IN VITRO STIMULATION OF STEROIDOGENESIS IN RAT TESTIS BY CHOLERA ENTEROTOXIN

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SUMMARY: Cholera enterotoxin increases C-AMP production, and stimulates testosterone secretion in the rat testis in vitro. This gonadotropic action of cholera enterotoxin is potentiated by theophylline. It is suggested that cholera enterotoxin acts on Leydig cells directly to activate their adenyl cyclase, and consequently stimulates steroidogenesis in the rat testis. However, the receptor of cholera enterotoxin seems to be located at a different site from that of human chorionic gonadotropin.

INTRODUCTION

Cholera enterotoxin, an exotoxin of Vibrio cholerae, is known to stimulate adenyl cyclase of the intestinal mucosa, to increase the intracellular C-AMP concentration and to stimulate intestinal fluid secretion, causing fulminant diarrhea (1).

Recently it was demonstrated that cholera enterotoxin also activated adenyl cyclase of other tissues, such as fat cells, liver, thyroid gland and adrenal tumor cells, stimulating lipolysis (2), glycogenolysis (3), colloid droplet formation (4) and steroidogenesis (5), respectively. In this communication, we have demonstrated that cholera enterotoxin also stimulates C-AMP production and steroidogenesis in the rat testis in vitro.

METHODS

A three-month old, Wistar-Imamichi rat was anesthesized with ether. The testis was removed, decapsulated and sectioned into slices of 50 mg each. Each testis slice was put into a glass homogenizer (13 mm x100 mm) filled with 0.5 ml of Krebs-Ringer-

bicarbonate buffer (pH 7.4), containing 1 mg/ml of glucose, 10 mM theophylline and an appropriate amount of cholera enterotoxin or human chorionic gonadotropin (hCG). Incubation was performed at 34°C for 3 hours unless otherwise described, with constant agitation in an atmosphere of oxygen 95% : carbon dioxide 5%.

At the end of the incubation, 50 µl of the incubation medium was pipetted for measurement of released testosterone, and 500 µl of 12% trichloroacetic acid was then added to the homogenizer. The testis was homogenized, and centrifuged, and the supernatant was extracted twice with water-saturated ether. The water phase was evaporated under N_2 gas, the residue was redissolved in 1 ml of 0.5 M sodium acetate buffer (pH 6.2) and aliquots of 100 µl were assayed for C-AMP.

In an attempt to determine the extracellular C-AMP level required to stimulate testosterone production, the testis was incubated as described above in media containing serial concentrations of C-AMP.

In another experiment, a testis slice was incubated with an appropriate dose of cholera enterotoxin or hCG in phosphate buffered saline (pH 7.4 1/15 M) containing ca. 10,000 cpm of ^{125}I-hCG, which was radioiodinated enzymatically by miyachi's lactoperoxidase method (6).

C-AMP was determined by Steiner's radioimmunoassay (7) modified by us (8), and testosterone by Nieschlag's (9).

Cholera enterotoxin which was purified from Vibrio Cholerae strain Inaba 569 B, was kindly supplied by Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan (10). HCG (CR 117) with a biologic activity of 10,000 - 15,000 IU/mg was provided by Dr. Canfield.

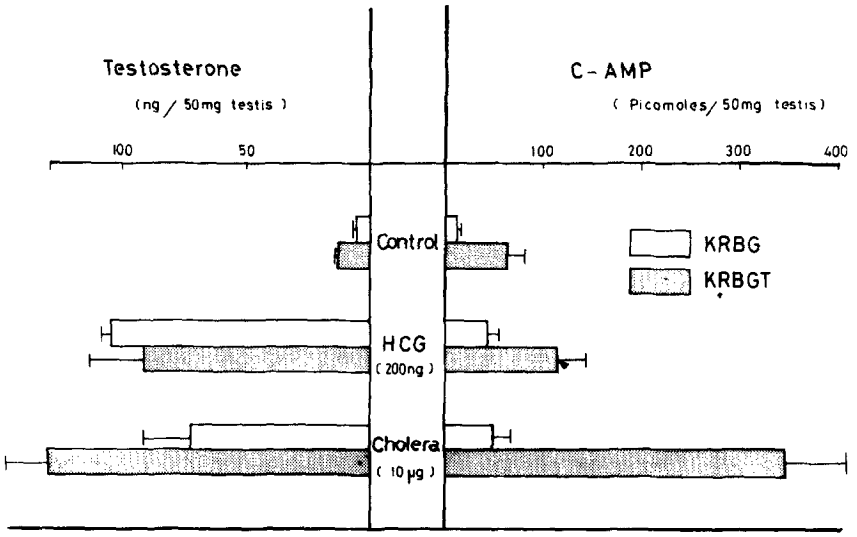

Fig. 1: Effects of hCG and cholera enterotoxin on C-AMP and testosterone production in the rat testis in vitro. Testis slice (50 mg) was incubated in Krebs-Ringer-bicarbonate glucose solution in the presence of theophylline (KRBGT) or its absence (KRBG). Values represent the mean ± standard error for six testis slices.

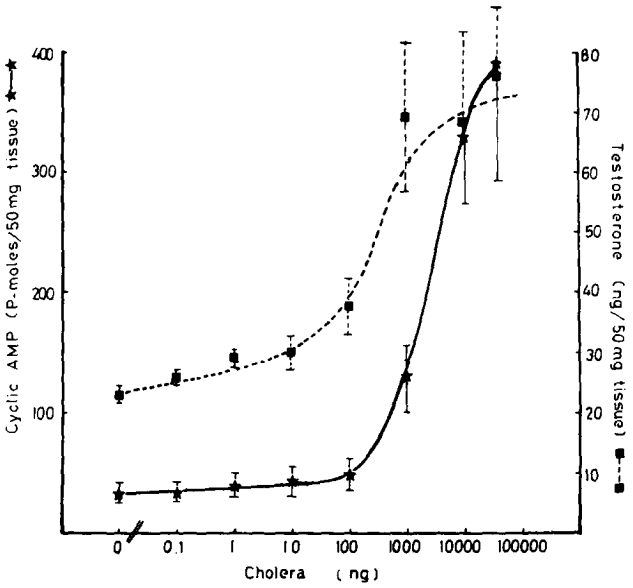

Fig. 2: Dose-response relationship of the action of cholera enterotoxin on C-AMP and testosterone productions in testis slice in KRBGT in vitro. Each point is the mean ± standard error for three testis slices.

RESULTS

As shown in Fig. 1, hCG increased C-AMP production in the rat testis in vitro during the 3 hour incubation in the Krebs-Ringer bicarbonate glucose (KRBG) solution. Cholera enterotoxin also increased C-AMP significantly. The increase in C-AMP production was further augmented, when the testis was incubated in a medium containing 10 mM theophylline (KRBGT).

In addition, hCG stimulated testosterone production, which seemed to be slightly decreased in the presence of 10 mM theophylline. Cholera enterotoxin, like hCG, also stimulated testosterone production, and this hCG-like activity was potentiated in the presence of theophylline. Therefore, all subsequent experiments were performed in KRBGT.

Fig. 2 indicates that increasing concentrations of cholera enterotoxin caused dose-responsive production of C-AMP and tes-

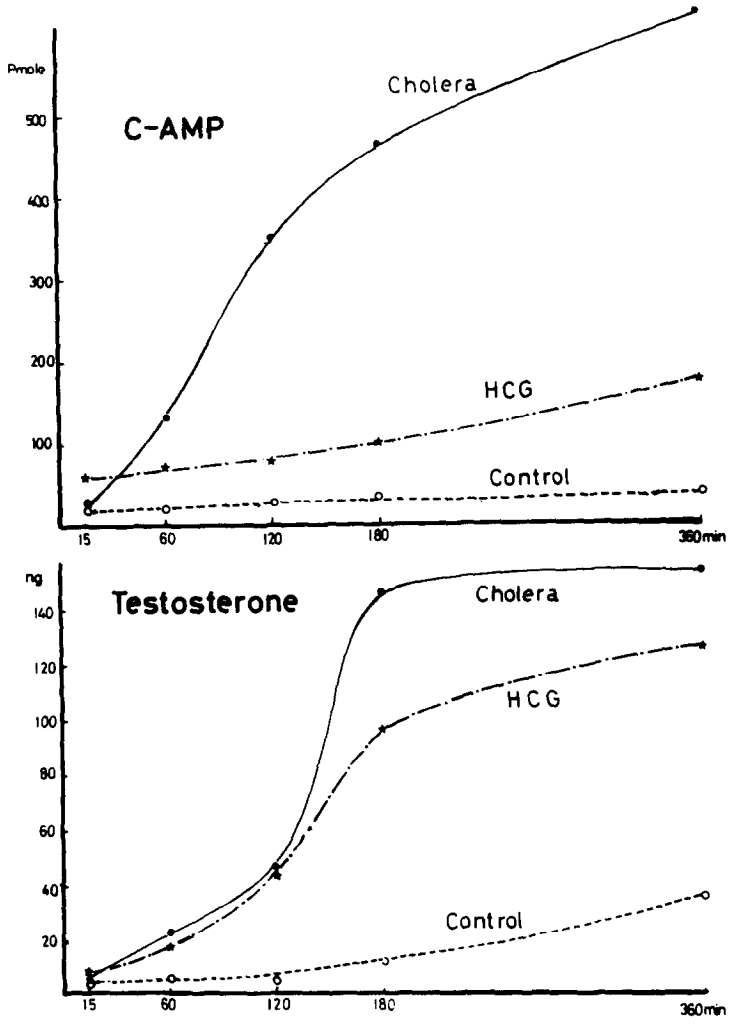

Fig. 3: Time courses of responses of the rat testis to cholera enterotoxin (10 μg) and hCG (1 μg) in KRBGT. Each point is the mean of duplicate determinations

tosterone. The minimal dose of cholera enterotoxin that stimulated C-AMP and testosterone productions was approximately 1 μg and 100 ng, respectively. Both productions appeared to be maximally stimulated by the addition of about 10 μg of cholera enterotoxin.

The time courses of the responses to hCG and cholera enterotoxin are shown in Fig. 3. 1 μg of hCG, which was proved most stimulative to C-AMP production in this series of experiments,

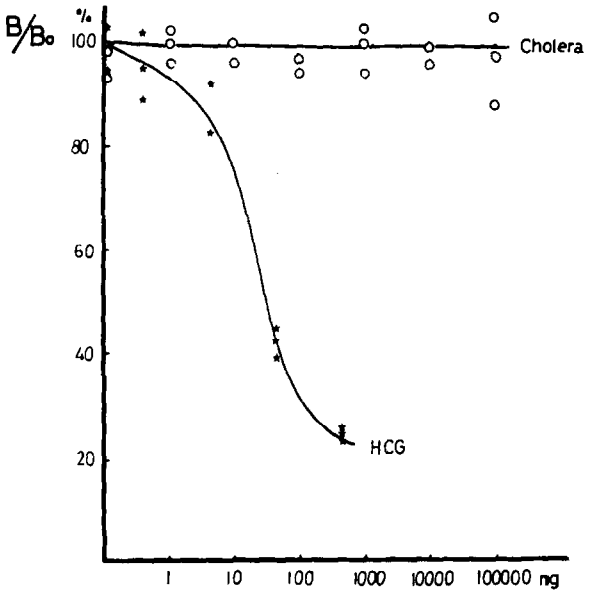

Fig. 4: Displacement of binding of ^{125}I-hCG to the rat testis in the presence of native hCG or cholera enterotoxin. The ordinate shows the bound radioactivity (B) relative to the bound for zero dose (Bo)

had caused distinct production of C-AMP in 15 min, while cholera enterotoxin did not affect C-AMP level with 15 min; cholera enterotoxin produced C-AMP progressively during 6 hours thereafter. The testosterone secretion gradually increased, and reached its maximum level in 3 hours of incubation.

In order to investigate whether cholera enterotoxin and hCG act on the same receptor in the rat testis, the inhibitory effect of cholera enterotoxin upon binding of radioiodinated hCG to its receptor sites was studied (Fig. 4). The ^{125}I-hCG bound to its specific receptor in the rat testis was displaced in the presence of native hCG, whereas cholera enterotoxin did not inhibit the binding at all, suggesting that their receptor sites were different from each other.

Another experiment was performed to determine the extacellular C-AMP concentration sufficient to stimulate testosterone

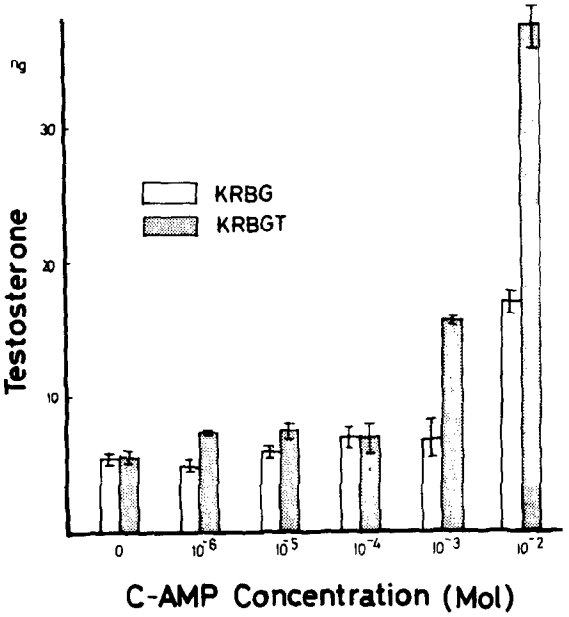

Fig. 5: Effect of exogenous C-AMP concentration on testosterone secretion in the rat testis in vitro.

release. Fig. 5 indicates that at least 10^{-3} M of exogenous C-AMP is necessary to stimulate testosterone production in the rat testis in vitro.

DISCUSSION

The data shown above indicate that cholera enterotoxin increases C-AMP and stimulates testosterone production in the rat testis in vitro, exhibiting hCG-like properties, and that this steroidogenic effect was potentiated by the addition of phosphodiesterase inhibitor, theophylline. The stimulatory effect of cholera enterotoxin in steroidogenesis in the rat testis appeared to be quite similar to the findings by Wolff, who demonstrated that cholera enterotoxin showd ACTH-like activities on adrenal tumor cells in vitro.

However, it was demonstrated that there were some difference in the steroidogenic action between hCG and cholera en-

terotoxin. Since the radioiodinated hCG bound to its specific receptor on Leydig cells (11) was not displaced by cholera enterotoxin, the acting site of cholera enterotoxin was presumed to be different from that of hCG. In addition, there was a lag of 15 min before C-AMP was increased by cholera enterotoxin, while hCG produced a distinct amount of C-AMP during the 15 min incubation. This lag phenomenon is consistent with the previous findings in other tissues (1. 2. 4. 5.).

It is of interest to consider the mechanism of steroidogenesis of cholera enterotoxin in the rat testis in vitro. As Leydig cells, known to secrete testosterone, account for only 10% of all testicular cells, cholera enterotoxin possibly activates adenyl cyclases of other testicular cells than Leydig cells, increases their intracellular C-AMP level, followed by the increase of C-AMP concentration in the medium and subsequent stimulation of Leydig cells. However, in view of the fact that the maximum C-AMP concentration attained in our experiments was 1.2×10^{-6} M at the most, when calculated as medium concentration (600 picomoles/tube), and that the extracellular C-AMP level required to stimulate steroidogenesis in the same environment was at least 10^{-3} M, such an indirect pathway was considered unlikely.

Recently it was proposed by Cuatrecasas (12) that the receptor of cholera enterotoxin was on membrane gangliosides, monosialoganglioside GM_1 in fat cells. Although it remains to be demonstrated that such gangliosides also exist on the cell membrane of Leydig cells, it is suggested that a portion of cholera enterotoxin acts directly on Leydig cells, stimulates adenyl cyclase, raises the intracellular C-AMP level, and stimulates testosterone production.

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