Tonic Inhibition of Adenylate Cyclase in Cultured Hamster Sertoli Cells

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Sertoli cells cultured from immature hamsters respond to FSH with a dose-related increase in cAMP accumulation. Pertussis toxin acts synergistically with FSH to stimulate cAMP accumulation. This effect of pertussis toxin indicates that Sertoli cell adenylate cyclase is under tonic inhibition due to the activity of the Ni inhibitory transducer. The acetylcholine receptor antagonists atropine or tubocurarine, or the opiod antagonist naltrexone, have no effect on the FSH-induced stimulation of cAMP accumulation, suggesting that neither acetylcholine nor opioids are responsible for the inhibition of Sertoli cell cyclase. While exogenous adenosine is inhibitory, adenosine deaminase augments the ability of FSH to stimulate cAMP accumulation, but not to the level of pertussis toxin. This indicates that the Sertoli cells produce endogenous adenosine that is at least partially responsible for the tonic inhibition of adenylate cyclase. Other possibilities for the tonic inhibition of cyclase include other Sertoli cell products, germ cell products, peritubular cell products or an action of FSH itself through both stimulatory and inhibitory transducers.

Key words: cAMP, hamster, Sertoli cell, adenylate cyclase culture, pertussis toxin.

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Sertoli cells respond to FSH with an increase in cAMP accumulation. This stimulation occurs as a result of an activation of adenylate cyclase via a stimulatory transducer Ns (Johnson et al, 1980; Frances et al, 1981; Heindel and Clark, 1982). We have recently shown that Sertoli cells also contain an Ni inhibitory transducer (Davenport and Heindel, 1986), suggesting the possibility that Sertoli cell adenylate cyclase may be physiologically regulated by both Ns and Ni. This mechanism would permit fine tuning of cyclase response during initiation and maintenance of spermatogenesis. To examine this possibility, we have used pertussis toxin as a probe of the activity of the Ni inhibitory transducer. Since pertussis toxin inactivates the Ni, if its presence leads to an augmentation of cAMP accumulation, this would indicate that the cyclase was under a tonic inhibition that was relieved by the toxin inhibiting signal transduction through the Ni (Murayama and Ui, 1983; Cote et al, 1984).

Materials and Methods

Hormones, Isotopes and Chemicals

Ovine FSH (NIH FSH-16) was provided by the NIADDK Pituitary Hormone Distribution Program. DNase, atro-

pine, d-tubocurarine, naltrexone, adenosine deaminase (180 U/mg protein), and adenosine were purchased from Sigma Chemical Co. (St. Louis, MO). The antibody to cAMP was prepared in collaboration with Biotek Research Inc. (St. Louis, MO). Tryspin (1/250), Eagles Minimal Essential Media with Earles Salts and essential amino acids, penicillin-streptomycin, fungizone, and MEM vitamins were purchased from GIBCO (Grand Island, NY). Collagenase (125 U/mg protein) was obtained from Worthington Diagnostics (Freehold, NJ). Carrier-free [^{125}I]Na was purchased from ICN Pharmaceuticals Inc. (Irvine, CA). Pertussis toxin was purchased from List Biological Lab Inc. (Campbell, CA). All other chemicals were reagent grade quality.

Animals

Syrian hamsters, originally obtained from Engle Labs, Farmersburg, IN, were randomly bred and maintained in the animal facility of the Department of Biology, University of Mississippi. Animals were housed in a photoperiodically-regulated facility (LD 14:10; lights on at 0500) and provided food and water *ad libitum*. All animals were 18 to 20 days of age with testis weights/pair averaging 180 ± 13 mg.

Sertoli Cell Preparation

Immature hamsters were sacrificed by decapitation. Each testis was removed under aseptic conditions, weighed and decapsulated. Sertoli cells were isolated and cultured according to protocols previously described in detail (Berkowitz and Heindel, 1984) with the following modifications. The dissociated peritubular, interstitial, and most germ cells were separated from clusters of Sertoli cells by three consecutive washes with MEM (MEM media plus penicillin streptomycin-fungisone and MEM vitamins), each wash being followed by centrifugation at 60 × g for 2 minutes. The Sertoli cells were cultured in stationary culture plates (16 mm: Costar Multiwell, Costar, Cambridge, MA) with 1 ml MEM. Medium was changed 24 hours before experimentation, usually on day 3 or 4 of culture.

Preincubation with Pertussis Toxin

Pertussis toxin at various doses was added to the culture dishes without a medium change. Incubation was for 3 hours.

Incubation Procedure

Cultures were rinsed with MEM to remove unattached cells and cellular debris. Cells were then incubated at 33 C for 30 minutes in 500 μl MEM containing various combinations of hormones and agents. Incubation was terminated by aspiration of the media and addition of 1 ml of absolute alcohol.

Cyclic Nucleotide Extraction and Assay

Cyclic AMP was released from the cells by disruption with a 3-second burst from a Branson Sonifier (Cell Disruptor 185) equipped with a microtip and the output controller set on 2. The alcohol/broken cell suspension was centrifuged at 1000 × g for 30 minutes at 4 C, and aliquots of the supernatants were dried for radioimmunoassay of cAMP as previously described (Heindel et al, 1975). Cell pellets were assayed for protein content by the method described by Schacterle and Pollack (1973). Levels of intracellular cAMP are expressed as pmol/mg protein.

Results

Figure 1 shows the dose dependence of pertussis toxin stimulation of FSH-induced cAMP accumulation in cultured Sertoli cells from immature hamsters. This effect of pertussis toxin was half maximal at 10 ng/ml and required a 3-hour preincubation period (data not shown). The effect of maximal pertussis pretreatment on the ability of FSH to stimulate cAMP accumulation is shown in Fig. 2. These results indicate that the Sertoli cell adenylate cyclase is under tonic inhibition.

Figure 3 shows that exogenous adenosine will inhibit the FSH-stimulated cAMP accumulation in a dose-dependent manner. We need incubated Sertoli cells in the presence of adenosine deaminase to determine if endogenously produced adenosine might be responsible for the tonic inhibition of Sertoli cell

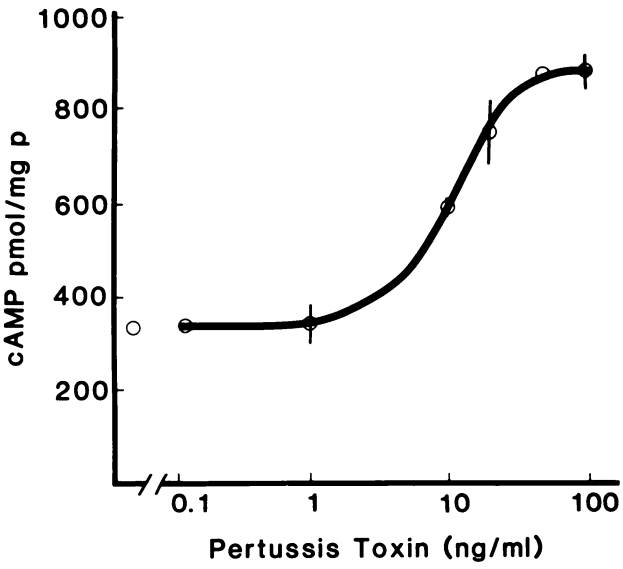

Fig. 1. Dose dependence of pertussis toxin stimulation of FSH-induced cAMP accumulation in Sertoli cells from immature hamsters. Cells were preincubated for 3 hours in the presence of pertussis toxin, washed and incubated with FSH (2 μg/ml) for 30 minutes. Each point is the average of quadruplicate determinations ± SEM from two separate experiments.

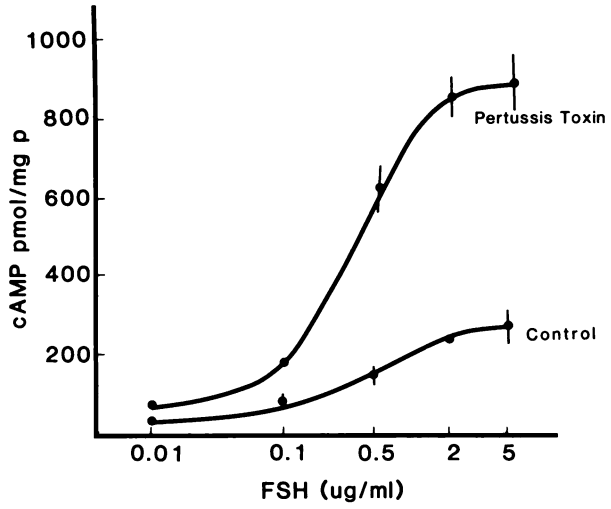

Fig. 2. Pertussis toxin augmentation of FSH-induced cAMP accumulation in Sertoli cells cultured from immature hamsters. Cells were either preincubated with pertussis toxin (100 ng/ml) or control media (5 μl MEM) for 3 hours, washed and incubated in MEM for 30 minutes with various doses of FSH. Each point is the average of quadruplicate determinations ± SEM from two separate experiments.

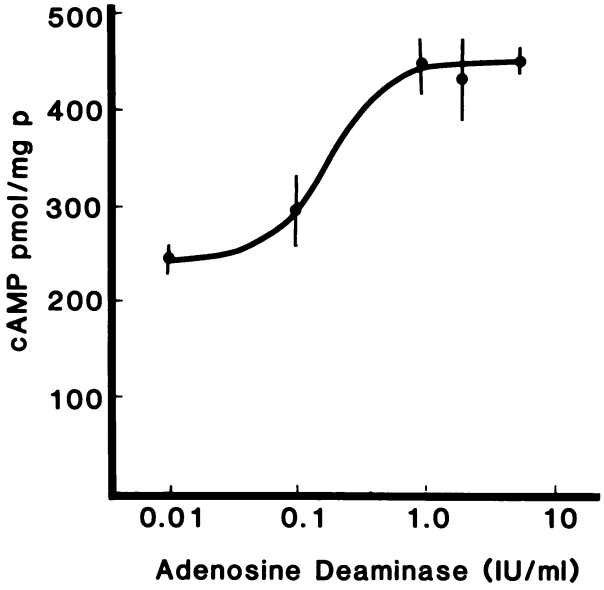

Fig. 4. Dose response of adenosine deaminase stimulation of FSH-induced cAMP accumulation in Sertoli cells cultured from immature hamsters. Cells were stimulated with FSH (2 μg/ml) in the presence of increasing doses of adenosine deaminase. Each point is the average of quadruplicate determinations ± SEM from two separate experiments.

adenylate cyclase. Indeed, the presence of adenosine deaminase in the incubation medium augmented the ability of FSH to stimulate cAMP accumulation (Fig. 4). However, as shown in Fig. 5, the removal of endogenously produced adenosine (by adenosine deaminase) could not account for all the inhibitory activity present in the cultured cells. Note that the effect of pertussis toxin is the same in the presence or absence of adenosine deaminase, but is significantly higher than adenosine deaminase alone.

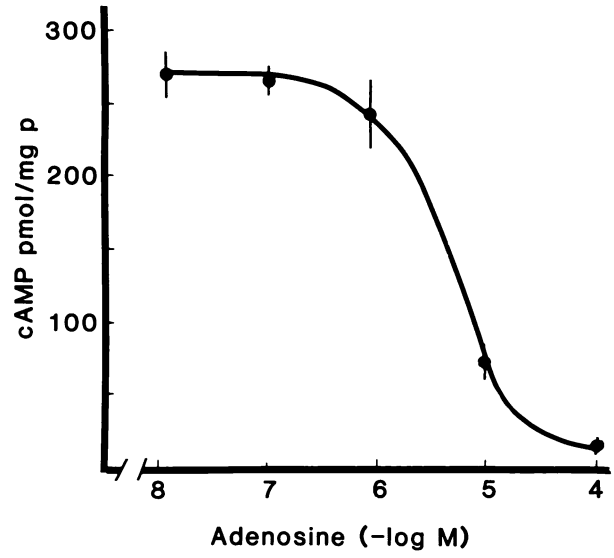

Fig. 3. Dose dependence of adenosine inhibition of FSH-stimulated cAMP accumulation. Sertoli cell cultures were incubated with FSH (2 μg/ml) and adenosine for 30 minutes. Each point is the average of triplicate determinations ± SEM from two separate experiments.

In order to determine if Sertoli cells secreted other products that might be responsible for this tonic inhibition of adenylate cyclase, the effects of atropine, tubocurarine, or naltrexone (100 μM) were examined. Tubocurarine and atropine, which would inhibit nicotinic or muscarinic agonist action, or naltrexone, which would antagonize endogenously produced opiates, had no effect (data not shown).

Discussion

We have previously shown that Sertoli cells are regulated by an adenylate cyclase coupled to both stimulatory Ns and inhibitory Ni transducers (Davenport and Heindel, 1986). Because pertussis toxin augments the ability of FSH to stimulate cAMP accumulation, we can conclude that under our culture conditions the adenylate cyclase system is under tonic inhibition by the inhibitory transducer, Ni. Per-

tussis toxin, a protein endotoxin from *Bordetalla* pertussis, has been shown to ADP ribosylate the Ni, thereby resulting in the inability of the Ni to couple to the adenylate cyclase catalytic unit (Murayama and Ui, 1983; Cote et al, 1984). Therefore, the ability of agents to act through Ni to inhibit cAMP accumulation is lost in the presence of pertussis toxin. A few years ago it was demonstrated that the cAMP-generating system of pituitary tumor cells (Reisine et al, 1983) and rat hepatocytes (Pushpendran et al, 1983) are also under tonic inhibition. It may be that all adenylate cyclases are normally under tonic inhibition by endogenous products.

We have also examined the nature of the agent(s) that normally act via the Ni to inhibit tonically Sertoli cell adenylate cyclase activity. As others have previously shown, we have confirmed that exogenous adenosine inhibits cAMP accumulation in Sertoli cells (Monaco et al, 1984; Eikvar et al, 1985). In addition, because FSH-stimulated cAMP accumulation is augmented significantly in the presence of adenosine deaminase (224 ± 13 to 488 ± 16 pmol/mg protein), the conclusion may be drawn that adenosine is produced endogenously by cultured Sertoli cells. However, the stimulation produced by FSH plus adenosine deaminase does not reach the level of the stimulation by FSH plus pertussis toxin. This indicates the presence of inhibitory effectors other than endogenously produced adenosine acting via Ni. Since Sertoli cells possess acetylcholine (Davenport and Heindel, 1986) and opiate receptors (Fabbri et al, 1986), we have used atropine, tubocurarine, and naltrexone to show that endogenously produced acetylcholine agonists or opioids are not responsible for the tonic inhibition of Sertoli cell cyclase.

Three basic possibilities remain. One is that other as yet unidentified agents are produced endogenously by Sertoli cells in culture; another is that the peritubular or germ cell contaminants in the culture produce agents that act on the cyclase of the Sertoli cell in a regulatory manner. The third possibility is that FSH itself may act through both stimulatory and inhibitory receptors.

While there is no direct evidence for any of these theories, indirect data supports the possibility that any one or a combination of these theories may be responsible for the effect. First, it is now known that Sertoli cells produce a variety of products that have not been identified and characterized (Cheng et al, 1986). Second, there is evidence that both peritubular (Hudson and Stocco, 1981; Skinner et al, 1985; Skinner and Fritz, 1986) and germ cells (Galdieri et al, 1984; Welsh et al, 1985) produce agents that influence Sertoli cell function. Third, there is precedence for the possibility of dual receptors coupled to adenylate cyclase (Friedholm, 1982; Creese et al, 1983; Exton, 1985). For example, catecholamines act through beta receptors to stimulate cyclase via Ns and act through alpha$_2$ receptors to inhibit cyclase via Ni (Exton, 1985). In both hepatocytes (Pushpendran et al, 1983) and pituitary tumor cells (Reisine et al, 1983), pertussis toxin has been shown to augment the ability of catecholamines to stimulate cAMP production. The presence of differing numbers of alpha$_2$ and beta receptors on a tissue therefore allows for fine tuning of the cAMP signal. Also, Michelangeli et al (1984) have presented data suggesting that PGE_2 acts on human breast cancer cells through both stimulatory and inhibitory transducers.

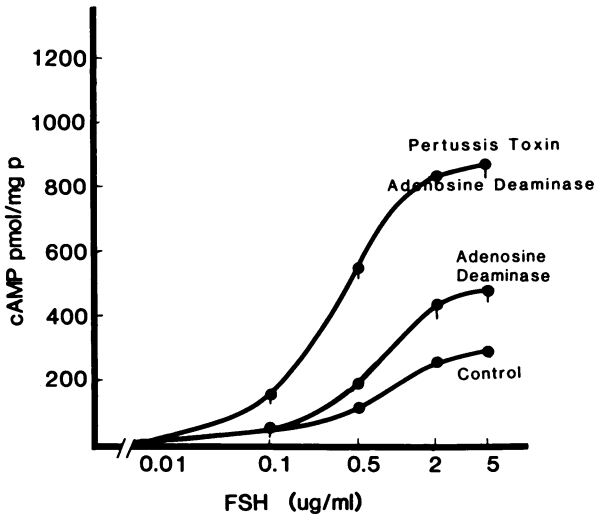

Fig. 5. Effect of pertussis toxin on the ability of the combination of maximal doses of FSH plus adenosine deaminase to stimulate cAMP accumulation in Sertoli cells. Cells were preincubated with pertussis toxin (100 ng/ml) for 3 hours, washed and incubated with FSH (2 μg/ml) plus adenosine deaminase (2 IU/ml) for 30 minutes. Each point is the average of quadruplicate determinations ± SEM from two separate experiments.

While the possibility of a dual action of FSH is speculative, it is very exciting, since it may also explain differential regulation of Sertoli cell adenylate cyclase at differing ages or stages of the spermatogenic cycle. This could occur via different receptors for FSH or differential coupling of the same receptor due to differing microheterogeneity of FSH activity. This last point is supported by recent data showing heterogeneity of FSH (Blum and Gupta, 1985; Chappel and Ramaley, 1985) and changes in the secreted forms of FSH with age (Chappel and Ramaley, 1985).

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