EFFECT OF CHOLERA ENTEROTOXIN ON PROSTAGLANDIN BIOSYNTHESIS AND METABOLISM IN THE RAT SMALL INTESTINE

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Until recently prostaglandin (PG) synthesis in the intestine under the influence of cholera and other toxins has been studied only with respect to PG of types E and F. It has recently been found that different types of PG (PGI_2, PGD_2, TXA_2-thromboxane, and other biologically active metabolites of arachidonic acid), found previously in other tissues [4], are normally synthesized in the intestine.

It was accordingly decided to study the synthesis and metabolism of various types of PG in the rat small intestine under the influence of cholera enterotoxin.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180-200 g. Cholera enterotoxin (150 μg per animal) was injected into an isolated loop of small intestine of the rats [8] under hexobarbital anesthesia (60 mg/kg). The animals were killed at various time intervals (30, 60, and 120 min), the proximal part of the small intestine was quickly removed and fixed in liquid nitrogen, and a tissue powder was prepared in a porcelain mortar in an atmosphere of liquid nitrogen. A weighed sample of the powder (200-250 mg) was rehomogenized in 0.1 M K-phosphate buffer, pH 8.0, containing 3.3 μM of the Na salt of ^{14}C-arachidonic acid (0.5 μCi, specific activity 55.5 mCi/mmole, England). The samples were incubated with constant shaking at 37°C for 20 min. After acidification of the specimens to pH 3.5 with formic acid the PG were extracted twice with four times their volume of ethyl acetate and the organic phases after centrifugation were evaporated in vacuo at 40°C, and chromatographed together with a mixture of PG standards (E_2, $F_{2\alpha}$, D_2, TXB_2, 6-keto-$PGF_{1\alpha}$, 15-keto-PGE_2, 15-keto-$PGF_{2\alpha}$) on a thin layer of mark G silica-gel (Silufol, Czechoslovakia) in an organic phase consisting of a mixture of solvents: ethyl acetate—iso-octane-acetic acid—water (110:50:20:100). The separated spots of standard PG were developed with a 10% solution of phosphotungstic acid and used to identify the labeled PG synthesized in vitro. Regions of the chromatograms were cut out and their radioactivity determined on a scintillation counter (RackBeta, Sweden). Total synthetase activity, and the quantity of synthesized PG and their metabolites were calculated by the usual method [5].

EXPERIMENTAL RESULTS

The time course of activity of the multienzyme PG-synthetase complex under the influence of different factors (cholera toxin, indomethacin, mock operation) is illustrated in Fig. 1. Synthesis and metabolism of PG were increased by 2.5 times 30 min after injection of cholera toxin into the intestinal lumen. Activity of the enzymes after 1 h was reduced, and after 2 h it reached the control level (in animals with a mock operation). The increase in enzyme activity in animals of the control group observed 1 h after the operation was evidently due to release of catecholamines, kinins, and other biologically active substances into the blood stream, activating PG biosynthesis [6].

Data on enzyme activity under the influence of cholera enterotoxin in animals premedicated with indomethacin (20 mg/kg, intraperitoneally, 1 h before injection of cholera toxin)

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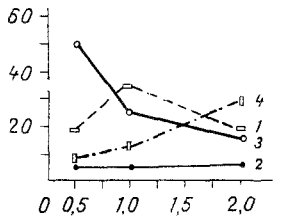

Fig. 1. Time course of activity of multienzyme PG-synthetase complex under the influence of cholera enterotoxin and indomethacin. 1) Control, 2) indomethacin, 3) cholera enterotoxin, 4) simultaneous action of cholera enterotoxin and indomethacin. Abscissa, time of action (in h); ordinate, content of ^{14}C-PG (in % of total quantity of ^{14}C-arachidonic acid added to the homogenate).

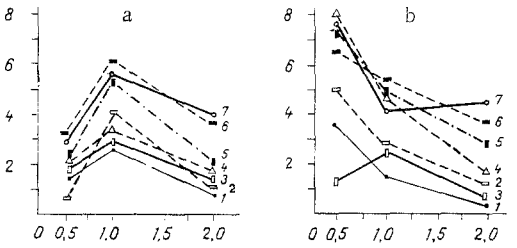

Fig. 2. Synthesis and metabolism of individual types of PG under normal conditions (a) and under the influence of cholera enterotoxin (b). 1) 6-keto-$PGF_{1\alpha}$, 2) PGA_2 + PGB_2, 3) 15-keto-$PGF_{2\alpha}$, 4) $PGF_{2\alpha}$, 5) PGE_2, 6) 15-keto-PGE_2, 7) PGD_2. Abscissa, time of action of toxin (in h); ordinate, quantity of ^{14}C-PG (in % of total quantity of injected ^{14}C-arachidonic acid).

are particularly interesting. Indomethacin is known to block synthesis of all types of PG after only 30 min [6]. We found that only 1 h after injection of the cholera toxin, PG synthesis and metabolism were activated. This activity reached a maximum after 2 h.

Data on synthesis and metabolism of the individual types of PG under the influence of cholera enterotoxin are given in Fig. 2a, b. Synthesis of "diarrheogenic" PG was activated considerably (PGE_2 by 3.4 times, $PGF_{2\alpha}$ by 4 times) and of "antidiarrheogenic" PG (PGI_2 by 2.5 times, PGD_2 by 3.5 times) was considerably activated 30 min after injection of the toxin. Activity of synthesis of prostacyclin (PGE_2; 6-keto-$PGF_{2\alpha}$ is its stable metabolite) fell sharply, and after 2 h it was close to the control level. The decrease in activity of enzymes synthesizing other types of PG (E_2, $F_{2\alpha}$, D_2, A_2, B_2) was not so sudden, but after 2 h their activity also reached the control level.

Activity of enzymes metabolizing PGE_2 and $PGF_{2\alpha}$ (PGE- and PGA-dehydrogenases) also was activated during the first 30 min after injection of the cholera enterotoxin. During the

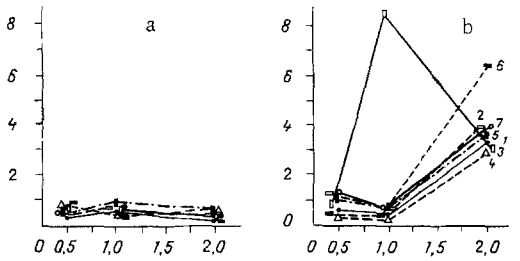

Fig. 3. Time course of synthesis and metabolism of individual types of PG under normal conditions (a) and under the influence of cholera enterotoxin and indomethacin (b). Legend as to Fig. 2.

next 30 min activity of these enzymes fell below the control values during this time interval, and after 2 h activity of the enzymes in both experimental and control series increased.

Data on the action of cholera enterotoxin on PG synthesis and metabolism after preliminary premedication of the animals with indomethacin (20 mg/kg, intraperitoneally, 1 h before injection of the toxin) are given in Fig. 3a, b. PG synthesis and metabolism were blocked by 80% (Fig. 3a) throughout the period of observation. The opposite picture was found if cholera enterotoxin was injected into the lumen of the intestine against this background. After a latent period of 1 h synthesis of all types of PG was sharply activated (E_2, $F_{2\alpha}$, D_2, and I_2 — approximately fivefold). After 60 min PG metabolism was increased almost sevenfold. After 2 h of observation activity of PG metabolism fell, but still remained at a comparatively high level (almost 2.7 times higher than initially). Activity of PGE_2 metabolism began to increase only after exposure of the mucous membrane of the small intestine to the toxin for 1 h, and after 2 h it was eight times higher than the control level.

This investigation revealed several new facts. First, during the action of cholera enterotoxin PG synthesis and metabolism reach a maximum during the first 30 min after injection of the toxin into the intestinal lumen. Second, in the course of action of the toxin there is a redistribution in the spectrum of synthesized PG toward predominance of the group of "diarrheogenic" PGE and PGF and their metabolites (PGA_2, PGB_2, 15-keto-$PGF_{2\alpha}$). Third, after premedication of the animals with indomethacin, activation of both synthesis and metabolism of the PG chosen for study was observed. This last fact is of great importance, for different opinions are held in the literature on the antidiarrheal effect of indomethacin. Some workers have observed an antidiarrheal action of indomethacin even in cases of diarrhea caused by cholera enterotoxin [3], whereas other [7] did not confirm these findings.

It is well known that PG, which have a diarrheogenic effect, like cholera enterotoxin, activate the adenylate cyclase system and promote accumulation of cAMP in the cell. According to the generally accepted hypothesis, cAMP is the principal mediator of diarrhea [3, 7]. In the light of the results now obtained, we can begin to understand why the second group of investigators could not confirm the results for the antidiarrheal effect of indomethacin. However, it is not quite clear why activation of both synthesis and metabolism of PG takes place under the influence of cholera toxin after premedication of the animals with indomethacin. Indomethacin is known not to inhibit completely the key enzyme of the PG-synthetase complex, cyclo-oxygenase [6], and we have no information whatever on the action of this substance on intermediate enzymes of PG synthesis and metabolism. There is even evidence that indomethacin inhibits 15-prostaglandin dehydrogenase (the principal enzyme of metabolism of all PG), phosphodiesterase, and other enzymes [2]. This action of indomethacin may evidently facilitate accumulation of PG and cAMP on account of its inhibitory effect on the corresponding enzymes of metabolism.

PG are known to possess a wide spectrum of regulating actions on metabolism of organs and tissues. For instance, they participate in ATP synthesis, regulate the activity of both adenylate and guanylate cyclase, phosphodiesterases, and various other enzymes [1], and through the protein kinase system they influence most physiological and biochemical effects in the cell. The action of cholera toxin may involve a disturbance of the normal relations between PG and the enzyme systems of the cell. The ensuing changes in enzyme activity may ultimately lead to the development of a diarrheal syndrome.

The results suggest that cholera toxin acts indirectly on many components in the enzyme chain of PG synthesis and metabolism, shifting their equilibrium in one direction or the other from the normal level.

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EXPERIMENTAL STUDY OF THE POSSIBILITY OF ISOLATED PERFUSION OF THE LYMPHATIC SYSTEM

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Among the many functions of the human lymphatic system, its role in transport has been the least adequately studied. It has become recognized that movement of lymph takes place only in the central direction on account of the valve system of the lymphatic vessels, rhythmic contractions of the diaphragm, pulsation of arteries, and active and passive movements. As regards relations between the lymphatic and blood systems, two diametrically opposite opinions are held. The supporters of lymphovenous junctions [1, 7, 9, 11, 13, 14] claim that anastomoses between lymphatic and venous vessels have now been proved reliably, but this is evidently true only of pathologically formed connections arising in the presence of chronic lymphatic stasis of varied origin. Other workers [2-4, 8, 10, 12], on the other hand, consider that the absence of any additional sites of junction of the blood and lymphatic vessels than the places of entry of the main lymphatic trunks into the venous system in the neck is an undisputed fact. Yet it is important to know whether lymphovenous anastomoses do or do not exist. Since under ordinary conditions no additional sites of drainage of lymphatic vessels into veins have been found, it would seem perfectly possible to create isolated perfusion of the lymphatic system by means of endolymphatic infusion with simultaneous drainage of the thoracic duct. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

The investigation was conducted in three directions: the volume flow rate of Evans' blue T-1824, injected endolymphatically was studied (by this method, passage of the dye into the blood system is not observed); in addition, the functional capacity of the lymphatic system to retain various drugs, low-molecular-weight substances, and microorganisms was tested, and the possibility of creation of an extracorporeal lymphatic circulation was investigated.

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