically significant ($p=0.11$). No direct effect of spiperone on the ovary was seen (OvM-Sp group compared to the OvM control).

During the post-reproductive period (Fig. 1), a significant ($p<0.05$) difference was seen in the preparation of ovary plus thoracic ganglion when spiperone was added (OvTG-Sp versus OvTG group). Again, no significant ($p>0.05$) effect was seen with spiperone added either to the preparation of ovary plus eyestalk tissue, or to ovary and muscle (as control tissue).

3.2. Effect of naloxone

Fig. 2 shows the mean values of leucine incorporation in the ovarian pieces, during the pre-reproductive period, together with the result of the statistical comparisons. As for spiperone, the addition of thoracic ganglion to the preparation of ovary was able to significantly ($p<0.05$) increase the uptake of leucine, regardless of the presence of naloxone. Besides, naloxone did not show any effect either on the ovary directly (OvM-Nx versus OvM group) or in the preparation of ovary plus eyestalk tissue.

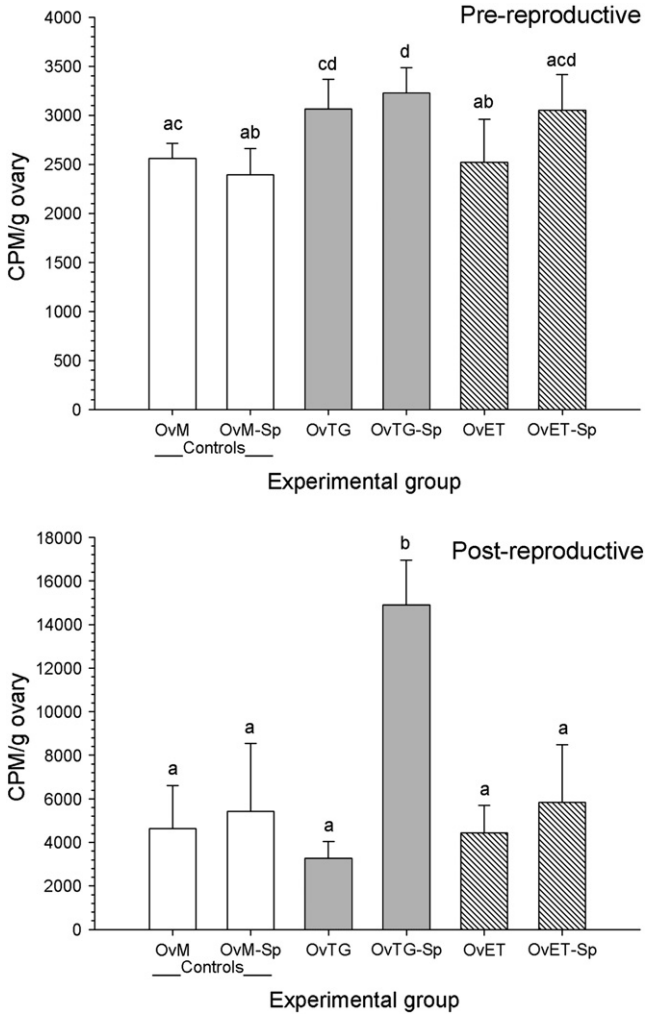

Fig. 1. Effect of spiperone (Sp, at 125 μM) on ovarian growth, in terms of leucine incorporation, for both pre- and post-reproductive periods. CPM: counts per minute (mean ± standard error). Ov: ovary, M: muscle, TG: thoracic ganglion, ET: eyestalk tissue. Different letters indicate significant differences ($p<0.05$).

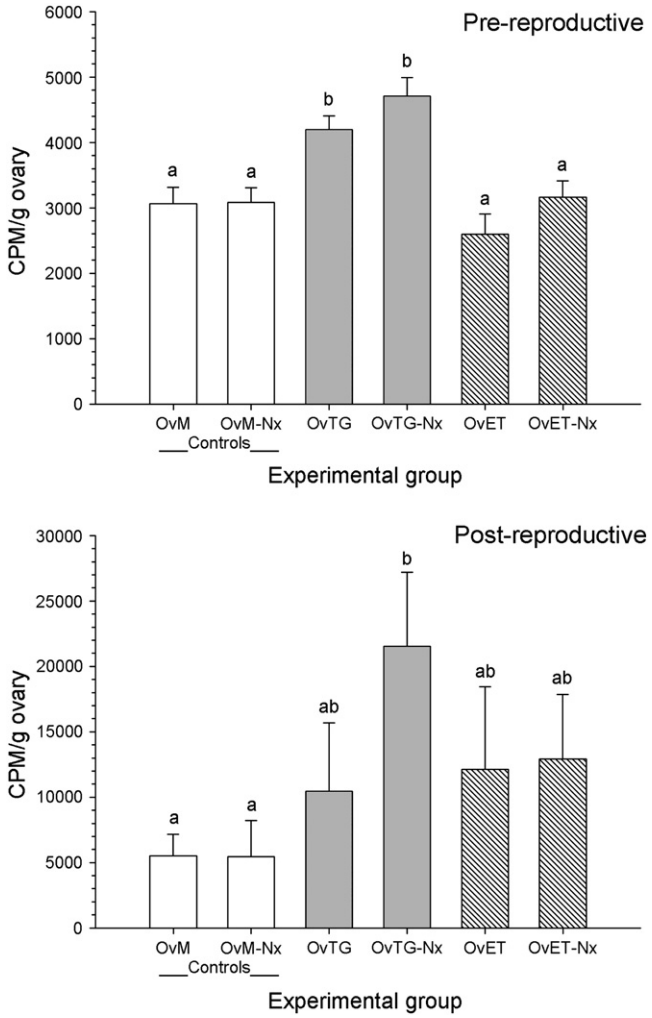

Fig. 2. Effect of naloxone (Nx, at 125 μM) on ovarian growth, in terms of leucine incorporation, for both pre- and post-reproductive periods. CPM: counts per minute (mean ± standard error). Ov: ovary, M: muscle, TG: thoracic ganglion, ET: eyestalk tissue. Different letters indicate significant differences ($p<0.05$).

Fig. 2 shows the results corresponding to the post-reproductive period. Significant ($p<0.05$) ovarian growth was seen when naloxone was added to the preparation containing thoracic ganglion (OvTG-Nx versus OvM-Nx group). As with the results obtained during the other period, no significant effects ($p>0.05$) of naloxone were seen in the preparation of ovary with either muscle or eyestalk tissue.

3.3. Crossed interaction between antagonist and endogenous ligands

In any trial, the assayed combinations of drugs produced a modification in the uptake of leucine by the incubated pieces of control ovary (Fig. 3). However, a significant ($p<0.05$) increase was noted when naloxone and dopamine were added to the preparation of ovary and thoracic ganglion (Fig. 3, first trial), but not when spiperone and met-enkephalin were added to the same preparation (Fig. 3, second trial).

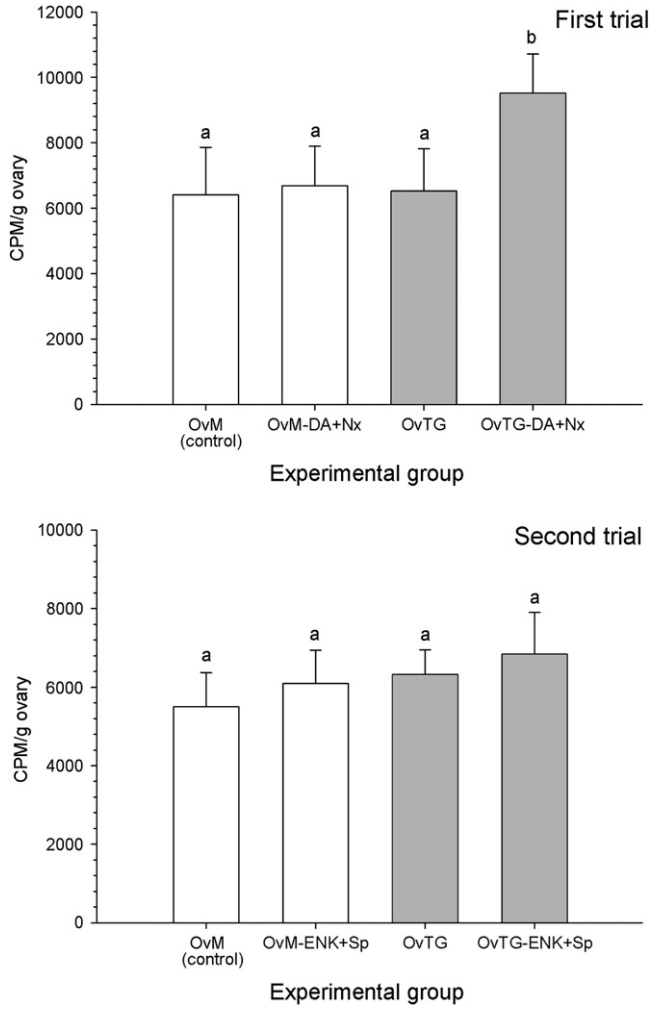

Fig. 3. Effect of the crossed interaction between antagonist and endogenous ligands on ovarian growth, in terms of leucine incorporation, for both experimental trials. CPM: counts per minute (mean ± standard error). Ov: ovary, M: muscle, TG: thoracic ganglion, ET: eyestalk tissue. DA: dopamine, Nx: naloxone, ENK: met-enkephalin, Sp: spiperone. Different letters indicate significant differences ($p<0.05$).

4. Discussion

By means of *in vitro* assays similar to those carried out in the present study (i.e., measuring incorporation or tritiated leucine to ovarian proteins), Kulkarni et al. (1991) have reported that endogenous protein synthesis seems to occur in the ovary of crayfish during the entire ovarian maturation process. Moreover, the same authors have shown that the addition of eyestalk tissue, brain or thoracic ganglion to the *in vitro* preparation of ovaries can change the protein synthesis of either early, mid- or late vitellogenic stages of *P. clarkii*.

In all the assays made in the present study, the absence of significant differences in ovarian growth when any of the assayed compounds, alone or in combination, was added to the control preparation (i.e. ovary plus muscle) indicates that there was no direct effect of those compounds on the ovary, at least under these experimental conditions.

Significant growth of the ovary co-incubated with thoracic ganglion was seen during the pre-reproductive period, regardless of the presence or absence of the tested antagonists. This result is in accordance with the expected increment in GSH secretion by the thoracic ganglion during this period, in order to stimulate the ovary for entering into the vitellogenic stage (Abdu et al., 2000; Tsukimura, 2001). However, the addition of spiperone or naloxone to the preparation of ovary plus thoracic ganglion did not enhance ovarian growth, which suggests that within the physiological context corresponding to the pre-reproductive period (i.e., a probably high and sustained GSH secretion rate aimed at stimulating vitellogenesis in the ovary, as mentioned above) GSH secretion would not be physiologically arrested, due to a normal decrement in the secretion of endogenous dopamine and/or enkephalins as neurotransmitters in the thoracic ganglion (Fingerman, 1997; Zapata et al., 2003). The possibility of a low responsiveness of the thoracic ganglion to dopamine and/or enkephalins due to down-regulation of their receptors, or to any change in the intracellular signal transduction mechanism, is also plausible. In any case, no significant effect of either dopamine or enkephalin antagonists would be expected.

According to the model proposed by Fingerman (1997), both dopamine and enkephalins would also be able to inhibit ovarian growth by stimulating GIH secretion in the eyestalks. According to our results, this stimulatory action would not be relevant during the pre-reproductive period of *C. quadricarinatus*. The fact that the preparation of ovary plus eyestalk tissue (with no antagonist added) was not different from the control (ovary plus muscle) supports that possibility. However, some other possibilities should be considered as plausible explanations, such as the irresponsiveness of the eyestalks to dopamine/enkephalins, as mentioned above for thoracic ganglion, as well as the insensitivity of the ovary to GIH due either to a possible down-regulation of GIH receptors or to changes in its transductional pathway, as suggested for the molt-inhibiting hormone (MIH) during premolt (Chung and Webster, 2003). Any of these considerations agree with the no statistically significant results obtained after the addition of either spiperone or naloxone to the preparation of ovary plus eyestalk tissue. Again, in the physiological

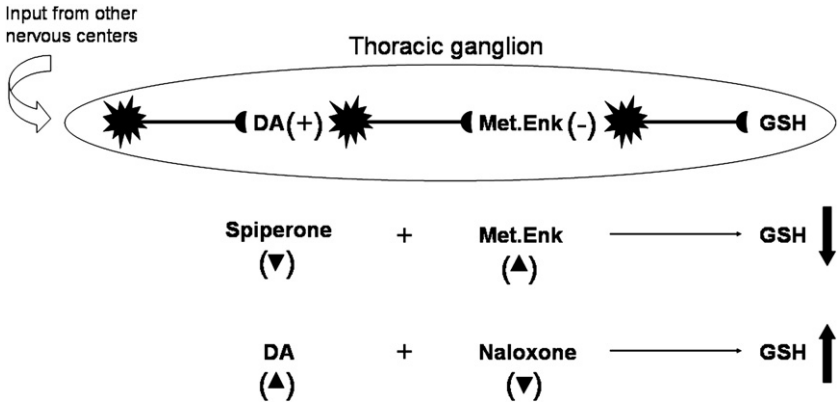

Fig. 4. Model proposed to explain the interaction between dopaminergic and enkephalinergic pathways in the regulation of GSH secretion by thoracic ganglion. (+): excitatory synapses; (−): inhibitory synapses; (▲): agonistic effect; (▼): antagonistic effect.

context corresponding to the pre-reproductive period (low levels of dopamine and enkephalins as endogenous neurotransmitters), the effects of both tested antagonists were negligible.

On the contrary, during the assays carried out during the post-reproductive period, the sole addition of thoracic ganglion to the incubation medium was not able to significantly enhance ovarian growth, in terms of leucine incorporation to proteins. This result was in accordance with the expected low or null secretion of GSH by the thoracic ganglion during this period (Adiyodi and Subramonian, 1983), this secretion being inhibited by either dopamine or enkephalins (Fingerman, 1997). Expectantly, when spiperone or naloxone was added to the preparation of ovary plus thoracic ganglion, a very clear and significant increment of ovarian growth was observed. Therefore, the effect of both antagonists seems to be related to the blocking they are exerting on the dopaminergic or enkephalinergic nervous pathways, which would be in turn inhibiting GSH secretion by neuroendocrine cells in the thoracic ganglion. Hence, the results of this study are confirming the model proposed by Fingerman (1997), at least with respect to the dopaminergic and enkephalinergic pathways involved in the neurohormonal control of reproduction carried out by the thoracic ganglion, in crustacean females.

As seen for the pre-reproductive period, the results obtained during the post-reproductive period did not stimulate ovarian growth when eyestalk tissue was added together with any of the tested antagonists. This result was also similar to the one observed in *Macrobrachium rosenbergii* by Chen et al. (2003), as well as to results reported by Tinikul et al. (2008, 2009) for the same species. These authors have proposed that dopamine regulates vitellogenin synthesis by only controlling GSH secretion by thoracic ganglia, but not GIH secretion by the eyestalks. With respect to the opioid system, Hanke et al. (1996) have reported that the highest concentration of enkephalinergic receptors is found in the thoracic ganglion of *Carcinus maenas*, while Sarojini et al. (1997) have proposed the presence of an endogenous opioid system in the thoracic ganglia of *P. clarkii*, responsible for the regulation of GSH release.

The roles of dopamine and enkephalins in different physiological processes have been studied in several crustacean species (Tinikul et al., 2008; Chen et al., 2003; Richardson et al., 1991; Sarojini et al., 1995a,b; Fingerman et al., 1985). For example, Sarojini et al. (1995a) have proved that injections of either dopamine or leucine-enkephalin produced hypoglycemia in intact crayfish *P. clarkii*. The administration of naloxone could suppress the inhibitory effect caused by either of the mentioned ligands on the secretion of CHH in the eyestalks. However, spiperone was only able to block the hypoglycemic effect caused by dopamine, but not by leucine-enkephalin. These results, taken together, suggest the existence of some kind of interaction between dopamine and leucine-enkephalin. Moreover, they are in accordance with the results obtained in the current study.

In the experiment where the crossed interaction between antagonist and endogenous ligands was evaluated, we have seen that naloxone was able to increase ovarian growth in the presence of thoracic ganglion, even when dopamine was also added to the incubation medium. On the other hand, spiperone did not show any effect on the ovary when administered together with met-enkephalin, in the presence of thoracic ganglion. In view of these results, we are proposing (as shown by the model of Fig. 4) that enkephalins would be acting (likely as neurotransmitters) in the last step of the nervous pathway that innervates the neurosecretory cells that produce and secrete GSH in the thoracic ganglia, while dopamine would be involved in a previous sequential synapses of the same pathway. In this way, naloxone would be counteracting the stimulating effect of dopamine on the secretion of endogenous enkephalin, the net result being an increase in GSH secretion. As for the other combination tested, even when spiperone would reduce the secretion of endogenous enkephalins, the addition of exogenous enkephalin (i.e., the last neurotransmitters acting in the pathway), would finally produce inhibition of GSH secretion by the thoracic ganglion.

Acknowledgments

This study was supported by grants from CONICET (PIP5652 and D845 program), and UBACYT 2008-2010 program (EX241). The authors wish to thank Dr. Milton Fingerman for his valuable comments and for helping with the translation of the manuscript.

References

Abdu, U., Yehezkel, G., Sagi, A., 2000. Oocyte development and polypeptide dynamics during ovarian maturation in the red-claw crayfish *Cherax quadricarinatus*. Invertebr. Reprod. Dev. 37, 75–83.

Abdu, U., Davis, C., Khalaila, I., Sagi, A., 2002. The vitellogenin cDNA of *Cherax quadricarinatus* encodes alipoprotein with calcium binding ability, and its expression is induced following the removal of the androgenic gland in a sexually plastic system. Gen. Comp. Endocrinol. 127, 262–272.

Adiyodi, R.G., Subramonian, T., 1983. Arthropoda-Crustacea. In: Adiyodi, K.G., Adiyodi, R.G. (Eds.), Reproductive Biology of Invertebrates. Oogenesis, Oviposition and Oosorption. John Wiley & Sons Ltd., New York, pp. 443–495.

Barki, A., Levi, T., Hulata, G., Karplus, I., 1997. Annual cycle spawning and molting in the red-claw crayfish, *Cherax quadricarinatus*, under laboratory conditions. Aquaculture 157, 239–249.

Cahansky, A.V., López Greco, L.S., Rodríguez, E.M., 2002. Inducción de crecimiento ovárico en *Cherax quadricarinatus*, mediante hormonas y neuroreguladores incorporados en el alimento. CIVA 2002, pp. 720–724. http://www.civa2002.org.

Cahansky, A.V., López Greco, L.S., Rodríguez, E.M., 2003. Incremento de la producción de hembras ovígeras de la langosta de agua dulce *Cherax quadricarinatus* durante el período reproductivo, mediante la administración de hormonas y neuroreguladores. CIVA 2003, pp. 512–517. http://www.civa2003.org.

Cahansky, A.V., Kaiser Dutra, B., Da Silva Castiglioni, D., Turcato Oliveira, G., Bond Buckup, G., Rodríguez, E.M., 2008a. Induction of ovarian growth in *Aegla platensis* (Crustacea, Aeglidae) by means of neuroregulators incorporated to food. Rev. Biol. Trop. 56, 1201–1207.

Cahansky, A.V., Medesani, D.A., Rodríguez, E.M., 2008b. Induction of ovarian growth in the red claw crayfish, *Cherax quadricarinatus*, by the enkephalinergic antagonist naloxone: *in vivo* and *in vitro* studies. Invertebr. Reprod. Dev. 51, 61–67.

Charniaux-Cotton, H., 1985. Vitellogenesis and its control in malacostracan Crustacea. Am. Zool. 25, 197–206.

Chen, Y.N., Fan, H.F., Hsieh, S.L., Kuo, C.M., 2003. Physiological involvement of DA in ovarian development of the freshwater giant prawn, *Macrobrachium rosenbergii*. Aquaculture 228, 383–395.

Chung, J.S., Webster, S.G., 2003. Molut cycle-related changes in biological activity of moult-inhibiting hormone (MIH) and crustacean hyperglycaemic hormone (CHH) in the crab, *Carcinus maenas*. Eur. J. Biochem. 270, 3280–3288.

De Kleijn, D.P.V., Van Herp, F., 1998. Involvement of the hyperglycemic neurohormone family in the control of reproduction in decapod crustaceans. Invertebr. Reprod. Dev. 33, 263–272.

Eastman-Reks, S.B., Fingerman, M., 1985. *In vitro* synthesis of vitellin by the ovary of the fiddler crab *Uca pugilator*. J. Exp. Zool. 233, 111–116.

Fingerman, M., 1995. Endocrine mechanism in crayfish, with emphasis on reproduction and neurotransmitter regulation of hormone release. Am. Zool. 35, 68–78.

Fingerman, M., 1997. Roles of neurotransmitters in regulating reproductive hormone release and gonadal maturation in decapod crustaceans. Invertebr. Reprod. Dev. 31, 47–54.

Fingerman, M., Kulkarni, G., 1991. Biologically active peptides of crustacean nervous system: their roles as neurohormones, neurotransmitters and neuromodulators. In: Thompson, M., Sarojini, R., Nagabhushanam, R. (Eds.), Bioactive Compounds from Marine Organism. Oxford & IBH Publishing Co., India, pp. 237–242.

Fingerman, M., Hanumante, M., Kulkarni, G., Ikeda, R., Vacca, L., 1985. Localization of substance P-like, leucine-enkephalin-like, methionine-enkephalin-like, and FMRFamide-like inmunoreactivity in the eyestalk of the fiddler crab, *Uca pugilator*. Cell Tissue Res. 241, 473–477.

Hanke, J., Willig, A., Jaros, P., 1996. Opioid receptor types for endogenous enkephalin in the thoracic ganglion of the crab, *Carcinus maenas*. Peptides 17, 965–972.

Jones, C.M., 1997. The Biology and Aquaculture Potential of the Tropical Freshwater Crayfish *Cherax quadricarinatus*. Department of Primary Industries, Brisbane, Australia.

Karplus, I., Gideon, H., Barki, A., 2003. Shifting the natural spring-summer breeding season of the Australian freshwater crayfish *Cherax quadricarinatus* into the winter by environmental manipulations. Aquaculture 220, 277–286.

Khalaila, I., Manor, R., Weil, S., Granot, Y., Keller, R., Sagi, A., 2002. The eyestalk-androgenic gland-testis endocrine axis in the crayfis *Cherax quadricarinatus*. Gen. Comp. Endocrinol. 127, 147–156.

Kulkarni, G., Glade, L., Fingerman, M., 1991. Oogenesis and effects of neuroendocrine tissues on *in vitro* synthesis of protein by the ovary of the red swamp crayfish *Procambarus clarkii* (Girard). J. Crust. Biol. 11, 513–521.

Lawrence, C., Jones, C., 2002. Cherax. In: Holdrich, D. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, Nottingham, pp. 635–669.

Meusy, J., Charniaux-Cotton, H., 1984. Endocrine control of vitellogenesis in Malacostraca crustaceans. Adv. Invertebr. Reprod. 3, 231–241.

Nagabhushanam, R., Sarojini, R., Reddy, P., Devi, M., Fingerman, M., 1995. Opioid peptides in invertebrates: localization, distribution and possible functional roles. Curr. Sci. 69, 659–671.

Richardson, H.G., Deecaraman, M., Fingerman, M., 1991. The effect of biogenic amines on ovarian development in the fiddler crab, *Uca pugilator*. Comp. Biochem. Physiol. 99C, 53–56.

Rodríguez, E.M., Medesani, D.A., López Greco, L.S., Fingerman, M., 2002a. Effects of some steroids and other compounds on ovarian growth of the red swamp crayfish, *Procambarus clarkii*, during early vitellogenesis. J. Exp. Zool. 292, 82–87.

Rodríguez, E.M., López Greco, L.S., Medesani, D.A., Laufer, H., Fingerman, M., 2002b. Effect of methyl farnesoate, alone and in combination with other hormones, on ovarian growth of the red swamp crayfish, *Procambarus clarkii*, during vitellogenesis. Gen. Comp. Endocrinol. 125, 34–40.

Sarojini, R., Nagabhushanam, R., Fingerman, M., 1995a. Dopaminergic and enkephalinergic involvement in the regulation of blood glucose in the red swamp crayfish, *Procambarus clarkii*. Gen. Comp. Endocrinol. 97, 160–170.

Sarojini, R., Nagabhushanam, R., Fingerman, M., 1995b. Evidence for opiod involvement in the regulation of ovarian maturation of the fiddler crab, *Uca pugilator*. Comp. Biochem. Physiol. 111A, 279–282.

Sarojini, R., Nagabhushanam, R., Fingerman, M., 1996. *In vivo* assessment of opiod agonist and antagonist on ovarian maturation in the red swamp crayfish, *Procambarus clarkii*. Comp. Biochem. Physiol. 115C, 149–153.

Sarojini, R., Nagabhushanam, R., Fingerman, M., 1997. An *in vitro* study of the inhibitory action of methionine enkephaline on ovarian maturation in the red swamp crayfish, *Procambarus clarkii*. Comp. Biochem. Physiol. 117C, 207–210.

Serrano-Pinto, V., Landais, I., Ogliastro, M.-H., Gutiérrez-Ayala, M., Mejía-Ruiz, H., Villarreal-Colmenares, H., García-Gasca, A., Vázquez-Boucard, C., 2004. Vitellogenin mRNA expression in *Cherax quadricarinatus* during secondary vitellogenesis at first maturation females. Mol. Reprod. Dev. 69, 17–21.

Sokal, R.R., Rohlf, F.J., 1981. Biometry, 2nd ed. Freeman, NY.

Tinikul, Y., Joffre Mercier, A., Soonklang, N., Sobhon, P., 2008. Changes in the levels of serotonin and dopamine in the central nervous system and ovary, and their possible roles in the ovarian development in the giant freshwater prawn, *Macrobrachium rosenbergii*. Gen. Comp. Endocrinol. 158, 250–258.

Tinikul, Y., Mercier, J.A.J., Sobhon, P., 2009. Distribution of dopamine and octopamine in the central nervous system and ovary during the ovarian maturation cycle of the giant freshwater prawn, *Macrobrachium rosenbergii*. Tissue Cell 41, 430–442.

Tsukimura, B., 2001. Crustacean vitellogenesis: its role in oocyte development. Am. Zool. 41, 465–476.

Villarreal Colmenares, H., Naranjo Páramo, J., 2008. Cultivo de langosta de agua dulce *Cherax quadricarinatus* (redclaw). Una oportunidad para la diversificación de la industria acuícola. Centro de Investigaciones Biológicas del Noroeste, México.

Wainwright, G., Rees, H.H., 2001. Hormonal regulation of reproductive development in crustaceans. In: Atkinson, D., Thorndyke, M. (Eds.), Environment and Animal Development. Bios Scientific Publishers Limited, Oxford, pp. 71–84.

Zapata, V., López Greco, L.S., Medesani, D.A., Rodríguez, E.M., 2003. Ovarian growth in the crab *Chasmagnathus granulata* induced by hormones and neuroregulators throughout the year. *In vivo* and *in vitro* studies. Aquaculture 224, 339–352.