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Effect of cholera toxin on the production of eicosanoids by rat jejunum

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1 Cholera toxin injected i.v. into rats stimulated the production of prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) and LTC_4 by segments of jejunum, but it had no effect when added to the tissue *in vitro*.

2 Pretreatment of the animals with the compound BW 755C reduced the increased production of PGE_2, LTB_4 and LTC_4 by i.v. cholera toxin. Pretreatment with indomethacin reduced the production of PGE_2.

3 These findings are consistent with the hypothesis that arachidonate metabolites are involved in the diarrhoea induced by cholera toxin.

Introduction

Cholera toxin and certain prostaglandins cause the same qualitative stimulation of intestinal secretion (Greenough *et al.*, 1969; Al-Awqati *et al.*, 1970). The hypothesis that prostaglandins might be involved in the mechanism of action of cholera toxin (Bennett, 1971) gained support when it was demonstrated that aspirin or indomethacin *in vivo* inhibited cholera-induced intestinal secretion (Finck & Katz, 1972; Jacoby & Marshall, 1972). However, doses of aspirin or indomethacin sufficient to inhibit prostaglandin synthesis did not inhibit the stimulation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) production by cholera toxin (Powell & Farris, 1975). The stimulatory effects of cholera toxin and prostaglandins on intestinal adenylate cyclase might be mediated by different receptors (Finck & Katz, 1972; Kimberg *et al.*, 1971). Furthermore, Hudson *et al.* (1975) and Smith *et al.* (1985) found that cholera toxin *in vivo* does not stimulate prostaglandin synthesis in rabbits and rats, and Bennett & Charlier (1977) found that cholera toxin did not release prostaglandins from guinea-pig isolated intestine. Thus the role of prostaglandins in mediating the action of cholera toxin in diarrhoea remains uncertain. We have examined this problem further by measuring the amounts of leukotriene B_4 (LTB_4) and LTC_4, as well as the amount of prostaglandin E_2 (PGE_2), synthesized by rat intestinal homogenates after stimulation with cholera toxin.

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Methods

Male Wistar-Nossan rats (130–140 g) were deprived of food overnight but allowed water *ad libitum*. Cholera toxin (*Vibrio cholera*, Sigma C-3012) 100 $\mu g\,kg^{-1}$, was administered i.v., and 2 h later the animals were killed by exposure to ether and bled. The jejunum was then removed, cut finely, rinsed in Krebs solution (composition of Krebs solution in $g\,ml^{-1}$: NaCl 6.9, KCl 0.35, $CaCl_2.2H_2O$ 0.36, $MgSO_4.7H_2O$ 0.29, KH_2PO_4 0.16, $NaHCO_3$ 2.1; dextrose 1.0), and immediately weighed. Samples of 20 mg tissue were homogenized in 1 ml of Krebs solution for 10 s at room temperature and incubated at 37°C for 30 min. Enzymic activity was then terminated with methanol/formic acid (1 ml: 40 µl), and the fatty acids were extracted into $CHCl_3$ by shaking twice with 2 ml quantities which were then evaporated to dryness under N_2 at room temperature. The extract was dissolved in 1 ml tricene buffer (0.1 M, pH 8) and stored at −20°C until assayed. Fatty acids were analysed by radioimmunoassay (Hennam *et al.*, 1974) with tritiated (LTB_4, LTC_4) or iodinated (PGE_2) standard (100 µl of 0.2 $\mu Ci\,ml^{-1}$ solution in phosphate buffer) and specific antiserum. Cross-reactions of the antibodies were as follows (%): PGE_2 antibody,

PGE_2 100, PGE_1 3.7, PGA_2 0.4, $PGF_{1\alpha}$ 0.03, Thromboxane B_2 (TXB_2) 0.02, $PGF_{2\alpha}$ < 0.01; LTB_4 antibody, LTB_4 100, LTC_4 0.03, LTD_4 0.03, 5, 12 dihydroxy-6, 8, 10-*trans*-14-*cis* eicosatetraenoic acid 3.3, 5, 6-diHETE 1.6, 5–12 diHETE 0.14, 5-HETE 0.03; LTC_4 antibody, LTC_4 100, LTD_4 8.8, LTE_4 8.6, 5-HETE 0.07, LTB_4 0.006, arachidonic acid 0.005. Some rats were pretreated with indomethacin (6 mg kg^{-1} i.p.) or BW 755C (10 mg kg^{-1} orally) 30 min before giving the cholera toxin. In other experiments segments of jejunum from untreated rats, were cut finely and homogenized in Krebs solution to give 20 mg ml^{-1}. Aliquots of 5 ml were incubated (30 min, 37°C) without (controls) or with cholera toxin (100 μg ml^{-1}), and the fatty acids extracted into chloroform before radioimmunoassay for PGE_2, LTB_4 and LTC_4. The sensitivity limit for PGE_2 was 2 pg; LTB_4 12 pg; LTC_4 10 pg. The mean recoveries (± s.d.) for PGE_2, LTB_4 and LTC_4 (by the chloroform extraction) were 87 ± 9.16%, 86 ± 7.4% and 84 ± 6.7% respectively. Estimates of precision (intra and interassay) were obtained by application of the procedure proposed by Coker *et al.* (1982). On 10 duplicate determinations performed in the same assay with values of eicosanoid ranging from 10–100 pg ml^{-1} of extract, the coefficient of variation (calculated as % CV = (standard deviation/mean) × 100) was (mean value): PGE_2 7.9%, LTB_4 8.7%; LTC_4 7.3%. In 10 duplicate determinations made on consecutive days, the coefficient of variation for each eicosanoid was as follows (mean value): PGE_2 13.8%; LTB_4 14.3%; LTC_4 12.9%.

Drugs

The following were used: cholera toxin and indomethacin (Sigma, Milano), [^{3}H]-LTC_4, and [^{125}I]-PGE_2 (NEN, Firenze), [^{3}H]-LTB_4 (Amersham, Milano), 3-amino-1[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline (BW 755C, Wellcome, Beckenham). Other chemicals were reagent grade.

Statistical test

The results were analysed statistically by Student's paired *t* test (2-tailed).

Results

Table 1 shows that homogenates of isolated jejunum from rats injected i.v. with cholera toxin (100 μg kg^{-1}) produced significantly more PGE_2, LTB_4 and LTC_4 than controls (increase of 95%, $P < 0.01$; 55% $P < 0.01$ and 60% $P < 0.05$, respectively). Pretreatment of the rats with indomethacin (6 mg kg^{-1}) reduced the increase in PGE_2 output by 43% ($P < 0.05$), while compound BW 755C (10 mg kg^{-1}) reduced the increased production of PGE_2 by 37% ($P < 0.01$), of LTB_4 by 40% ($P < 0.01$) and of LTC_4 by 44% ($P < 0.01$). In rats not given cholera toxin, indomethacin reduced only the PGE_2 output (by 60%, $P < 0.01$), while compound BW 755C, a dual cyclooxygenase/lipoxygenase inhibitor, reduced the outputs of PGE_2 (by 50%, $P < 0.01$), LTB_4 (by 56%, $P < 0.01$) and LTC_4 (by 62%, $P < 0.01$%).

Cholera toxin added to the organ bath (100 μg ml^{-1}) had little or no effect on the basal output of PGE_2, LTB_4 and LTC_4 by the jejunum (pg mg^{-1} wet weight; mean ± s.e.mean, $n = 4$); control, PGE_2 58.0 ± 8.4; LTB_4 18.7 ± 3.7; LTC_4 20.5 ± 5.0; cholera toxin-treated, PGE_2 55.4 ± 10.4; LTB_4 19.7 ± 5.7; LTC_4 18.0 ± 3.7.

Discussion

Cholera toxin and some prostaglandins stimulate intestinal secretion, and increase gut adenylate cyclase activity (Kimberg *et al.*, 1971) and the cyclic AMP content (Kimberg *et al.*, 1974). These findings, together with the reduction of the secretory effects of cholera toxin by prostaglandin synthesis inhibitors (Finck & Katz, 1972; Jacoby & Marshall, 1972) suggest a primary role for prostaglandins in the action of the cholera toxin. Some studies indicate that prostaglandins and cholera toxin stimulate intestinal secretion by different mechanisms (Kimberg *et al.*, 1971; Finck & Katz, 1972), refuting the concept of an intermediary role for prostaglandins in the pathogenesis of the diarrhoea (Hudson *et al.*, 1975; Smith *et al.*, 1985). However, Speelman *et al.* (1985) produced evidence that PGE_2 may play a pathophysiological role in human cholera.

Indeed our results indicate that the production of PGE_2 by rat isolated jejunum is stimulated by i.v. injection of cholera toxin, whereas no such stimulation is seen after addition of cholera toxin to the tissue *in vitro*. This difference *in vivo* and *in vitro* could depend upon the necessity of cholera toxin to interact with a blood or plasma component (Vaughan-Williams *et al.*, 1969; Gawurz *et al.*, 1970) to produce an intermediary which in turn can stimulate prostaglandin synthesis. This may explain why cholera toxin has no effect when added to normal tissue *in situ* (Smith *et al.*, 1985). Another explanation is the use of crude or pure toxin with different constituents (Bennett, 1976; Bennett & Charlier, 1977). The present study also showed that cholera toxin given i.v. to rats increases the formation of LTB_4 and LTC_4 by the jejunum. LTB_4 selectively increases the vascular permeability in the rat caecum (Sharon & Stenson, 1985), and this could contribute to the characteristic diarrhoea produced by cholera toxin. In addition 5-hydroxy- and 5-hydroperoxy-

Table 1 Effects of cholera toxin (100 $\mu g\ kg^{-1}$ i.v.), indomethacin (6 $mg\ kg^{-1}$, i.p.) and BW 755C (10 $mg\ kg^{-1}$, orally) on mean amounts ($pg\ mg^{-1}$ tissue wet weight) of prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) and LTC_4 produced by rat jejunum homogenates

Treatment	*PGE_2*	*LTB_4*	*LTC_4*
None	100.4 ± 7.20	36.0 ± 2.10	35.2 ± 1.99
Cholera toxin	195.6 ± 9.10[b]	55.9 ± 2.08[b]	56.6 ± 3.70[a]
Indomethacin	40.5 ± 5.10[b]	36.1 ± 3.10	36.9 ± 1.40
BW 755C	50.6 ± 6.10[b]	15.8 ± 2.67[b]	13.1 ± 2.21[b]
Cholera toxin + indomethacin	110.5 ± 6.50[c]	54.7 ± 3.40	55.8 ± 4.20
Cholera toxin + BW 755C	125.8 ± 5.73[c]	33.0 ± 2.67[c]	31.4 ± 2.78[c]

Values are mean ± s.e.mean, $n = 8$.
[a] $P < 0.05$; [b] $P < 0.01$ compared with control:
[c] $P < 0.01$ compared with cholera toxin. Student's t test for paired data, 2-tailed.

eicosatetraenoic acids have strong secretory effects in rabbit distal colon and guinea-pig ileum (Musch *et al.*, 1982), and the laxative effect of phenolphthalein and other secretagogues might involve the formation of prostanoids, LTB_4 and 5-HETE (Capasso *et al.*, 1984; 1985). Thus although lipoxygenase products do not affect intestinal electrolyte transport similarly and uniformally in all species (Donowitz, 1985), they may act together with arachidonate cyclo-oxygenase products in producing cholera toxin-induced diarrhoea. If so, drugs that inhibit both types of pathway may be therapeutically useful.

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