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Immunohistochemical localization of cyclic AMP and ultrastructural demonstration of adenylate cyclase activity in the testis of *Esox lucius* at time of spermiation

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Summary. In the testis of *Esox lucius* at the time of spermiation, activity of cyclic adenosine 3′,5′-monophosphate (cAMP) was immunocytochemically localized at the level of the Sertoli cells. In these cells adenylate cyclase activity was also ultracytochemically demonstrated by using adenylyl imidodiphosphate as a substrate. Reaction products of adenylate cyclase were primarily detectable on the basal and adluminal plasma membranes and on the surface of protrusions of the cell body into the lumen.

Key words: Cyclic adenosine monophosphate – Adenylate cyclase – Sertoli cells – *Esox lucius* (Teleostei)

In the testis of *Esox lucius* spermatogenesis occurs entirely in cysts formed by Sertoli cell processes (Grier et al. 1980). Spermatogonia are confined to small peripheral cysts which subsequently enlarge, extending toward the tubule lumen as spermatogenesis takes place. After spermatogenesis Sertoli cell processes separate, the lumina of cysts and tubules becoming continuous with ripe sperm being voided therein. In the majority of teleosts, testicular hormone-dependent hydratation usually accompanies the process of sperm fluidification (Clemens et al. 1964; Clemens and Grant 1965; Billard 1983). Billard et al. (1971) suggested that this hydration can be due to seminal fluid secreted by Sertoli cells. It is known that in teleostean testis a blood-testis barrier is formed after meiosis (Abraham et al. 1979, 1980; Marcaillau and Szollosi 1980). Abraham et al. (1980) suggest that probably the Sertoli cells themselves, while thus forming intracellular junctional complexes, are supplied with metabolites and hormones that they discharge into the lumen.

Cyclic adenosine 3′,5′-monophosphate (cAMP) is recognized as the intracellular mediator (second messenger) for the effect of a wide variety of hormones (Davoren and Sutherland 1963). cAMP is formed in part by the catalytic action of the membrane-bound enzyme, adenylate cyclase (AC), on adenosine 5′-triphosphate (ATP) (Rall and Sutherland 1962). For the mammalian testis biochemical evidence suggests that the AC-cAMP system mediates the response of Sertoli cells to FSH (Dorrington et al. 1962). Furthermore, AC has been demonstrated cytochemically in the basal aspect of Sertoli cells of guinea pigs (Pascolini et al. 1983).

The present paper reports at the time of spermiation in *E. lucius* both immunocytochemical localization of cAMP and ultracytochemical demonstration of AC activity at the level of intratubular somatic cells, commonly designated as Sertoli cells. For cytochemistry, we used the modified method of Howell and Whitfield (1972), which is considered valid for localization of AC and which has been employed for cytochemical studies in a variety of cells and tissues (see review by Cutler 1983). For immunocytochemical localization of cAMP we used a specific immunocytochemical method (Sternberger 1979).

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Materials and methods

The testes of 10 specimens of *Esox lucius*, captured in lake Trasimeno during February when they were "ripe," were investigated.

Immunocytochemical localization of cAMP

Small pieces of the testes were fixed by immersion in Bouin's solution and embedded in paraffin. Sections 5-μm thick were processed for the unlabeled antibody peroxidase-antiperoxidase (PAP) method (Sternberger 1979). Tissue slices were subsequently incubated in a damp chamber with a highly sensitive rabbit anti-cAMP antiserum, with a goat anti-rabbit antiserum (Miles-Yeda Ltd) and finally with the PAP complex raised in the rabbit. The peroxidase was detected by a 3′-3′ diaminobenzidine-H_2O_2 solution in Tris-HCl buffer pH 7.4. In each experiment, controls were prepared omitting the anti-cAMP antiserum.

Cytochemical localization

Testes were dissected and fixed by immersion in 1% glutaraldehyde in a 0.1 M cacodylate buffer pH 7.4 for 15 min. Then the pieces were washed 3 times, for 10 min each, in the same buffer. Thin slices were then cut with a razor blade under a dissecting microscope and incubated at 30° C for 45 min in 80 mM Tris-maleate buffer pH 7.4 containing 8% sucrose 4 mM $MgSO_4$, 2 mM theophylline, 2 mM $Pb(NO_3)_2$, 10 mM NaF, and 0.5 mM 5′-adenylyl imidodiphosphate (App(NH)p) (Sigma).

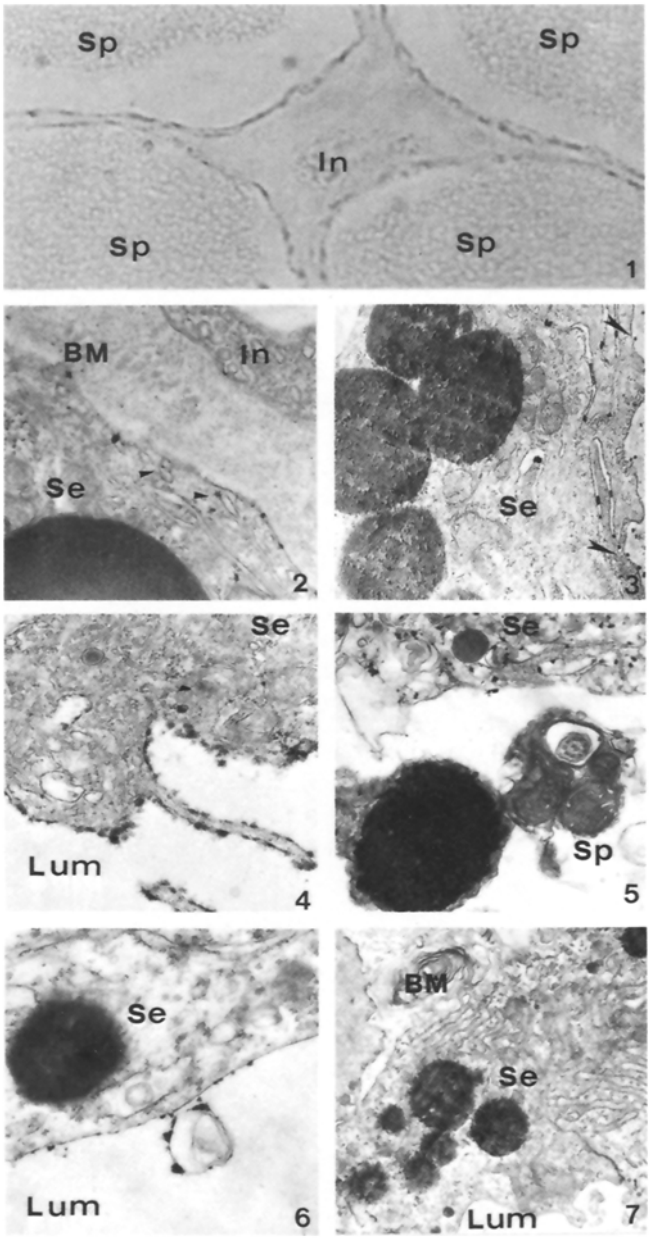

Fig. 1. Immunocytochemical localization of cAMP in Sertoli cells outlining tubules filled with sperm (*Sp*). *In* interstitium. ×960

Fig. 2. Deposits of lead imidodiphosphate, indicative of AC activity, along the basal surface of Sertoli cell (*Se*) and in pinocytic vesicles (*arrows*). *In* interstitium; *BM* basement membrane. ×18400

Fig. 3. Reaction products of AC on the basal surface of Sertoli cell (*arrows*) and its foldings. ×12000

Fig. 4. Deposits of lead imidodiphosphate on the adluminal surface of the Sertoli cell (*Se*) and along the surface of protrusions of Sertoli cells into the lumen; *lum* lumen. ×14400

Fig. 5. Reaction products on the adluminal surface of a Sertoli cell (*Se*) and in subsurface vesicles. Note the absence of deposits of lead imidodiphosphate in the spermatozoa (*Sp*) of the lumen. ×16000

Fig. 6. Reaction products at the adluminal plasma membrane of the Sertoli cell and at the surface of a small portion detaching from the cell body; *lum* lumen. ×16000

App(NH)p is a substrate for AC that produces cAMP together with imidodiphosphate (PNP), which is precipitated by lead. Control samples were heat-inactivated at 70° C for 5 min before incubation. The reaction was stopped by washing the tissue in cacodylate buffer and post-fixed in 1% OsO_4 for 2 h. Testicular material was then dehydrated in a graded series of alcohols and embedded in Epon-Araldite. After a brief staining in a saturated solution of uranyl acetate in 50% ethanol, sections were examined with a Philips EM 400 operating at an accelerating voltage of 60 Kv.

Similar results were obtained in all examined specimens.

Results

Immunocytochemical observations

Immunohistochemical studies employing antibodies against cAMP indicated that the cAMP was localized only at the level of the Sertoli cells (Fig. 1).

Cytochemical observations

The ultracytochemical analysis of the distribution of AC activity demonstrated that the precipitation sites of this enzyme were only in Sertoli cells. Reaction products of AC were detectable on the basal plasma membrane, on its foldings (Figs. 2, 3) and at the level of the adluminal membrane (Figs. 4–6). Reaction products were also associated with subsurface vesicles (Fig. 5) with the surface of thin projections of the Sertoli cells into the lumen (Fig. 4) and with the surface of small portions detaching from the cell body (Fig. 6). Deposition of a non-enzymatic reaction product did not occur in any of the cells of the testis after incubation of the control specimens in the reaction mixture without App(NH)p (Fig. 7). Heat-inactivated tissue was also free of reaction product.

Discussion

The demonstration, in the testes of *Esox lucius* at the time of spermiation, that both cAMP and AC occur only in the Sertoli cells is of interest. During spermiation Sertoli cells showed pinocitic vesicles, extensive foldings of basal plasma membranes and microvillous projections into the lumen, observations similar to those of Abraham et al. (1980) in *Aphanius dispar*. We agree with the hypothesis of these authors regarding the possibility of transfer by Sertoli cells of metabolites and hormones from the basement membrane spaces into the lumen. The occurrence of AC at the level of basal surface, subsurface vesicles, adluminal surface and its projections into the lumen, suggests that the cAMP-AC system plays an important role in regulating this transfer of material.

Although the endocrine pattern of spermatogenesis and spermiation in fish requires further studies, there is nevertheless biochemical evidence that gonadotropin stimulates the production of androgen and eventually controls both

Fig. 7. Portion of a testis incubated in a substrate-free medium. *Se* Sertoli cell; *lum* lumen of the tubule; *BM* basement membrane. ×6400

spermatogenesis and spermiation (Idler and Ng 1979, Ng and Idler 1980, Billard et al. 1982). The presence of cAMP and AC at the level of Sertoli cell leads us to suppose that these cells are the targets for action of gonadotropins.

Finally, the observation that ripe spermatozoa contain neither cAMP nor AC activity agrees with results of Morisawa et al. (1982, 1983) who demonstrated that, in salmonid fish (rainbow trout and chum salmon) and freshwater cyprinid fish (goldfish), cAMP is the factor that induces initiation of sperm movement after spawning.

References

Abraham M, Rehamim E, Tibika H (1979) The blood-testis barrier in teleosts. Abstr I Congr Eur Comp Endocrinol Soc (Sorrento)

Abraham M, Rehamim E, Tibika H, Golenser E (1980) The blood-testis barrier in *Aphanius dispar* (Teleostei). Cell Tissue Res 211:207–214

Billard R (1983) A quantitative analysis of spermatogenesis in the trout *Salmo trutta fario*. Cell Tissue Res 230:495–502

Billard R, Breton B, Jalambert B (1971) La production spermatogénique chez la truite. Ann Biol Anim Biochem Biophys 11:190–212

Billard R, Fostier A, Weil C, Breton B (1982) Endocrine control of spermatogenesis in teleost fish. Can J Fish Aquat Sci 39:65–79

Clemens HP, Grant FB (1965) The seminal thinning response of carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdnerii*) after injections of pituitary extracts. Copeia 2:174–177

Clemens HP, Ciereszko LS, Shoemaker JD, Grant FB (1964) Partial characterization of the gonadal hydration principle in the pituitaries of carp (*Cyprinus carpio*). Gen Comp Endocrinol 4:503–507

Cutler LS (1983) Cytochemical methods for the localization of adenylate cyclase. J Histochem Cytochem 31:85–93

Davoren PR, Sutherland EW (1963) Cellular localization of adenyl cyclase in the pigeon erythrocyte. J Biol Chem 238:3016–3023

Dorrington JH, Vernon RG, Fritz IB (1972) The effect of gonadotropins on the 3′,5′-cyclic AMP levels of seminiferous tubules. Biochem Biophys Res Commun 46:1523–1528

Grier HJ, Linton JR, Leatherland F, De Vlaming VL (1980) Structural evidence for two different testicular types in teleost fishes. Am J Anat 159:331–345

Howell SL, Whitfield M (1972) Cytochemical localization of adenylate cyclase activity in rat islets of Langherhans. J Histochem Cytochem 20:873–879

Idler DR, Ng TB (1979) Studies on two types of gonadotropins from both salmon and carp pituitaries. Gen Comp Endocrinol 38:421–440

Marcaillou C, Szollosi A (1980) The blood-testis barrier in a nematode and a fish: a generalizable concept. J Ultrastruct Res 70:128–136

Morisawa M, Okuno M (1982) Cyclic AMP induces maturation of trout sperm axoneme to initiate sperm motility. Nature 295:703–704

Morisawa M, Okuno M, Suzuki K, Morisawa S, Ishida K (1983) Initiation of sperm motility in teleosts. Submicrose Cytol 15:61–65

Ng TB, Idler DR (1980) Gonadotropic regulation of androgen production in flounder and salmonids. Gen Comp Endocrinol 42:25–38

Pascolini R, Vagnetti D, Spreca A, Marinelli M, Lorvik S (1983) Cytochemical study on the distribution of adenylate cyclase in guinea pig testis. Anat Rec 207:4 (in press)

Rall TW, Sutherland EW (1962) Adenyl cyclase II: Enzymatically catalyzed formation of adenosine 3′,5′-phosphate and inorganic pyrophosphate from adenosine triphosphate. J Biol Chem 237:1228–1235

Sternberger LA (1979) The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In: Immunocytochemistry. John Wiley & Sons eds, New York, pp 104–169

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