STIMULATION BY CYCLIC GMP OF PROSTAGLANDIN E PRODUCTION IN ISOLATED GRAAFIAN FOLLICLES

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SUMMARY

Rat Graafian follicles isolated intact responded to 8-Br-cyclic GMP (0.3 and 1.0 mM) with increased prostaglandin E (PGE) production (4-fold and 8-fold, respectively) during a 6 h incubation. The effect of 8-Br-cyclic GMP was noted after a lag period of 2-4 h. 8-Br-cyclic AMP (1.0 mM) also stimulated PGE production (4-fold increase), while 8-Br-cyclic IMP, 8-Br-5'GMP and 8-Br-5'AMP were inactive in this respect. Actinomycin D (10 µg/ml) and cycloheximide (10 µg/ml) given simultaneously with 8-Br-cyclic GMP prevented the stimulatory effect of the cyclic nucleotide. The results suggest that cyclic GMP induces *de novo* synthesis of a macromolecular component of the ovarian prostaglandin synthetase system, and that this cyclic nucleotide, along with cyclic AMP, may play a role in the known stimulatory action of luteinizing hormone on follicular prostaglandin production.

INTRODUCTION

Several hormones such as angiotensin II (1,2), bradykinin (2,3), TSH (4), FSH (5), LH (5,6) and ACTH (7) stimulate prostaglandin production in the appropriate target tissue. Since these hormones are well known as activators of adenylate cyclase, it seems possible that cyclic AMP may mediate the stimulatory action of these hormones on prostaglandin production. Recently, cyclic AMP has been found to augment prostaglandin accumulation in several tissues (4,8), including the ovary (6).

Insulin, acetylcholine and $PGF_{2\alpha}$ increased cyclic GMP concentration in several tissues (9-11). Conflicting observations regarding the influence of LH and FSH on ovarian cyclic GMP production have been reported, both stimu-

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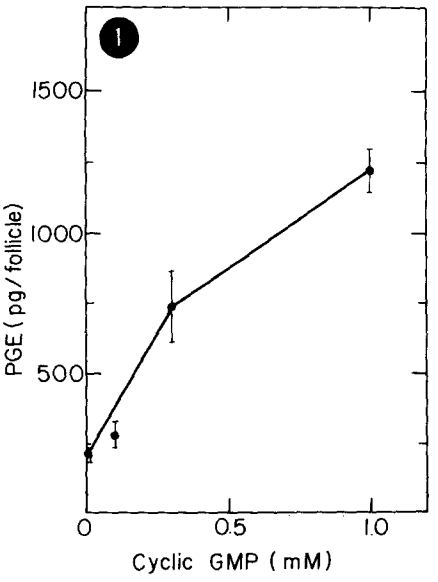

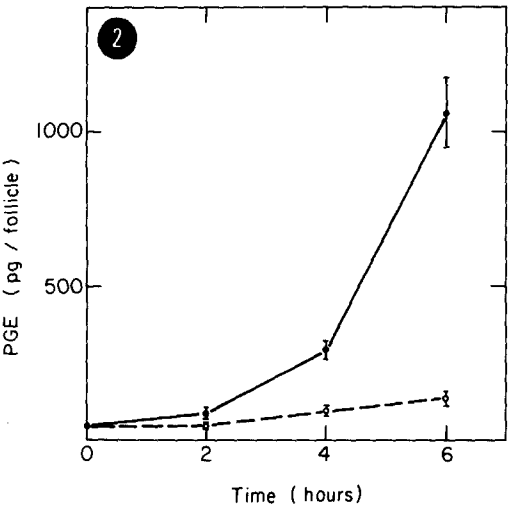

Fig. 1. Stimulation by 8-Br-cGMP of PGE production by isolated Graafian follicles: dose-response curve. Incubation for 6 h; for conditions see Materials and Methods. Vertical brackets, ± S.E.M. (n = 15).

Fig. 2. Time-course of 8-Br-cGMP action on PGE formation by cultured Graafian follicles; ●—●, cGMP (1 mM); o- - -o, control. Vertical brackets, ± S.E.M. (n = 10).

latory (12) and negative (13,14) or even inhibitory effects (14) having been noted. The β-subunit of hCG was reported to stimulate ovarian cyclic GMP production (13). The role of cyclic GMP in the regulation of prostaglandin production has not been examined. The purpose of the present study was to examine whether cyclic GMP derivatives can affect the rate of PGE accumulation in cultured Graafian follicles.

MATERIALS AND METHODS

Materials. Bromo-derivatives of the cyclic and non-cyclic nucleotides, cycloheximide and arachidonic acid were purchased from Sigma Chemical Co., St. Louis, Mo., actinomycin D from Merck, Sharpe and Döhme, Rahway, N.J., and the thio-derivatives of cyclic GMP (8-thio-cGMP and 8-methyl-thio-cGMP) from ICN Pharmaceuticals, Cleveland, Ohio. PGE_2 was a generous gift from Dr. Pike of the Upjohn Co., Kalamazoo, Michigan.

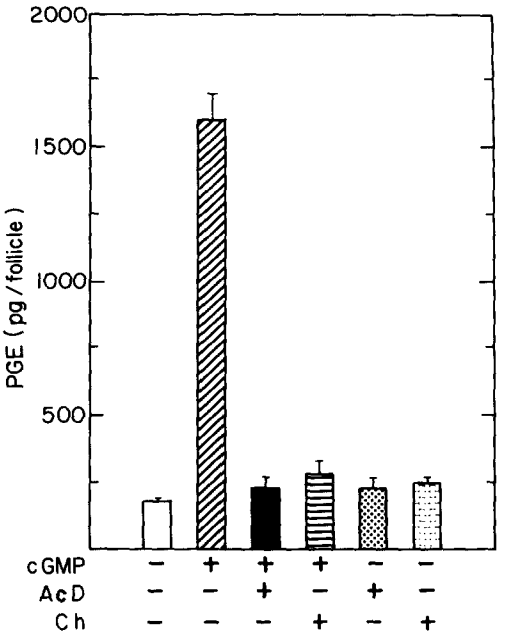

Fig. 3. Effect of inhibitors of macromolecular synthesis on 8-Br-cGMP-stimulated PGE production by follicles. cGMP, cyclic GMP (1.0 mM); AcD, actinomycin D (10 µg/ml); Ch, cycloheximide (10 µg/ml). Incubation for 6 h under conditions described in Materials and Methods section. Vertical brackets, ± S.E.M. (n = 15).

Methods. Three-month-old Wistar derived rats of the departmental colony were sacrificed between 8.00-12.00 h on the day of proestrus. Large intact Graafian follicles were isolated (15) and incubated at 37°C in Krebs-Ringer bicarbonate buffer containing 1 mg/ml glucose and 1 mg/ml fat-free BSA (Pentex-Kentaky), under an atmosphere of 95% O_2 5% CO_2 for 6 h or for the time specified.

Assay of PGE. After incubation, follicles were homogenized in Tris-EDTA buffer pH 7 and PGE was determined in homogenate and medium by radioimmunoassay as previously described (16). The total PGE produced represents the amount found in the incubation medium and in the follicular homogenate and is expressed as pg PGE/follicle.

RESULTS

Addition of 8-Br-cyclic GMP (0.3 or 1.0 mM) to the medium induced a 4 and 6-fold increase, respectively, in PGE production by isolated Graafian follicles during a 6-h incubation (Fig. 1). Thio derivatives of cyclic GMP (8-Me-S-cGMP and 8-HS-cGMP; each at 1.0 mM) also induced a 2-3 fold increase

(data not presented). 8-Br-cyclic IMP, 8-Br-5'GMP and 8-Br-5'AMP were inactive at 1.0 mM, while 8-Br-cyclic AMP (1.0 mM) also stimulated PGE production 4-fold (data not shown). The effect of 8-Br-cyclic GMP was noted after a lag period of about 2-4 h (Fig. 2).

Actinomycin D and cycloheximide (10 µg/ml each) given simultaneously with 8-Br-cyclic GMP prevented the stimulatory effect on PGE production (Fig. 3). Concomitant addition of arachidonic acid (2 µg/ml) did not overcome the effects of these inhibitors.

DISCUSSION

The Yin-Yang hypothesis proposed by Goldberg and associates (17,18) suggests that cyclic GMP has an antagonistic effect to that of cyclic AMP on cell growth (17), muscle contractility (19), lisosomal stability (20), protein phosphorylation (21) and heart-phosphofructokinase activity (22). However, both cyclic GMP and cyclic AMP stimulated adrenal steroidogenesis (23) and ovarian PGE production (this report).

The exact mechanism by which cyclic GMP induces PGE production is still unclear. Two main possibilities exist: 1) in several tissues prostaglandin synthesis is limited by substrate availability (24). Thus cyclic GMP, which is known as a labilizer of lysosomes (20), may enhance the release of phospholipase A and as a consequence cause an increase in free arachidonate level; 2) stimulation of *de novo* synthesis of microsomal PG synthetase system. The second possibility is more likely, since the effect of cyclic GMP is characterized by a lag period of about 3 h and is susceptible to inhibitors of macromolecular synthesis. Moreover, it seems that the level of endogenous free arachidonic acid is not rate-limiting in ovarian PGE production (data not shown); and concomitant addition of arachidonic acid with cyclic GMP did not overcome the inhibitory action of actinomycin D and cycloheximide on PGE production.

An essential role of PGs in the ovulatory action of LH has been estab-

lished (25, for review see 26). Stimulation of PG production by LH is characterized by a lag period and is susceptible to inhibitors of protein synthesis (27), similar to the action of cyclic GMP reported in the present study. Although the stimulatory effect of LH on ovarian cyclic AMP production was extensively investigated (for review see 28), only fragmentary studies on the effect of LH on ovarian cyclic GMP formation are available and the results are conflicting. Surprisingly, the β-subunit of hCG stimulated cyclic GMP production in rat luteal tissue, while the intact hormone was inactive in this respect (13). On the other hand, a marked reduction in cyclic GMP level was found in follicular tissue after LH and FSH addition (14). It is obvious that further study is required before one can conclude whether cyclic GMP is a physiological mediator of gonadotropin action on ovarian PGE production.

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