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CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE IN THE CHOROID PLEXUS: STIMULATION BY CHOLERA TOXIN

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SUMMARY - Cholera toxin was found to induce high accumulations of cyclic AMP in the isolated choroid plexus of the rabbit and in the incubation medium. The accumulation showed a characteristic lag phase of at least 30 min and continued for at least 3 hours. Inactivated cholera toxin was unable to increase cyclic AMP levels. There was only a moderate effect of cholera toxin on cyclic AMP "low K_m" phosphodiesterase activity in homogenates. The effect of cholera toxin on cyclic AMP levels confirms the existance of a potent cyclic AMP generating system in the choroid plexus which is activated also by ß-adrenergic agonists, histamine and prostaglandin E_1.

The choroid plexus of the cerebral ventricles is a major site of cerebrospinal fluid production (1). In the rabbit choroid plexus we have described a cyclic AMP generating system, suggesting a physiological role of cyclic AMP in the function of this organ (2). The measurement of cyclic AMP accumulation in tissues during incubation with putative neurotransmitters and neuromodulators is currently used to study the regulation of cyclic AMP systems (3). Recently cholera toxin has been shown to be a powerful stimulator of adenylate cyclase in a variety of tissues and a useful probe to study the cyclic AMP producing capacity (4,5). Of special interest is the effect of cholera toxin in fluid-secreting organs where the activity of adenylate cyclase appears to be related to electrolyte transport (6-8). In a series of experiments designed to study the regulation of adenylate cyclase activity and the possible significance of cyclic AMP levels in the choroid plexus we have studied the effect of cholera toxin on the accumulation of cyclic AMP in this tissue and compared the action of cholera toxin to that of biogenic amines and prostaglandins.

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MATERIALS AND METHODS

Randomly bred male rabbits of 2 to 3.5 kg body weight were killed by cervical dislocation. After rapid removal of the brain the lateral ventricles were opened, their choroid plexus excised and transferred into chilled Krebs-Henseleit solution of pH 7.4. The incubation medium contained Na^+ 141 mM, K^+ 5.8 mM, Ca^{++} 2.5 mM, Mg^{++} 1.16 mM, Cl^- 122 mM, HCO_3^- 25 mM, PO_4^{---} 116 mM, SO_4^{--} 116 mM and in addition glucose 5 mM and theophylline 10 mM. The tissue was preincubated for 20 minutes, then the drugs were added and the incubation continued for various periods of time. Cholera toxin and inactivated cholera toxin (inactivated by boiling for 5 minutes) were used at 2×10^{-7} M, saline was added instead of cholera toxin in control experiments. After the incubation choroid plexuses were removed from the medium and transferred into icecold 5 % trichloroacetic acid, homogenized and centrifuged for 10 minutes at 10 000 g. The protein content of the pellet was determined according to the method of Bensadoun et al. (9), bovine serum albumine serving as a standard. The supernatant was extracted four times with five volumes of water-saturated diethylether to remove the acid and then analyzed for cyclic AMP by radioimmunoassay (10). The incubation medium was analyzed directly for cyclic AMP.
Cyclic AMP phosphodiesterase activity was assayed according to Thompson and Appleman (11). Plexuses were homogenized in 0.04 M imidazolbuffer pH 7.6, 100 µl of the homogenate were incubated at 30° C in a solution composed of 20 µl 0.4 M tris-HCl pH 8.0, 20 µl 0.05 M $MgSO_4$, 20 µl labelled and 20 µl unlabelled cyclic AMP as well as 50 µl of snake venom nucleotidase (Crotalus atrox, Sigma Company, St. Louis, MO). The effect of cholera toxin on phosphodiesterase activity was determined either by adding 50 µl of cholera toxin (final concentration 2×10^{-7} M) or by measuring phosphodiesterase activity in plexuses which were preincubated in the presence of cholera toxin for two and four hours with ampicillin and oxacillin

(100 I.E./ml) added to the Krebs-Henseleit solution. After incubation for ten minutes the reaction was stopped by boiling for two minutes. The labelled nucleoside was seperated from labelled nucleotide by 500 µl of a Dowex 1 x 2 suspension and counted in Instagel. Results were expressed as pmol cyclic AMP hydrolyzed per mg protein per minute.

RESULTS

As shown in table 1 isoprenaline, histamine and PG E_1 were able to increase the cyclic AMP content of choroid plexus rapidly within minutes. The most rapid increase of cyclic AMP was seen with isoprenaline, the maximum accumulation was higher upon incubation with histamine then with isoprenaline and PG E_1. Cholera toxin increased cyclic AMP only after 30 minutes but, on further incubation, induced an extremely marked accumulation of cyclic AMP which continued for at least three hours (figure 1). Inactivated cholera toxin was unable to increase cyclic AMP levels (figure 2). With a delay of about 60 minutes cyclic AMP concentrations increased also in the incubation medium during the incubation with cholera toxin (figure 1). After 120 minutes about half of the total cyclic AMP was found in the incubation medium where it continued to rise on further incubation. As is shown in figure 3 cholera toxin at the concentration used, had only a moderate inhibitory effect on phosphodiesterase activity (26 % inhibition in the homogenate) which makes an effect on phosphodiesterase activity unlikely as a cause of the cyclic AMP accumulation. Preincubation of choroid plexuses with cholera toxin for four hours did not reveal an enzyme inducing effect of the toxin or the cyclic AMP accumulation but rather showed a moderate inhibition of phosphodiesterase activity.

DISCUSSION

Our findings confirm that cyclic AMP accumulation in the choroid plexus can be induced by a beta-adrenergic agonist, by histamine and by

Table 1 Maximum effects on cyclic AMP accumulation in choroid plexus after the substances' characteristic incubation times

Conditions		Incubation time	N	Substance (pmol ± S.E.M. per mg protein)	Control
Isoprenaline	10^{-5} M	5 min	4	107.5 ± 8.2	54.8 ± 3.6
PG E_1	10^{-6} M	10 min	4	136.9 ± 22.6	29.1 ± 3.8
Histamine	5×10^{-4} M	20 min	4	258.0 ± 64.0	48,3 ± 4.1
Cholera toxin	2×10^{-7} M	180 min	4	1500.0 ± 162.0	23.1 ± 2.7

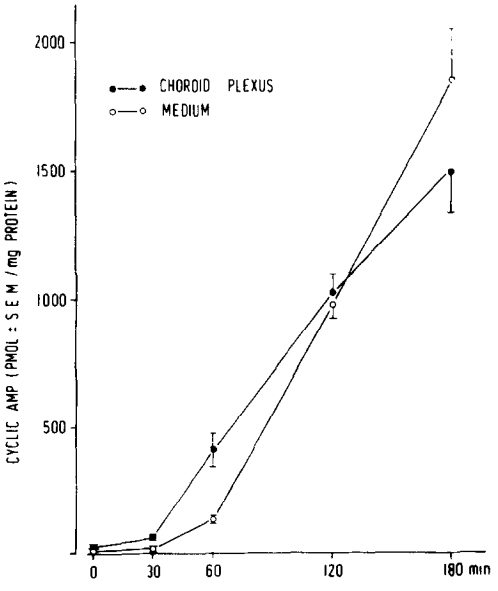

Fig. 1 - Time course of accumulation of cyclic AMP induced by cholera toxin.
Cholera toxin was used at a concentration of 2×10^{-7} M, cyclic AMP was determined according to Cailla et al. (10). •, cyclic AMP content of the choroid plexus; o, cyclic AMP appearing in the medium. Each point represents the mean of four experiments. Vertical bars show standard error of the mean.

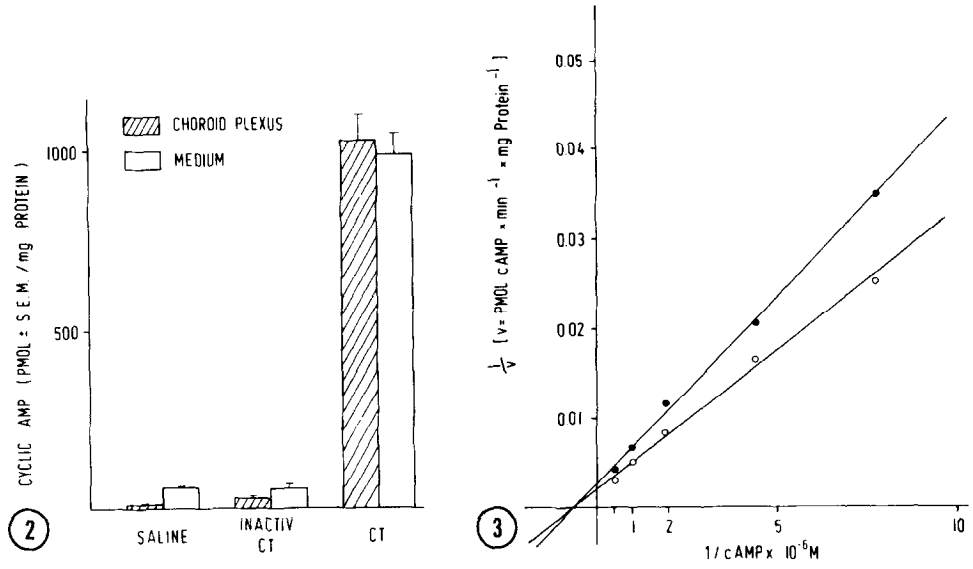

Fig. 2 - Effects of cholera toxin, inactivated cholera toxin and saline on cyclic AMP accumulation.
Incubation time in presence of the drug was 120 min. Shaded bars represent cyclic AMP content in the choroid plexus, open bars represent the cyclic AMP content in the incubation medium. Brackets show the standard error of the mean; N = 4 in all groups.

Fig. 3 - Effect of cholera toxin on phosphodiesterase activity.
"Low K_m" phosphodiesterase was assayed in choroid plexus homogenates, cholera toxin was added to the homogenate and incubation continued for 5 min. Results were plotted according to Lineweaver-Burk. K_m = 500 pmol x min^{-1} x mg $protein^{-1}$. Non-competitive inhibition of the enzyme of about 26% by cholera toxin (2 x 10^{-7} M). •, homogenate, incubated with cholera toxin; o, homogenate, incubated without cholera toxin.

prostaglandin E_1. Both, catecholamines and histamine also stimulate cyclic AMP production in the superior cervical ganglion (12) which innervates the choroid plexus (13). While cyclic AMP accumulation appeared to occur instantaneously upon addition and penetration of biogenic amines and PG E_1, cyclic AMP levels started to rise only after about 30 minutes of incubation in the presence of cholera toxin. This lag-phase of the activation is in accordance with findings in other systems (4-6). The lag-phase is explained by the necessity of the B-subunit of cholera toxin to bind to plasma membranes and the subsequent penetration of the A_1 fragment of the

A-unit through the membrane, although no agreement has been reached as to the molecular mechanisms leading to increased cyclase activity (4,5). The activation of the adenylate cyclase by cholera toxin generally is irreversible and persists after addition of anti-toxin (5). In our experiments cyclic AMP levels continued to rise for at least three hours of incubation time. The possibility that the accumulation of cyclic AMP was due to an effect on cyclic AMP phosphodiesterase was made unlikely since cholera toxin had only a moderate inhibitory effect on phosphodiesterase activity measured in the homogenates.

Strikingly large amounts of cyclic AMP generated through the effect of cholera toxin apparently were released into the incubation medium where after three hours of incubation more than half of the total cyclic AMP was recovered. Release of cyclic AMP from an intact tissue in relationship to the amount of intracellular accumulation was also observed in the superior cervical ganglion (12). In cultured cells excretion of cyclic AMP appears to be a common finding (14). That the choroid plexus is able to release cyclic AMP is relevant also with respect to measurement of cyclic AMP in the cerebrospinal fluid of patients in morbid conditions (15,16), and after application of drugs (15,17).

Since cholera toxin in several other secreting organs enhances physiological secretion of electrolytes and fluid, an effect which can be mimicked by cyclic AMP analogues or other cyclic AMP elevating drugs (6), adenylate cyclase may be involved in the production of cerebrospinal fluid in the choroid plexus. This hypothesis is supported by recent findings of Epstein et al. (18), who reported an up to five fold enhanced bulk secretion of cerebrospinal fluid in dogs following intraventricular application of cholera toxin two hours prior to the flux measurements. The fluid secreted after cholera toxin administration was isotonic with respect to the cerebrospinal fluid.

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