Effect of Adrenaline on Hypothalamic Neurohormone Stimulation of the Functional Activity of the Sterlet Interrenal Gland *In Vitro*

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In vitro experiments on sterlet kidney fragments with interrenal glands showed that vasotocin and oxytocin activated the function of these glands, while simultaneous exposure to these neurohormones and adrenaline virtually did not modify the functional activity of the glands.

Key Words: *interrenal glands; vasotocin; oxytocin; adrenaline; Acipenseridae*

It is no longer doubted that hypothalamic (vasotocin and mesotocin) and catecholamine neurohormones affect the function of the interrenal gland (IRG) in amphibians. *In vitro* experiments demonstrated that the effects of these neurohormones on IRG function are realized via specific receptors [5,7-10].

Only indirect evidence on the stimulating effect of vasotocin (VT) on IRG in intact and hypophysectomied fishes has been obtained [1].

Previously we demonstrated a stimulatory effect of VT and oxytocin (OT) on functional activity of IRG in sturgeon hybrids *in vitro*. By contrast, incubation of kidney fragments with IRG in the presence of adrenaline markedly inhibited their function.

We investigated *in vitro* effects of hypothalamic neurohormones VT and OT and their combinations with adrenaline on IRG function in sterlets and compared the reaction of specimens from natural and artificial fish populations.

MATERIALS AND METHODS

Experiments were carried out on 10 adult (weighing about 700 g) sterlets of both sexes from the Volga river at the end of November in Astrakhan (Ikryaninsk Fish Breeding Plant) at water temperature 7°C and air temperature 9°C. Before the experiment the sterlets were kept for 7 days in a reservoir with flowing Volga water for adaptation.

Kidney fragments with IRG were incubated in 3 ml culture medium. The bottom well was coated with bactoagar (1% bactoagar solution was prepared on HEPES adjusted to pH 7.4 with 8 mM NaOH) [2]. Culture medium was normal saline for cold-blooded animals with ionic composition adjusted to sturgeon serum [4] (g/liter): 6.72 NaCl, 0.23 KCl, 0.12 $MgCl_2$, 0.1 NaH_2PO_4, 0.004 Na_2HPO_4, 0.3 $CaCl_2$, 0.67 $NaHCO_3$, and 0.99 glucose, pH 7.4. Before the experiment, the medium was aerated for 10 min with an acquarium air vent.

Preincubation was carried out for 60 min in 2 portions of culture medium, after which the samples were incubated with (experiment) and without (control) hormones for 60 min.

The reaction of IRG to VT (1 nM) and OT (1 nM) alone or in combination with adrenalin (100 ng/ml) was evaluated. The hormone concentrations previously used in experiments [3,11] were selected with consideration for their concentration in the blood of cold-blooded animals.

After incubation the material was fixed in Bouin's fluid and treated routinely for histological analysis. Paraffin sections (5-6 μ) were stained with azane according to Heidenhain. The morphology of the glands was examined and morphometric analysis was performed. The diameters of steroidogenic cell nuclei

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were measured and their volume was estimated by the formula for rotation ellipsoid: $V=\pi/4D\times d^2$, where D and d are the long and short axes of the nucleus, respectively. The significance of differences was evaluated using Student's t test.

RESULTS

Preliminary methodological experiments showed that incubation of IRG in culture medium for up to 120 min did not notably modify their structure. IRG cells were arranged in cords separated from each other by sinusoidal capillaries. Cell nuclei were round or oval, nucleoli were clearly seen, no pyknomorphic cells were detected.

Nonapeptide neurohormones considerably activated the function of IRG. The nuclei of steroidogenic cells increased in size under the effect of VT or OT by 47 and 28%, respectively ($p<0.05$).

Combined exposure to adrenaline and OT increased the size of nuclei of steroidogenic cells by only 3% in comparison with the control, while adrenaline+VT caused no changes (Fig. 1).

Therefore, adrenaline abolished the stimulating effect of both VT and OT.

There are no published data about combined effects of adrenaline and nonapeptide neurohormones on IRG function in fishes, but dopamine and adrenaline are known to suppress the stimulatory effect of VT in amphibians [3,6].

Thus, our findings are in line with previous reports and suggest that nonapeptide and catecholamine neurohormones are involved in the regulation of IRG in sturgeon hybrids and sterlet under natural conditions. These neurohormones are released into the blood during stress, prevent exhaustion of IRG, and maintain the optimal level of corticosteroids in the organism.

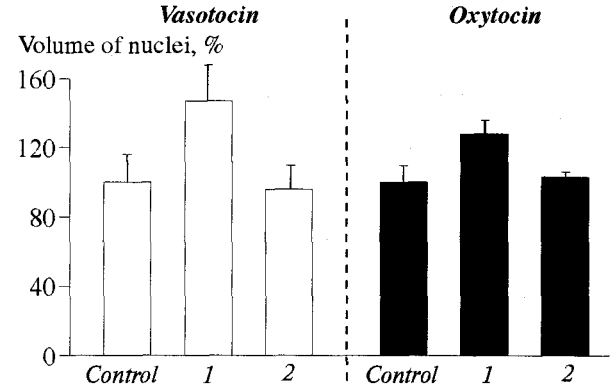

Fig. 1. Changes in the size of cell nuclei in the interrenal gland after incubation in the presence of hormones alone (*1*) and in combination with adrenaline (*2*).

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