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NICOTINE STIMULATES *IN VITRO* GtH2 RELEASE FROM COMMON CARP (*Cyprinus carpio* L.) PITUITARY CELLS.

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Introduction

Maturational gonadotropin (GtH2) secretion in teleost fish is under complex hormonal and neurohormonal control. There is more and more evidence that beside two main regulators: GnRH and dopamine also other neuromediators such as NPY (1), GABA (2), and serotonin (3) are involved in different manner in the control of GtH2 release. Up to now there is no data about influence of the cholinergic system on GtH2 secretion in fish. In this study we investigated the effect of different cholinergic drugs on the release of GtH2 from dispersed carp pituitary cells in culture as well as in dynamic perifusion system.

Material & methods

For enzymatical dispersion of the pituitary cells the glands were taken from seven year old female carps during their reproductive period. After dispersion the cells were placed in thermoregulated perifusion columns or plated into culture wells for primary culture. Perifusion experiments started after 16 hours equilibration period and were performed at 18°C, at a flow rate of 15 ml/h. In the culture experiments cells were plated in 96-well plates (5 x 10^{-4} cells/well) precoated with poly-L-lysine (5ug/cm^2) and maintained at 24°C. After 60 h of culture they were washed and then reincubated in medium with or without tested drugs. Specific RIA (perif. exps) or ELISA (cult. exps) were used to measure GtH2 levels in the medium.

Results and disscussion

In the perifusion exps increasing doses of nicotine applied in a form of 12.5 min pulses provoked a dose dependent stimulation of GtH2 release. Concentrations of nicotine of 10^{-8}, 10^{-7} and 10^{-6}M increased the GtH2 secretion level by 39, 122 and 521 % respectively (mean GtH2 level was 28ng/ml). Other cholinergic drugs such as muscarine and carbachol had very weak effect on the GtH2 secretion in comparison with nicotine. In culture experiments nicotine significantly increased GtH2 secretion after 30 min of stimulation, starting from a concentration 10^{-10}M and reaching the plateau at the concentration of 10^{-8}M. Nicotine present in culture medium for longer time (1,3,6 hr) had no effect on GtH2 secretion as well as all the other cholinergic drugs at any sampling time. Our results bring an evidence that cholinergic system could be directly involved in the neuroendocrine control of GtH2 secretion in carp. However, very weak effect of ACh, carbachol and muscarine shows that structure-funcional differences could exist between cholinergic receptors in mammals and fish. Another possible explaination is that nicotine stimulates GtH2 secretion by activating its own noncholinergic receptor. Existence of such a type of receptor has been already demonstrated in mammals (4).

References

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