

## The control of oocyte maturation in the starfish and amphibians by serotonin and its antagonists

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**ABSTRACT** Serotonin acts as antagonist of progesterone on amphibian oocytes. Serotonin antagonists inhibit maturation of starfish oocytes and stimulate or trigger maturation in amphibians. We propose that endogenous serotonin participates in the control of oocyte maturation.

**KEY WORDS:** oocyte maturation, starfish, amphibians, serotonin

Neurotransmitters (acetylcholine, catecholamines, 5-HT) are active throughout ontogenesis including pre-nervous development. In particular, they play the part of triggers and regulators of cleavage divisions (Buznikov, 1967, 1990; Renaud *et al.*, 1983). However, almost nothing is known about their function during oocyte maturation. It is found only that acetylcholine stimulates oocyte maturation in amphibians (Dascal *et al.*, 1985) and 5-HT triggers oocyte maturation in the surf clam *Spisula* (Krantic *et al.*, 1991). In this paper we report the possible role of 5-HT in maturation of the full-grown oocytes of starfish and amphibians. We used 5-HT antagonists IHC and IPHC (the lipophilic tertiary indoleamines which may enter the cytoplasm via the cell membrane), IIM and IPIM (their hydrophilic quaternary analogs which practically do not enter the cytoplasm from incubation medium) (Buznikov, 1990).

5-HT (10-100  $\mu$ M) does not induce the maturation of starfish oocytes but potentiates the effect of 1-MeAde. This effect of 5-HT is retained after removal of the follicular envelope. 5-HT antagonists in concentrations from 1 to 100  $\mu$ M inhibit the oocyte maturation induced by 1-MeAde. Tertiary amines (IHC and IPHC) act similarly on the maturation of intact and denuded oocytes. Their quaternary analogs (IIM and IPIM) affect the denuded oocytes much more strongly, and the intact ones much more weakly than the corresponding amines (Fig. 1). The effect of IIM and IPIM is reversible and can be partly eliminated by 5-HT.

5-HT and its agonists (5-CIT and 5-ET) added to RS or injected into the cells inhibit progesterone-induced maturation of both intact and denuded oocytes of *B. viridis* and *X. laevis*. The sensitivity of *B. viridis* oocytes to 5-HT, 5-CIT and 5-ET begins to rise simultaneously with the rise of sensitivity to progesterone up to maximal level in May-June.

IIM induces true maturation of *B. viridis* and *X. laevis* oocytes without the addition of progesterone. The sensitivity of intact *B. viridis* oocytes to IIM grows from September to February-March and

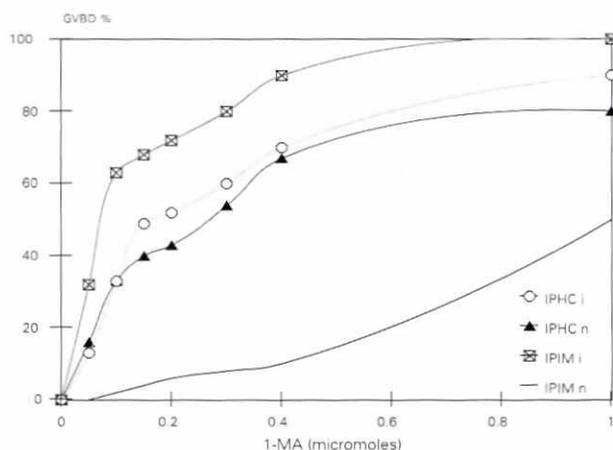
decreases in May-June. Denuded oocytes are insensitive to the maturation-inducing action of IIM from September to March and show maximum sensitivity in May and sometimes in June, i.e. during the breeding season of *B. viridis* (Fig. 2). The sensitivity of denuded oocytes to IIM is higher than that of intact ones (minimally effective concentrations below 20-50 and 100-200  $\mu$ M, respectively). IHC has similar activity. 5-HT weakens the action of both progesterone and 5-HT-antagonists (Fig. 3).

Thus, 5-HT enhances the action of 1-MeAde and inhibits the action of progesterone. In other words, 5-HT can act as a positive modulator of maturation hormone for the starfish and negative modulator or even functional antagonist of such hormone for amphibians. Incidentally, the intact oocytes of the starfish and amphibians contain 5-HT (Buznikov, 1990). 5-HT antagonists IIM (our data) and mianserin (Hanocq-Quertier and Baltus, 1981) induce maturation of denuded amphibian oocytes, i.e. have a progesterone-like action.

5-HT-receptors (or, cautiously speaking, 5-HT-sensitive sites) seem to be located on the surface of the starfish oocyte but neither in its cytoplasm nor in the follicular cells. Evidence of this is the difference between the action of lipophilic and hydrophilic 5-HT antagonists on the intact and denuded starfish oocytes (Fig. 1). A more complicated situation was found for amphibians. High sensitivity of intact *B. viridis* oocytes to IIM is observed already in December-

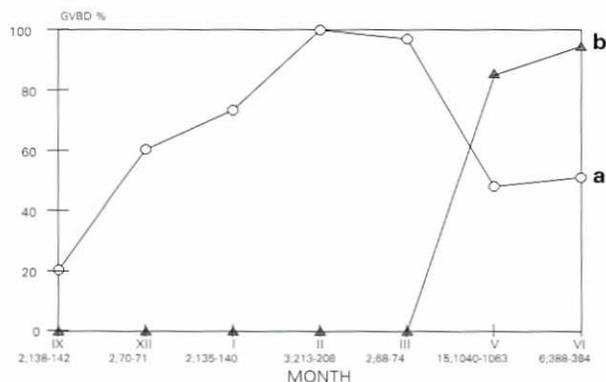
*Abbreviations used in this paper:* ASW, artificial sea water; 5-CIT, 5-chlorotryptamine hydrochloride; 5-ET, 5-ethyltryptamine hydrochloride; GVBD, germinal vesicle breakdown; 5-HT, serotonin; IHC, inmecarb hydrochloride; IIM, inmecarb methiodide; IPHC, indolopyridoazepine hydrochloride; IPIM, indolopyridoazepine methiodide; 1-MeAde, 1-methyladenine; RS, Ringer solution.

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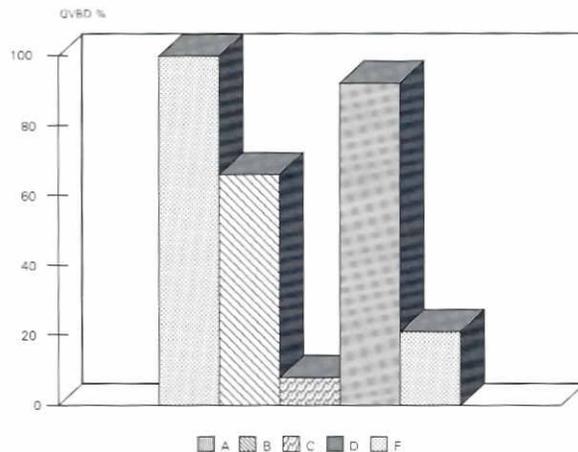


**Fig. 1.** The influence of 5-HT antagonists on the 1-MeAde induced maturation of intact (i) and denuded (n) oocytes of *P. pectinifera* 90 min after the introduction of drugs). Concentrations: IPHC 80  $\mu$ M, IPIM 80  $\mu$ M.

March. In the case of denuded oocytes such sensitivity is not detected until after March (Fig. 2). Therefore, IIM can act on the oocytes via the follicular cell receptors and the oocyte surface receptors. Moreover, there is a third group of receptors (or functional receptor equivalents) — the intracellular ones responsible for the sensitivity of oocytes to the anti-progesterone action of 5-HT and its agonists injected into the cytoplasm. All three groups can participate in 5-HT action on oocyte maturation. We propose that amphibians have a complex multilevel serotonergic system for maintaining the meiosis block in the full-grown oocyte and for the control of meiosis. Different levels of this system are switched on at different times.



**Fig. 2.** The seasonal changes of the sensitivity of intact (a) and denuded (b) *B. viridis* oocytes to 5-HT antagonist IIM, 200  $\mu$ M. The number of females, intact oocytes and denuded oocytes are given for each month (first, second and third number, respectively).



**Fig. 3.** The influence of 5-HT on the progesterone (3.2  $\mu$ M) or IIM (200  $\mu$ M) induced maturation of *B. viridis* denuded oocytes. (A) Progesterone. (B) Progesterone+5-HT 1.2  $\mu$ M. (C) Progesterone+5-HT 2.4  $\mu$ M. (D) IIM. (E) IIM+5-HT 100  $\mu$ M. 120 oocytes (6 females) were used for each variant.

**Experimental Procedures**

The full-grown oocytes of starfish *Patiria (Asterina) pectinifera* and *Aphelasterias japonica* and of anuran amphibians *Bufo viridis* and *Xenopus laevis* were used. The obtaining and handling of these cells, including the composition of incubation medium (ASW for starfish, modified RS for amphibians) and pharmacological techniques used, have been described in our earlier publications. The follicular envelope was removed by means of 3-4-fold washing of oocytes with Ca-free ASW (starfish) and by 40 min collagenase treatment (amphibians); the efficiency of removal was determined *in vivo* by means of light microscopy. The rate of maturation (percentage of GvBD) was evaluated for starfish *in vivo* and for amphibians on stained preparations as also described previously (Nikitina et al., 1988; Buznikov, 1990).

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