PHOSPHODIESTERASE INHIBITION OF DNA SYNTHESIS IN THE TESTIS OF THE SPINY DOGFISH SQUALUS ACANTHIAS

J. Michael Redding and Kathryn A. Dahmus
Department of Biology
Tennessee Technological University
Cookeville, TN 38505

The shark testis model developed by G.V. Callard and her associates has proven to be a valuable tool for the study of vertebrate spermatogenesis (Callard et al., J. Exp. Zool. Suppl. 2:23, 1989). In particular, the model offers a sensitive system for analyzing the regulation of spermatogonial and Sertoli cell function in vitro under conditions that approximate the natural testicular environment better than current mammalian models. Previous studies using in vitro culture methods identified several factors, both extracellular and intracellular, that stimulate or inhibit cell proliferation in spermatocysts (germ cell-Sertoli cell units) as indicated by the incorporation of radioactive thymidine (3H-T) into newly synthesized DNA. Of particular interest to the present work is the observation that DNA synthesis is inhibited by isobutyl-methylxanthine (IBMX) and other non-specific inhibitors of a class of enzymes collectively termed phosphodiesterases (PDE)(Redding and Callard, Bull. MDIBL 30:30, 1991). Commonly, inhibitors of PDE are assumed to increase the availability of intracellular cyclic AMP by decreasing its degradation rate. However, in preliminary studies, inhibition of DNA synthesis with micromolar concentrations of permeable analogs of cAMP was not evident, suggesting the possibility of alternative PDE regulated pathways. For example, certain types of PDE have greater affinity for cGMP as a substrate; thus, IBMX-induced effects may be mediated by cGMP or other pathways sensitive to non-specific PDE inhibitors (see Weishaar et al., J. Med. Chem. 28:537, 1985). The present study was designed to elucidate the intracellular pathway by which PDE activity mediates DNA synthesis in the Squalus testis. Specifically, we tested the effectiveness of specific PDE inhibitors that are known to selectively inhibit different intracellular regulatory pathways.

Premeiotic spermatocysts were isolated from the testes of 2-6 sharks and maintained in culture in 350 ul of media at 17-18^{o} C following procedures described by Callard and Dubois (Bull. MDIBL 27:30,1988). Inhibitors of PDE were tested for their inhibitory effects on DNA synthesis by exposing spermatocysts to the test substances for 3-18 h. During the last 3-6 h of treatment, spermatocysts were exposed to 2.5 uCi/ml 3H-T. After washing, the spermatocysts were then treated with 10% cold trichloroacetic acid to precipitate all macromolecules including the cellular DNA, with 3H-T incorporated into the newly synthesized molecules.

Results shown in Table 1 indicate that the type IV PDE inhibitors, Rolipram and RO-20-1724, inhibit DNA synthesis with a potency approximating that of the non-specific inhibitor IBMX. In dispersed mammalian germ cells these agents are potent inhibitors of cAMP-specific PDE with ED50 concentrations of 0.5 and 1-2 uM, respectively (M. Conti, personal communication). Lower sensitivity in shark spermatocysts may be due to the relatively isolated position of cells within the cysts, making them less accessible to the drugs. Type III PDE inhibitors, such as Piroximone and Millirone, are more specific for the insulin regulated PDE pathway and were ineffective in our system, despite the fact that in mammalian germ cells Millirone also inhibits type IV PDE at concentrations above 1 uM (M. Conti, personal

communication). Interestingly, the most effective agents were the non-specific PDE inhibitors, papaverine and dipyridamole. Explanations for the high potency of these agents are lacking at this time. Our findings generally support the hypothesis that enhancement of intracellular cAMP by the inhibition of type IV PDE may be a negative regulatory mechanism for DNA synthesis in shark spermatocysts. The seemingly paradoxical finding that micromolar concentrations of permeable analogs of cAMP have no inhibitory effect may be explained either by a lack of accessibility to mitotic cells within the cysts or the presence of extremely active intracellular PDE. In support of the latter possibility we have found that very high concentrations (5-10 mM) of one analog, dibutyryl-cAMP, can inhibit DNA synthesis, and lower concentrations (1 mM) can be effective if sub-maximal levels of IBMX are applied simultaneously.

In other experiments we tested the relative potency of various non-specific PDE inhibitors (dipyridamole, DIPY; papaverine, PAPA; IBMX; theophyllin, THEO) for their inhibitory effects on DNA synthesis. In order of their inhibitory potency we found DIPY > PAPA >> IBMX > THEO, with minimal effective concentrations in the range of 0.1, 10, 200, and 2000 uM, respectively. We also demonstrated the reversibility of IBMX- and PAPA-induced inhibition of DNA synthesis. Spermatocysts were able to synthesize DNA at normal rates within a few hours after removing the inhibitory substances from the media. Such reversibility indicates that the inhibition was not simply a reflection of cell death induced by the substances.

Table 1. Dose dependent effects of various PDE inhibitors on the rate of DNA synthesis in shark spermatocysts. Data are expressed as a percentage of the mean control value, standard errors are shown in parentheses, n=4. Asterix indicates significant ($P<0.05$) difference. ND indicates no data.

	Concentration		
PDE Inhibitor	5 uM	50 uM	500 uM
Dipyridamole	36.2 (0.5)*	20.4 (2.4)*	ND
Papaverine	87.7 (13.4)	13.1 (3.3)*	7.6 (0.2)*
Rolipram	96.7 (10.2)	93.1 (14.7)	46.4 (4.5)*
IBMX	113.7 (10.9)	99.5 (4.3)	61.9 (6.1)*
RO-20-1724	119.0 (11.0)	85.6 (7.8)	62.2 (10.9)*
Piroximone	84.7 (20.2)	80.8 (13.6)	95.2 (6.1)
Millirone	110.5 (15.6)	107.2 (4.1)	128.0 (7.4)

Supported by a fellowship from the Burroughs Wellcome Fund.