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

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Abstract. Dopamine inhibits 5-hydroxytryptamine-stimulated maturation of the ovaries of the red swamp crayfish, *Procambarus clarkii*, in vitro just as it does in vivo. This in vitro inhibition appears to be due to inhibition of release of the gonad-stimulating hormone from the brain and thoracic ganglia. However, it is possible that in vivo dopamine also triggers release of the gonad-inhibiting hormone.

Key words. Crayfish; dopamine; 5-hydroxytryptamine; ovary; *Procambarus clarkii*.

Biogenic amines, such as dopamine (DA) and 5-hydroxy-tryptamine (5-HT), function as neurotransmitters in many animals, both invertebrate and vertebrate[1–4]. More specifically, both DA and 5-HT are present in nervous organs of crustaceans, including crayfishes[5–11]; and among the roles of DA and 5-HT in crustaceans is that of stimulating release of several neurohormones[12]. Aside from possible roles of DA and 5-HT in reproduction, which will be discussed below, DA triggers release of chromatophorotropic red and black pigment-concentrating hormones[13,14] and the distal retinal pigment dark-adapting hormone[15], whereas 5-HT is known to stimulate release of the crayfish hyperglycemic hormone[16], red pigment-dispersing hormone[17], neurodepressing hormone[18] and molt-inhibiting hormone[19].

In decapod crustaceans, the major neuroendocrine complex of the eyestalk, the medulla terminalis X-organ-sinus gland complex, is the source of the gonad-inhibiting hormone (GIH)[20], whereas 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]. However, this stimulatory effect of 5-HT on the ovary is an indirect one, 5-HT presumably triggering GSH release from the brain and thoracic ganglia[26]. 5-HT has no stimulatory effect on ovarian explants in the absence of brain or thoracic ganglia from the incubation medium. In an in vivo study with *Procambarus clarkii*, Sarojini et al.[27] demonstrated that DA antagonizes the ovary-stimulating action of 5-HT. The study described below was designed to investigate further this antagonistic action of DA through in vitro experiments with ovarian explants from *Procambarus clarkii*.

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Materials and methods

Red swamp crayfish, *Procambarus clarkii*, were purchased from a local seafood dealer and maintained in freshwater tanks where the water circulated through sand filtration units. The animals were fed commercially prepared crayfish food daily. Intermolt females having a carapace length of 30–35 mm and a body weight of 15–16 g were selected from the stock for this study. An in vitro ovarian bioassay was used. Ovary refers herein to all the ovarian tissue in one crayfish. The ovary of each crayfish used was dissected from the cephalothorax, washed in crayfish physiological saline[28] containing 60 mg/100 ml penicillin G and cut into four or five pieces that were then placed into a culture vial containing 2 ml of culture medium (Medium 199) with 6 mg/100 ml penicillin G. The tissues were then incubated in darkness at 24 ± 2 °C in an orbital shaker (50–60 rpm) for 24 h. All of the reagents used were purchased from Sigma Chemical Co. Solutions of 5-HT creatinine sulfate and DA hydrochloride were prepared in crayfish saline. After incubation, the explants were fixed in aqueous Bouin's fluid for 24 h, dehydrated in an alcoholic series and embedded in paraffin (m.p. 56–58 °C). Sections 7 µm in thickness were cut and stained with Delafield's hematoxylin followed by counterstaining in alcoholic eosin[29]. The diameters of 100 oocytes in each ovary were then measured using a compound microscope with an ocular micrometer. The data presented in the figure represent the means of 30 replicates. The measurements were analyzed by means of Student's *t* test with significance set at the 95% confidence interval. Standard errors of the means (SEM) were also calculated. The four experiments were done concurrently.

Experiments and results

Experiment 1. The aim of this experiment was twofold: (1) to confirm that brain and thoracic ganglia stimulate

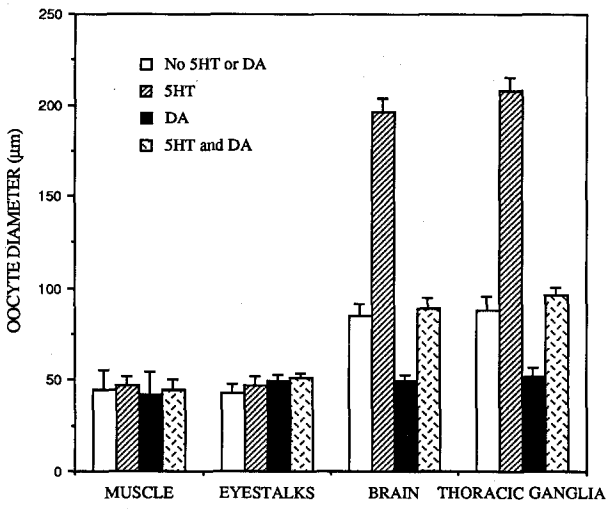

Figure. The mean oocyte diameters (µm) of ovarian explants from *Procambarus clarkii* after 24 h of incubation with muscle, eyestalk tissue, brain or thoracic ganglia in culture medium alone, or with 5-HT, DA or 5-HT plus DA. Error bars are SEM.

ovarian maturation in vitro and (2) to obtain baseline data with which to compare with the results of the three following experiments. Into a vial were placed the tissues removed from two eyestalks, one brain, the thoracic ganglia of one crayfish or a piece of muscle approximately the same size as the other tissues along with the cut-up ovary, the culture medium and 100 µl of crayfish saline. The oocytes in the ovarian explants that had been incubated with muscle alone were in the immature stage[25] with a mean diameter of 42 µm (fig.). At this stage the oocytes are surrounded by ovarian stromatal tissue. The oocyte nucleus is centrally located, with a well-defined nuclear membrane, and is large in relation to the cytoplasm. No follicle cells occur around the oocytes. The oocytes of the ovarian tissue incubated with eyestalk tissue averaged 40 µm in diameter and had the same cytological characteristics as the oocytes incubated with muscle. The ovarian explants that were incubated with brain or thoracic ganglia showed significant development and had entered the avitellogenic phase, with their oocytes averaging 80 µm and 86 µm respectively, presumably due to GSH released from these nervous tissues.

Experiment 2. The aim of this experiment was not only to provide additional evidence that the ovary-stimulating effect of 5-HT is not due to direct action on the ovary but also to lay the basis for comparison with the effect of DA on ovarian maturation. The protocol for this experiment was same as that of experiment 1 except that to each culture vial in experiment 2 10^{-6} mol of 5-HT creatinine sulfate in 100 µl of saline was added. This is the amount of 5-HT added to each vial, not the final concentration of 5-HT in each vial. The ovarian explants that were incubated with 5-HT and muscle did not show significant development as compared with the explants exposed to muscle alone (fig.). Similarly, the ovarian explants incubated with the eyestalk tissue, with or without 5- HT, showed no significant difference in oocyte diameter compared with each other or with any of the explants incubated with muscle. In contrast, the ovarian explants that were incubated with brain and 5-HT or thoracic ganglia and 5-HT showed significant development as compared with the explants incubated with brain or thoracic ganglia alone, presumably due to 5-HT-stimulated release of GSH. The oocytes exposed to brain plus 5-HT or thoracic ganglia plus 5-HT, having entered the early vitellogenic phase, contained yolk granules.

Experiment 3. The aim of this experiment was to learn whether the inhibitory action of DA on ovarian maturation that was observed in the previous in vivo study[27] could be duplicated in vitro. The protocol for this experiment was same as for experiment 1 except that to each culture vial in experiment 3 10^{-6} mol of DA hydrochloride in 100 µl of saline was added. As with 5-HT, this is the amount of DA added to each vial, not the final concentration of DA in each vial. The ovarian explants that were incubated with DA and muscle did not show significant change. Likewise, the ovarian explants incubated with the neuroendocrine tissues, eyestalk, brain or thoracic ganglia, and DA showed no significant differences in mean oocyte diameter compared with each other or with any of the explants incubated with muscle (fig.). Furthermore, the oocytes in the explants incubated with DA and brain or thoracic ganglia were significantly smaller than the oocytes incubated with just brain or thoracic ganglia. Presumably, DA inhibited GSH release from these nervous tissues.

Experiment 4. The aim of this experiment was to determine in vitro whether DA can antagonize the stimulatory action of 5-HT. The experimental protocol for this experiment was same as in experiment 1 except that to each culture vial in experiment 4 10^{-6} mol 5-HT in 50 µl of saline and 10^{-6} mol DA in 50 µl of saline were added. The ovarian explants that were incubated with muscle and 5-HT plus DA did not show significant development as compared with the explants incubated with muscle alone or with muscle and 5-HT or DA. Similarly, the ovarian explants incubated with the eyestalk tissue and 5-HT plus DA showed no significant change in mean oocyte diameter. The ovarian explants that were incubated with DA plus 5-HT and brain or thoracic ganglia contained oocytes in the avitellogenic stage which were significantly larger than the oocytes of the ovarian explants incubated with these tissues and DA alone, but not significantly different from the ovaries incubated with brain or thoracic ganglia alone (fig.). However, the oocytes incubated with 5-HT plus DA and brain or thoracic ganglia were significantly smaller than the oocytes of the ovarian explants that were incubated with just 5-HT and brain or thoracic ganglia, presumably due to DA inhibition of 5-HT-stimulated release of GSH.

Discussion

Organ incubation techniques for studying the effects on target organs in crustaceans have not been used frequently. With respect to in vitro studies of crustacean ovaries, Eastman-Reks and Fingerman[23] showed that the thoracic ganglion of *Uca pugilator* stimulated endogenous ovarian vitellin synthesis, and Kulkarni et al.[25] observed that brain and thoracic ganglia of *Procambarus clarkii* stimulated incorporation of leucine into ovarian proteins.

In the present study, when the ovarian explants of *Procambarus clarkii* were incubated with muscle and 5-HT, ovarian development did not take place. However, in the presence of brain or thoracic ganglia not only did ovarian development occur, as evidenced by significant increases in the oocyte diameters, but this effect was significantly enhanced by 5-HT (fig.). These results are consistent with previously derived data that showed 5-HT triggers release of GSH from neuroendocrine centers of *Procambarus clarkii* to bring about ovarian maturation[25,27].

In contrast, DA inhibited ovarian maturation, as evidenced by the significantly smaller diameters of the oocytes of the ovarian explants that were incubated with DA and brain or thoracic ganglia as compared with the explants incubated with brain or thoracic ganglia alone (fig.). Furthermore, when DA and 5-HT were both added to the culture vials, DA inhibited the stimulatory action of 5-HT (fig.). The amount of 5-HT and DA used in this study is quite like the amounts of biogenic amines used with the shore crab, *Carcinus maenas*[30,31], the American lobster, *Homarus americanus*[32], and the crayfish used in the present study, *Procambarus clarkii*[33].

As mentioned above, Sarojini et al.[27] in an in vivo experiment showed an antagonism between DA and 5-HT with respect to ovarian maturation in *Procambarus clarkii*. On the basis of the in vivo data it was suggested that the inhibitory action of DA on ovarian maturation was due to (1) inhibition of GSH release, thereby directly counteracting the GSH-releasing action of 5-HT, (2) stimulation of the release of the GSH antagonist, GIH, or (3) both (1) and (2)[27]. While one of the in vivo actions of DA may be to stimulate GIH release from the eyestalks, the in vitro data presented here that show the combination of DA plus 5-HT and brain or thoracic ganglia produced significantly less ovarian development than did just 5-HT with either of these tissues provide evidence that DA does indeed at least inhibit GSH release.

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