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In vivo inhibition by dopamine of 5-hydroxytryptamine-stimulated ovarian maturation in the red swamp crayfish, *Procambarus clarkii*

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Abstract. In vivo dopamine antagonizes the ovary-stimulating action of 5-hydroxytryptamine in the red swamp crayfish, *Procambarus clarkii.*
Key words. Crayfish; dopamine; 5-hydroxytryptamine; ovary; *Procambarus clarkii.*

Biogenic amines function as neurotransmitters in a wide array of animals[1-4]. Several such amines have been found in nervous organs of crustaceans, including crayfishes[5-11], where one of their roles as neurotransmitters is the regulation of release of several neurohormones[12]. Dopamine (DA) stimulates release of chromatophorotropic red and black pigment-concentrating hormones[13,14] and the distal retinal pigment dark-adapting hormone[15], whereas among the roles of 5-hydroxytraptamine (5-HT) is stimulation of release of the crayfish hyperglycemic hormone[16], red pigment-dispersing hormone[17], neurodepressing hormone[18] and molt-inhibiting hormone[19].

With specific regard to reproduction, in decapod crustaceans the major neuroendocrine component of the eyestalk, the medulla terminalis X-organ-sinus gland complex, is the source of gonad-inhibiting hormone (GIH)[20]. A gonad stimulating hormone (GSH) has been found in the brain and thoracic ganglia[21-23]. In addition to the roles of 5-HT mentioned above, in vivo it stimulates ovarian maturation in the fiddler crab, *Uca pugilator*[24], and the red swamp crayfish, *Procambarus clarkii*[25]. This stimulatory effect of 5-HT on the ovary is an indirect one. 5-HT does not have a stimulatory effect on ovaries incubated in vitro[25]. DA and 5-HT have antagonistic actions on color changes of the fiddler crab, *Uca pugilator*; 5-HT stimulates the release of red pigment-dispersing hormone whereas DA stimulates release of its antagonist, red pigment-concentrating hormone. It was therefore of interest to determine whether DA might also antagonize the in vivo ovary-stimulating action of 5-HT in *Procambarus clarkii.*

Materials and methods

Red swamp crayfish, *Procambarus clarkii,* were procured from a local seafood dealer and maintained in freshwater tanks where the water was recirculated through sand filtration units. During the experimental period the animals were fed commercially prepared crayfish food daily. Intermolt females with a carapace length of 30–35 mm and a body weight of 15–16 g were used. 5-HT creatinine sulfate and DA hydrochloride were purchased from Sigma Chemical Corp. For each experiment 75 female crayfish were removed from the stock supply and divided into five groups of 15 crayfish each. The first group, which served as the initial control and did not receive any treatment, was sacrificed on the first day of the experiment. The second group, which served as a simultaneous control, was treated the same as the experimental groups but received injections of crayfish physiological saline[26]. Groups three and four received injections of 10^{-6} mol/crayfish of DA or 5-HT respectively in a dose of 100 µl per injection. Group five received injections of 10^{-6} mol/crayfish of DA in 50 µl followed by 10^{-6} mol/crayfish of 5-HT in 50 µl 30 minutes after the DA injection. The drugs were dissolved in crayfish physiological saline. Groups 2–5 were given injections on the first, fifth and tenth day, and were sacrificed on day 15.

The amount of each drug used in this study is similar to the amounts of 5-HT used with the shore crab, *Carcinus maenas* (up to 1.1×10^{-6} mol/crab[27]) and the American lobster, *Homarus americanus* (up to 5.7×10^{-5} mol/lobster[28]) and of DA used with *Carcinus maenas* (up to 2.5×10^{-7} mol/crab[29]). The concentration of 5-HT in the hemolymph of the American lobster is 1.8×10^{-9} M (ref. 28). After sacrifice the crayfish were weighted and their ovaries were removed, weighed and fixed in aqueous Bouin's fluid. After 24 hours of fixation, the tissues were dehydrated in a series of alcohols and then embedded in paraffin (m.p. 56–58 °C). Sections 7 µm thick were cut and stained with Delafield's hematoxylin and counterstained with alcoholic eosin[30]. Oocytes (50 per crayfish) were measured using an ocular micrometer. Ovarian indexes were determined using the standard formula:

$$\text{Ovarian index} = \frac{\text{wet weight of ovary}}{\text{wet weight of crayfish}} \times 100$$

Here the term ovary refers to all ovarian tissue in a single crayfish. The experiment was repeated once. The results of both experiments were consistent. They were

averaged and the means were used to prepare the figures.

Results and discussion

The oocytes of the initial control crayfish were in the immature stage, according to the stages of Kulkarni et al.[31], whereas by the end of the experiment the oocytes of the simultaneous control crayfish had advanced to the avitellogenic stage. 5-HT administration produced a much larger mean ovarian index than was found for the simultaneous control, whereas treatment with DA alone resulted in a somewhat smaller mean ovarian index than in the simultaneous control specimens (fig.). The crayfish given DA plus 5-HT had a significantly smaller mean ovarian index than the crayfish that received 5-HT alone, but a larger one than the crayfish that received DA alone. The changes in the mean oocyte diameters of groups 2–5, as compared with the initial control group, were qualitatively identical to the changes observed in the ovarian indexes of the same group. An increase of the ovarian index reflects an increase in oocyte diameter. 5-HT treatment increased the oocyte diameters significantly ($p < 0.05$) compared with the simultaneous controls. The oocytes of the crayfish given 5-HT developed from the avitellogenic stage to the mid-vitellogenic stage. The crayfish that received the combination of DA and 5-HT had oocytes that were in the avitellogenic stage, but were larger than those in the crayfish that received DA alone, though they were significantly smaller than those of the crayfish that received 5-HT alone. With DA treatment alone, the oocytes were in the avitellogenic stage, but were smaller than those of the simultaneous control crayfish. The increases that occurred in oocyte diameter and ovarian index of the simultaneous control crayfish, compared with the corresponding values for the initial controls, are evidence that the ovaries were developing slowly during the course of the study.

5-HT had a stimulatory effect on the ovary, evidenced by the larger oocyte diameters and ovarian indexes in the crayfish given only 5-HT, as compared with both control groups. These results are consistent with previous data that showed that 5-HT triggers release of GSH from neuroendocrine centers of *Procambarus clarkii*, which would bring about ovarian maturation[31].

As stated above, the effects of 5-HT and DA on the erythrophores of the fiddler crab, *Uca pugilator*, are mutually antagonistic[13]. The present results for *Procambarus clarkii* revealed for the first time with any crustacean a similar antagonism between DA and 5-HT with respect to ovarian maturation. DA has an inhibitory effect on ovarian maturation induced by 5-HT in vivo.

Because 5-HT exerts its effect on the ovary indirectly, by stimulating GSH release, the inhibitory action of DA on ovarian maturation induced by 5-HT could have been due to 1) inhibition of GSH release, thereby directly counteracting the action of 5-HT, 2) stimulation of release of the GSH antagonist, GIH, or 3) both 1) and 2). Experiments are in progress to establish which of these alternatives is correct. Nevertheless, it is clear from the figure that DA does indeed inhibit the in vivo ovary-stimulating action of 5-HT when both DA and 5-HT are injected into the same crayfish.

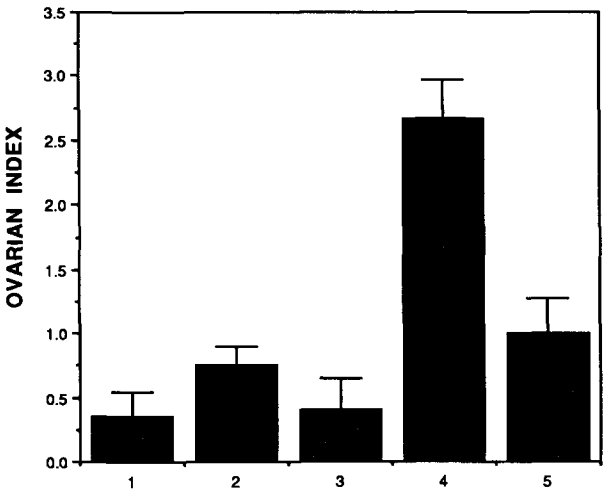

Figure. Effects of injections of DA, 5-HT and DA plus 5-HT on the mean ovarian index of the crayfish, *Procambarus clarkii*. *1* Initial control; *2* Simultaneous control; *3* DA; *4* 5-HT; *5* DA plus 5-HT. Error bars are SEM.

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