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REGULATORY FUNCTIONS OF CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE IN 1-METHYLADENINE PRODUCTION BY STARFISH FOLLICLE CELLS

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SUMMARY: The biosynthesis of 1-methyladenine (1-MeAde) in follicle cells of the starfish, *Asterina pectinifera*, occurred in response to a gonad-stimulating substance (GSS). Simultaneously with 1-MeAde production, the intracellular cAMP level immediately increased following the administration of GSS. This level in follicle cells markedly depended on GSS concentration. Although 1-MeAde production was also induced by 1-methyladenosine, it caused no increase in cAMP content. It thus appears that the effect of GSS on starfish follicle cells results in the receptor-mediated formation of cAMP.

In most animals, oocytes in the ovary are arrested at the prophase of the first maturation division. Immature oocytes can not be fertilized and developed. Hormonal control is required for the resumption of meiosis. In starfish, maturation division can be resumed by 1-MeAde (1-4), which is produced in follicle cells by the action of a GSS secreted from the neural system (5). GSS is a single peptide (6) and its reactions seem to correspond to those of a pituitary gonadotropin in vertebrates. 1-MeAde does not accumulate in follicle cells but rather is newly synthesized under the influence of GSS (7). Starfish follicle cells are also capable of producing 1-MeAde when incubated with 1-MeAde-R (8). The presence of the enzyme, 1-MeAde-R ribohydrolase, has been reported in starfish follicle cells (9,10). 1-MeAde-R may possibly be a precursor of 1-MeAde.

However, the action of GSS on 1-MeAde biosynthesis in follicle cells is yet unknown. The stimulatory effects of many peptide hormones on the functions of target cells and tissues are considered to occur as a result of increase in cAMP content (11). In the present study, an attempt is made to determine the relation between 1-MeAde production and intracellular cAMP level in starfish follicle cells.

Abbreviations used: 1-MeAde, 1-methyladenine; 1-MeAde-R, 1-methyladenosine; GSS, gonad-stimulating substance; GVBD, germinal vesicle breakdown.

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MATERIALS AND METHODS

The follicle cells of the starfish, *Asterina pectinifera*, were prepared as previously described (12). The amount of follicle cells was expressed in terms of number of oocytes, since an oocyte is enclosed with approximately fifty follicle cells (personal information from Shirai). GSS was prepared from the lyophilized radial nerves of *A. pectinifera* (13,14). The amount of GSS was expressed by original nerve weight (weight of dry nerve equivalent). The sea water used was modified Van't Hoff's artificial sea water; the pH was adjusted to 8.2 with 0.02 M borate buffer (15). 1-MeAde and 1-MeAde-R were purchased from Sigma Chemical Co., St. Louis, MO.

The isolated follicle cells were incubated with sea water containing GSS at 20°C with occasionally shaking. After incubation, the cell suspension was centrifuged at 5,000 rpm for 1 min. The supernatant was assayed with respect to the amount of 1-MeAde. The sedimented cells were used to estimate cAMP concentration. The amount of 1-MeAde was determined by a biological assay according to the method of Shirai (14,16), using an authentic 1-MeAde solution as the standard reference. The cAMP content was determined with a commercial radioimmunoassay kit (Yamasa Shoyu Co., Chiba, Japan)(17), following extraction of the cells with 6% trichloroacetic acid.

RESULTS

When isolated follicle cells were incubated with GSS, the 1-MeAde produced was found in the incubation media (Fig. 1). The continual presence of GSS in these media brought about an almost linear 1-MeAde production for 4 h. However, the follicle cells never produced 1-MeAde without GSS, or

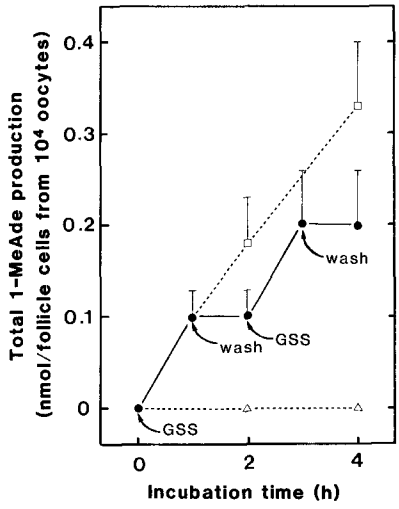

Fig. 1. Effects of GSS on 1-MeAde production by follicle cells. (●): The follicle cells were incubated with GSS (0.05 mg/ml). After incubation for 1 h, the cells were washed with sea water and incubated without GSS for 1 h. GSS was then added to the cell suspension followed by incubation for 1 h. The cells were washed again and incubated without GSS for 1 h. (□): The follicle cells were continuously incubated with GSS (0.05 mg/ml). (△): The follicle cells were incubated without GSS. Each point shows the mean ± S.E.M. of three separate experiments.

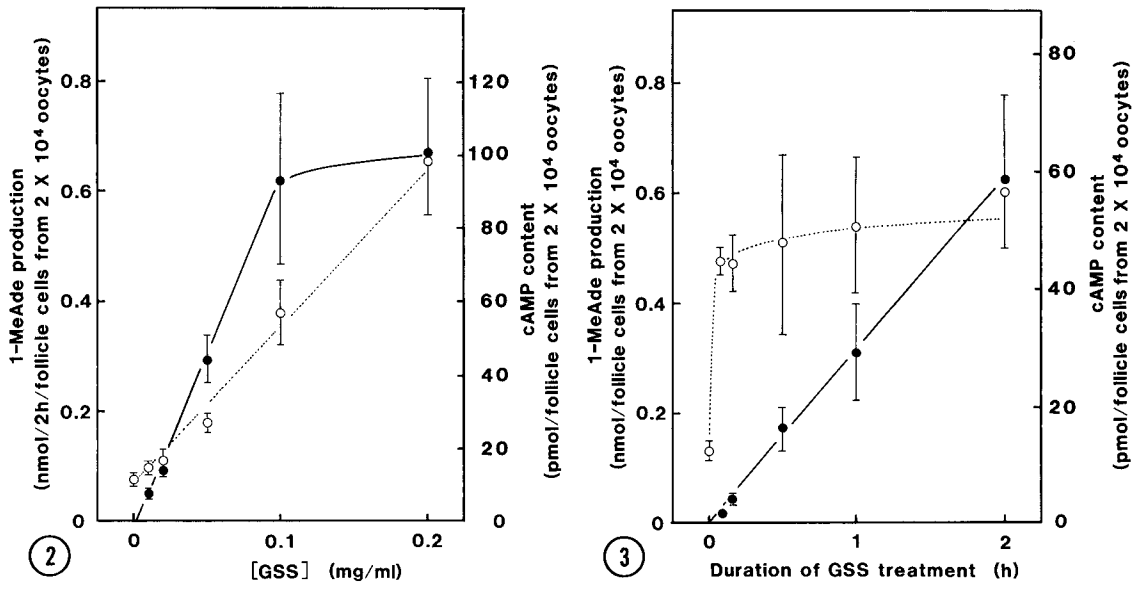

Fig. 2. Dose-response curves for 1-MeAde production and the cAMP level in follicle cells with GSS. The follicle cells were incubated with GSS at the indicated concentrations for 2 h. Each point shows the mean ± S.E.M. of four separate experiments. (●): 1-MeAde production, (o): cAMP content.

Fig. 3. Effects of GSS on 1-MeAde production and the cAMP level in follicle cells. The follicle cells were incubated with GSS (0.1 mg/ml) for specified periods. Each point shows the mean ± S.E.M. of four separate experiments. (●): 1-MeAde production, (o): cAMP content.

following its removal from the media. The addition of GSS to the cell suspension caused 1-MeAde to be produced. It follows from these findings that follicle cells are incapable of producing 1-MeAde without GSS.

The production of 1-MeAde in follicle cells has been shown to be markedly dependent on GSS concentration (14,18,19). The amount of 1-MeAde produced by follicle cells increased as GSS concentration rose to as much as 0.1 mg/ml (Fig. 2). At the same time, cAMP content in follicle cells increased following the administration of GSS. 1-MeAde production by follicle cells in the presence of 0.2 mg/ml GSS was almost the same as that in the presence of 0.1 mg/ml GSS. On the other hand, the cAMP level in the former was about twice that in the latter. It is likely that a relatively higher level of cAMP is sufficient to cause maximal activation of 1-MeAde biosynthesis.

The content of cAMP in follicle cells increased within 5 min following treatment with GSS (Fig. 3). In the presence of GSS (0.1 mg/ml), it was five times that in the control (Figs. 2 and 3). The continual presence of GSS maintained relatively higher levels of cAMP.

Follicle cells have been shown to produce 1-MeAde from 1-MeAde-R (8). Though the presence of 1-MeAde-R (1 mM) led to the production of 1-MeAde, it

Table 1. Effects of GSS and 1-MeAde-R on 1-MeAde production and the cAMP level in follicle cells

Condition	1-MeAde production (nmol/2 h/10^6 follicle cells)	cAMP content (pmol/10^6 follicle cells)
Zero time control	——	12.3 ± 1.8
Control	<0.01	11.2 ± 2.2
1-MeAde-R (1 mM)	0.27 ± 0.01	12.6 ± 2.0*
GSS (0.1 mg/ml)	0.62 ± 0.16	60.8 ± 12.0**

The follicle cells from 2 x 10^4 oocytes were incubated with GSS or 1-MeAde-R for 2 h. The values are the means ± S.E.M. obtained in four separate experiments. P values compared with the zero time control value. *: $P > 0.5$, **: $P < 0.01$.

caused no change in the intracellular content of cAMP (Table 1). Thus, 1-MeAde-R-induced 1-MeAde production may not necessarily be related to the formation of cAMP.

DISCUSSION

The data of the present study indicate the biosynthesis of 1-MeAde in starfish follicle cells to require stimulation by GSS (Fig. 1). Without GSS, follicle cells, even following pretreatment with GSS, never produced 1-MeAde. 1-MeAde production in follicle cells remarkably depends on GSS concentration (14,18,19)(Fig. 2), strongly suggesting GSS to be a trigger for 1-MeAde biosynthesis.

The intracellular level of cAMP was also found to increase immediately following administration of GSS (Fig. 3). Thus, the first step of the stimulatory effect of GSS on 1-MeAde production by follicle cells may be receptor-mediated activation of adenylate cyclase and the formation of cAMP. The cAMP level was also noted to be correlated to GSS concentration (Fig. 2). However, 1-MeAde production reached a plateau in the presence of more than 0.1 mg/ml GSS, even with linear increase in the level of cAMP. This indicates that cAMP indirectly regulates 1-MeAde production in follicle cells. It is likely that cAMP-dependent protein kinase activated by cAMP stimulates 1-MeAde biosynthesis.

It also become evident that 1-MeAde production induced by 1-MeAde-R is not related to cAMP level (Table 1). 1-MeAde-R ribohydrolase which catalyzes the reaction from 1-MeAde-R to 1-MeAde (9,10) may not be regulated by cAMP. It is assumed that, as soon as 1-MeAde-R is produced in follicle cells, it is metabolized to 1-MeAde by 1-MeAde-R ribohydrolase. This would confirm that 1-MeAde-R is a precursor of 1-MeAde.

Methionine has been reported to enhance 1-MeAde production in the presence of GSS (20,21). The radioactive methyl group of methionine is incorporated into 1-MeAde in follicle cells treated with GSS (21). Thus, transmethylation is considered to be involved in biosynthesis of 1-MeAde. cAMP may possibly activate transmethylation through the action of protein kinase.

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