ACTIVATORS OF PROTEIN KINASE C STIMULATE MEIOTIC MATURATION OF RAT OOCYTES

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Agonistic analogs of gonadotropin releasing hormone can induce oocyte maturation in rat follicle-enclosed oocytes (1-5). Cyclic AMP does not rise following exposure of the ovarian follicle to GnRH (3) suggesting that cAMP-dependent protein kinase is not involved in the mechanism of GnRH action in this system. Protein kinase C, which is independent of CAMP, has recently been reported to mediate GnRH action in the pituitary (6-8). The possible involvement of this enzyme in the regulation of oocyte maturation has been tested in the present study. We report here that phospholipase C and direct activators of protein kinase C can mimic the response of rat oocytes to GnRH. These results suggest that GnRH-induced meiotic maturation of rat oocytes is mediated by the phospholipid-dependent protein kinase, protein kinase C.

Maturation of the mammalian oocyte is physiologically stimulated by luteinizing hormone but can be effectively induced also by GnRH (1-5). Beyond the hormonal stimuli required, the mechanism for induction of oocyte maturation in mammals is completely obscure. The transduction of the hormonal signal which finally leads to meiotic maturation has not been investigated as yet. It is known, however, that maturation of rat, mouse and bovine oocytes can be blocked by cAMP (9-11). It has recently been shown that GnRH action in the pituitary involves activation of the phospholipid-dependent protein kinase, protein kinase C (6-8). Protein kinase C is not only independent of cAMP but rather is inhibited by the presence of this

ABBREVIATIONS USED:
GnRH, gonadotropin releasing hormone; GnRHα, GnRH analog; cAMP, cyclic adenosine 3':5'-monophosphate; TPA, 12-0-tetradecanoyl phorbol-13-acetate; OAG, 1-oleoyl-2 acetyl-glycerol.
cyclic nucleotide (12). Taken together, these findings led us to examine the possible involvement of protein kinase C in the regulation of oocyte maturation. For this purpose the response of rat oocytes to direct activators of protein kinase C, such as a phorbol ester, a diacylglycerol derivative and phospholipase C has been tested.

MATERIALS AND METHODS

Sexually immature Wistar female rats (26 days old) were injected subcutaneously with 15 IU of pregnant mare's serum gonadotropin (PMSG, Gestyl, Organon, Holland) in 0.1 ml 0.9% NaCl. The rats were killed by cervical dislocation 48 h after the injection. The ovaries were removed and placed in Leibovitz's L-15 medium (Gibco USA), supplemented with 10% fetal bovine serum (Sera-Lab, England) penicillin (100 U/ml) and streptomycin (100 ug/ml) (Gibco). Ovarian follicles were dissected under a stereoscopic microscope, rinsed and placed in 2 ml of the above medium in 25 ml flasks, gassed with 50% O₂ and 50% N₂. Incubations were carried out at 37°C in an oscillating water bath in the presence of the indicated concentrations of phospholipase C (Sigma, USA), TPA (Sigma, USA) OAG (Avanti Polar Lipids, Inc. USA), GnRHα ([D-ser(t-Bu)]des-Gly-GnRH-N-ethylamide) or combination of these agents. After 20 h the follicles were incised and the oocytes were recovered and analyzed for maturation by Nomarski interference contrast microscopy. Resumption of meiosis was indicated by the germinal vesicle breakdown (GVB) in the individual oocytes. The presence of the first polar body in the oocytes was also examined.

RESULTS AND DISCUSSION

Tumor promoting phorbol esters are known to activate protein kinase C by intercalating into the membrane and substituting for the physiological substrate of this enzyme (13,14). We found that TPA induced the resumption of meiosis in follicle-enclosed oocytes in a dose dependent manner with an ED₅₀ of 1.2 ug/ml (Fig. 1). TPA not only induced initiation of the meiotic process which is indicated by the disappearance of the germinal vesicle (GV) in the oocyte, but stimulated the oocytes to proceed to the second metaphase forming the first polar body. When TPA and GnRHα were added together at doses that by themselves induced meiosis resumption of only 20% of the examined oocytes, maturation was significantly enhanced (Fig. 2, p<0.01, student's t-test).
Fig. 1: Concentration dependency of TPA-induced maturation of follicle-enclosed rat oocytes. Maturation of the oocyte recovered from the follicles following 20 h of incubation was examined as described in Materials and Methods. The data of 7 individual experiments is combined and the results are reported as the fraction of oocytes with GVB. At least 100 oocytes were examined for each experimental point.

Fig. 2: Augmentation of the effect of a GnRH agonistic analog on follicle-enclosed rat oocytes by TPA. Maturation of the oocytes recovered from the follicles following 20 h of incubation was examined as described in Materials and Methods. The data of 4 individual experiments is combined and the results are reported as the fraction of oocytes with GVB. Over 150 oocytes were examined for each experimental point.

Protein kinase C is directly linked to the inositol phospholipid turnover. This enzyme is activated by diacylglycerol that is transiently produced in membranes during the signal-induced turnover of inositol phospholipids (15,16). We tested the possible effect of this biological activator of C-kinase on oocyte maturation. We found that the membrane permeable derivative of diacylglycerol, OAG, induced meiosis resumption in a dose dependent manner with an ED₅₀ of 120 µg/ml (Fig. 3). Similar to TPA, OAG-induced maturation was not only initiated but also completed as indicated by the formation of the first polar body in the oocytes. OAG also potentiated the effect of GnRHα in a very significant manner (p<0.001, student’s t-test). Concentrations of OAG and GnRHα which alone stimulated about 20% of the oocyte together provoked maturation of 80% of the examined oocytes (Fig. 4).

Activation of protein kinase C is dependent on the inositol phospholipid turnover. This reaction is initiated by the cleavage of
Fig. 3: Concentration dependency of OAG-induced maturation of follicle-enclosed rat oocytes. Maturation of the oocytes recovered from the follicles following 20 h of incubation was examined as described in Materials and Methods. The data of 4 individual experiments is combined and the results are reported as the fraction of oocytes with GVB. At least 110 oocytes were examined for each experimental point.

Fig. 4: Augmentation of the effect of GnRH on follicle-enclosed rat oocytes by OAG. Maturation of the oocytes recovered from the follicles after 20 h of incubation was examined as described in Materials and Methods. The data of 3 individual experiments is combined and the results are reported as the fraction of oocytes with GVB. Over 150 oocytes were examined for each experimental point.

The phosphodiester linkage which is catalyzed by phospholipase C. The possible effect of this enzyme on follicle-enclosed oocytes has also been tested. We found that in follicles incubated in the presence of 0.5 U/ml of phospholipase C, 100% of the oocytes were stimulated to complete their maturation.

The results presented here suggest that in the process of rat oocyte maturation the effect of GnRH is probably mediated by protein kinase C, since the two most potent activators of this enzyme and phospholipase C mimicked GnRH stimulation. It has been shown in tissues such as platelets, lymphocytes, neutrophils and mast cells that the hormonal signal inducing inositol phospholipid breakdown usually promotes activation of cellular function and proliferation, whereas the trigger that produces cyclic AMP normally antagonizes such activation (12). Since many studies have established that cAMP inhibits meiosis resumption in mammalian oocytes (9-11), it is possible that GnRH activation of phospholipids turnover and of protein
kinase C could reverse the negative control exerted by cAMP on the oocyte leading to resumption of meiosis.

REFERENCES