Ann. Zool. Fennici 34: 213–216
Helsinki 18 September 1997
ISSN 0003-455X

Commentary

Effect of triiodthyronine on *in vitro* maturation of vendace (*Coregonus albula*) oocytes under unfavourable influences

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Received 1 April 1997, accepted 7 July 1997

The ultimate control of fish oocyte final maturation by gonadotropin appears to be mediated through follicular synthesis of a steroid mediator. The mediator, identified in most teleosts as 17α-hydroxy-20β-dihydroprogesterone (17,20P), is synthesized in follicular (granulosa) cells and it directly acts on the oocyte, triggering nuclear and cytoplasmic transformations leading to a fertilizable egg (Dettlaff 1977, Masui & Clarke 1979, Nagahama 1987abc). Both of these hormones, acting on the different structures of the follicle (oocyte + follicular envelope), can induce oocyte final maturation in a variety of fish species *in vivo* and *in vitro* (review: Saat 1985b). This allows experimental investigation of the responsiveness of each follicular component at different conditions. This approach has been used to study the differential sensitivity of follicular components to unfavourable influences in the chondrostean, *Acipenser stellatus* Pall. (Davydova 1972, Dettlaff & Davydova 1974, 1979ab, 1981) and in the warm-water teleost, *Carassius auratus gibelio* (Bloch) (Saat 1985a). In both these species, the follicular epithelium cells lost their capacity to respond to hypophyseal hormones earlier than oocytes to progesterone, suggesting that the formers are more sensitive to unfavourable influences. In the stellate sturgeon *A. stellatus*, the follicular response to gonadotropin can be restored by the administration of triiodthyronine (T_3) (Dettlaff & Davydova 1974, 1979ab, 1981). Thyroid hormones have been shown to enhance the oocyte final maturation also in teleosts (Hurlburt 1977, Epler & Bieniarz 1983, Sullivan *et al.* 1989), and therefore one can expect the same phenomenon in these fish. The aim of the present study was to investigate the effect of unfavourable influences and T_3 on the follicular sensitivity to hormones in the cold-water teleost, *Coregonus albula* (L.), *in vitro*.

Methods

Female vendace *Coregonus albula* (L.) between 18.2–19.2 cm in total length were caught in Lake Kallavesi (Central Finland) during the prespawning period in October 1993, when the water temperature had decreased to 7–8°C. The animals were killed by decapitation and the ovaries were immediately excised and placed in cooled medium 199 (10°C) with Earle's salts (Sigma Chemical Co.) supplemented with Gibco Europe Antibiotic Antimycotic Solution (penicillin, 10^6 U; streptomycin, 10^6 µg; fungizone, 250 µg per litre). Ovarian fragments containing approximately 10 fully grown follicles were separated from the ovary using forceps under a stereo dissecting microscope. Follicles with a diameter of

~ 1.2–1.3 mm with no signs of atresia were selected. The fragments were pooled, rinsed in medium 199 and dispensed into 25 × 5-mm plastic Petri dishes (approximately 50–60 follicles per dish containing 5 ml of medium 199).

17α-hydroxy-20β-dihydroprogesterone (4-pregnen-17α,20β-diol-3-one, 17,20P; Sigma) and triiodthyronine (3,5,3´-triiodo-L-thyronine, T_3; Sigma) were dispensed in ethanol (0.01 ml/dish) at a final concentration of 1 and 10 µg ml^{-1}, respectively; control incubates received a vehicle only. Salmon pituitary extract (SPE; Argent Chemical Laboratory) was dissolved in culture medium at 10 µg ml^{-1}.

Follicles from each of five females were dispensed into 20 Petri dishes.

Five dishes (without hormones; 17,20P; SPE; SPE + T_3; T_3) were immediately placed at 10°C for 5 days.

Five dishes per female were preincubated without hormones in medium 199 at 10°C for 20 hours. After hormone administration (17,20P; SPE; SPE + T_3; T_3), the follicles were incubated for 5 days at 10°C.

Ten dishes per female were preincubated in medium 199 for 6 hours at 20°C with or without hormones (SPE; T_3; SPE + T_3; 17,20P; 17,20P + T_3) and, thereafter, for 5 days at 10°C in the presence of SPE or 17,20P (with or without T_3; see below).

At the end of the incubation period, the number of mature, immature, and dead (cytolysing) oocytes was counted. The number of dead oocytes (about 20%) was similar in control and experimental dishes and in different females; this figure is therefore excluded from further calculations. No ovulation was observed during 5 days of incubation. Incubation of mature oocytes for an additional 5 days at 10°C did not result in ovulation either.

Results

Both SPE and 17,20P appeared to be highly effective, inducing 96–98 and 98–100% of unaffected oocytes, respectively, to mature. In dishes containing SPE + T_3, 98–100% of oocytes matured. There was no maturation in control dishes (without hormones) or in dishes with T_3 alone in this and further experiments.

Preincubation without hormones (20 h at 10°C) had no effect on 17,20P-induced oocyte maturation. However, oocytes from 3 females (of 6) did not mature in the presence of SPE. The addition of T_3 to the culture medium restored follicular sensitivity to SPE, and 58–96% of oocytes (depending on the female) matured (Fig. 1A).

Preincubation for 6 h at 20°C (with and without SPE in the culture medium) lead to a decreased number of maturing oocytes under the effect of SPE in all females as compared with oocytes not preincubated at all or preincubated at low temperature, especially when SPE was added to the medium during preincubation. In the presence of T_3, the number of mature oocytes usually remained the same or was slightly less than in oocytes not preincubated (Fig. 1B); Only the follicles of female 2 preincubated in the presence of T_3 showed a week response (19%) to SPE + T_3 (Fig. 1).

Again, preincubation (with and without T_3 or 17,20P in the medium) had no remarkable effect on the 17,20P-induced *in vitro* maturation (86–100% of mature oocytes in different females).

Discussion

Preincubation conditions applied in this study did not affect the 17,20P-induced oocyte maturation but lead to a rather dramatic decrease in follicular response to SPE. As the effect of pituitary gonadotropin(s) is mediated by processes in follicular investments, and 17,20P acts directly on the oocyte (Nagahama 1987ab), we can suggest that the processes in follicular investments are more sensitive to unfavourable conditions than the oocyte itself. Such differential sensitivity of the oocyte and its investments has been documented earlier in special experiments, both in the acipenserid fish *A. stellatus* (Davydova 1972, Dettlaff & Davydova 1974, 1978, 1979ab, 1981) and the warm-water teleost *C. a. gibelio* (Saat 1985a).

As it has been proved in acipenserid fish, both *in vivo* and *in vitro* (Dettlaff & Davydova 1974, 1979ab, 1981), alterations in follicular tissue are reversible, and competence to gonadotropin can be restored by the administration of T_3. The same has been demonstrated for the cold-water teleost, *C. albula*, in our *in vitro* experiments. These findings are in accordance with evidence that thyroid hormones act by increasing ovarian sensitivity to gonadotropin, both during vitellogenesis (Cyr & Eales 1988) and oocyte final maturation (Sullivan *et al.* 1989).

It has been suggested that T_3 acts on the follicular envelope, and does not affect the state

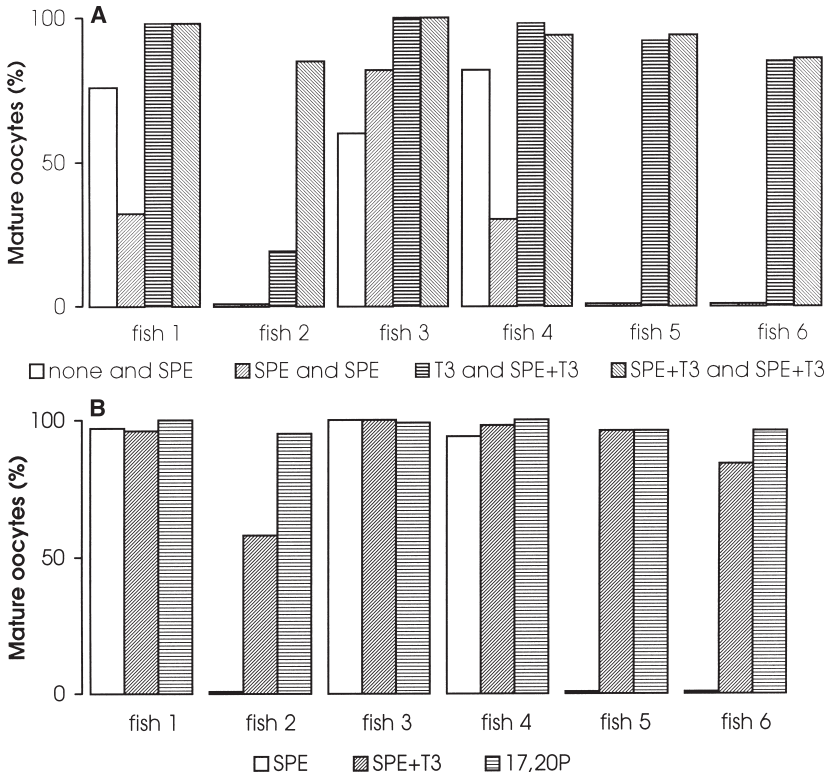

Fig. 1. Maturation of oocytes of 6 females following preincubation in medium 199. A: Preincubation in the medium without hormones for 20 hours at 10°C, followed by SPE, SPE + T_3, and 17,20P treatment. B: preincubation for 6 hours at 20°C. Hormones during preincubation and maturation: none and SPE, SPE and SPE, T_3 and SPE + T_3, SPE + T_3 and SPE + T_3. No maturation in control dishes (without hormones or T_3 alone).

of the oocyte. Injecting T_3 into female stellate sturgeons collected at the end of the breeding season did not improve the quality of the oocytes (Dettlaff & Davydova 1978, 1979b). Sullivan *et al.* (1989) studied the mechanism of T_3 action on steelhead salmon (*Oncorhyncus mykiss* Walb.) maturation and found that T_3 potentiates the steroidogenic response of the ovary to gonadotropin. However, Epler and Bieniarz (1983) demonstrated the synergistic effect of T_3 to the carp *Cyprinus carpio* L. oocyte *in vitro* maturation not only in combination with gonadotropin, but also with several steroid hormones, including 17,20P, suggesting the direct effect of thyroid hormones on oocytes.

The role of thyroid hormones in natural spawning, however, remains unclear (Sullivan *et al.* 1989). In the white sucker, *Catastomus commersoni*, plasma levels of T_3 and thyroxine (T_4) decrease in preovulatory fish (Stacey *et al.* 1984). In the gravid *O. mykiss*, plasma levels of T_3 are also reduced as compared with immature fish. At the same time, mature females exhibit increased levels of T_4 (Sullivan *et al.* 1989). Also, in the same species, handling stress causes a rapid elevation in plasma T_4 with no accompanying increase in plasma T_3 (Brown *et al.* 1978, Himick & Eales 1990). Hurlburt (1977) found that T_4 increases ovarian sensitivity to gonadotropin stimulation in goldfish *C. auratus in vivo*. Thus, it would be interesting to reveal the effect of T_4 on oocyte final maturation before and after unfavourable influences.

Our data also suggest that follicles from different females have a different resistance to unfavourable influences. After preincubation, oocytes from some females did not mature in the presence of SPE, while oocytes of other females retained this capacity. This may result from the different physiological state of prespawning females. During this period, a fluent transition to maturation occurs (Saat 1993), accompanied by changes of circulating hormone levels and pattern (Scott *et al.* 1984, Stacey *et al.* 1984, Nagahama *et al.* 1991). It has been shown that hormonally stimulated follicles of acipenserid fish are more sensitive to unfavourable influences than unstimulated follicles (Dettlaff & Davydova 1978). Also in our experiments on vendace (Fig. 1B) and steelhead salmon (Tambets 1993), preincubation in the presence of SPE had more severe effect on further maturation, than preincubation without hormones. However, increased resistance to unfavourable conditions would be of great importance, and in certain circumstances, may have evolutionary

consequences. It has been shown that follicles of the triploid (unisexual, gynogenetic) *C. a. gibelio* are more resistant to unfavourable influences than follicles of the diploid (bisexual) *C. a. gibelio*. Gynogenetic females reproduce with males of other species, and their spawning time is dependent on that of other species; it is probable that a longer period of maturation competence may increase the reproduction success of gynogenetic fish (Saat 1985a).

Acknowledgements. Financial support from the University of Kuopio, Finland, is greatly appreciated. We thank Mr. Petri Muje for kind assistance in obtaining fish for experiments.

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