INFECTION AND IMMUNITY, Oct. 1978, p. 290-291
0019-9567/78/0022-0290$02.00/0

Vol. 22, No. 1
Printed in U.S.A.

Effect of *Escherichia coli* Heat-Stable Enterotoxin on Cyclic GMP Levels in Mouse Intestine

P. M. NEWSOME,* M. N. BURGESS, AND N. A. MULLAN

Beecham Pharmaceuticals Research Division, Nutritional Research Centre, Walton Oaks, Tadworth, Surrey, England

Received for publication 17 July 1978

Partially purified heat-stable enterotoxin obtained from *Escherichia coli* strain F11/P155 caused an accumulation of cyclic GMP in the intestines of 8-day-old mice.

Enterotoxigenic strains of *Escherichia coli* produce two types of enterotoxin which alter fluid and electrolyte transport in the small intestine (5, 9). One type (ST) is relatively heat stable with a rapid onset of action and a short duration, and the other type (LT) is heat labile with a slower onset of action and a longer duration (2). LT appears, like cholera toxin, to stimulate adenyl cyclase, leading to accumulation of cyclic AMP which causes electrolyte secretion and loss of water (3, 4).

We have shown previously that ST toxin comprises two distinct toxins (1), but little is known about the mechanism of action of either. This study was designed to characterize the effect of methanol-soluble ST on cyclic nucleotide levels in mice.

In order that effects on cyclic nucleotide levels could be unambiguously related to the effect of heat-stable enterotoxin, studies were performed with toxin from *E. coli* strain F11/P155, which W. Smith (10) produced by donation of the Ent^+ plasmid to the non-enterotoxigenic strain F11. Thus, it was possible to perform control experiments with material obtained from strain F11. Partially purified ST (80 ml) was obtained by methanol extraction and gel chromatography of a 64-h, 5-liter culture supernatant of *E. coli* strain F11/P155, essentially by the procedure we have described for strain P16 (8). Control material was similarly obtained from strain F11.

Freeze-clamped, full-thickness intestinal biopsies were taken from 8-day-old mice under ether anaesthesia, at 40 min after oral dosing with toxin. Biopsies were extracted with 8% (wt/vol) trichloroacetic acid containing a tracer quantity of [^{3}H]cyclic AMP as a recovery marker. Trichloroacetic acid-insoluble protein was determined by the method of Lowry (7), and, after removal of trichloroacetic acid by ether extraction, cyclic AMP was determined by the method of Tovey et al. (12). Cyclic GMP was determined by radioimmunoassay by an adaptation of the method of Steiner et al. (11).

Results in Table 1 show that both the F11/P155 and F11 strains produced substantial quantities of extracellular cyclic nucleotides, but that both cyclic AMP and cyclic GMP were reduced to negligible concentrations after gel chromatography. The effect of ST toxin at 40 min after oral dosing to infant mice can be seen in Table 2. There was a significant ($P < 0.001$) elevation of cyclic GMP concentration in the intestine, but cyclic AMP levels were not increased. The failure of ST to raise cyclic AMP levels in mouse intestine is consistent with a recent report by Giannella (R. A. Giannella, Gastroenterology **72**:1062, 1977).

Our observation that ST raised cyclic GMP concentration in the infant mouse intestine was unexpected and indicates that this nucleotide may be involved in the mechanism of action of this toxin. During the preparation of this manuscript, Hughes et al. (6) reported that crude culture filtrates from two enterotoxigenic strains of *E. coli* raised cyclic GMP in ligated intestinal segments of adult rabbits.

We have demonstrated previously that ST may be fractionated, on the basis of methanol solubility, into two components: a methanol-soluble toxin, STa, which is active in neonatal mice but not in the rabbit model used by Hughes et al. (6), and a methanol-insoluble toxin, STb, which is active in the rabbit model but not in infant mice (1). The culture filtrates used by Hughes et al. presumably contain STa and STb. By using a methanol-soluble toxin from strain F11/P155 we have shown that STa causes both fluid secretion and cyclic GMP accumulation in the intestines of infant mice. Further work will be needed to clarify the effect of isolated STb on cyclic GMP in these systems.

However, the accumulation of cyclic GMP in two animal models, responding to different en-

TABLE 1. *Cyclic nucleotide concentrations in toxin and control materials before and after partial purification*

Material	Concn (pmol/ml)			
	Before purification		After purification	
	Cyclic AMP	Cyclic GMP	Cyclic AMP	Cyclic GMP
F11/P155 toxin	35,000	111	24	18
F11 control	>50,000	210	29	22

TABLE 2. *Cyclic nucleotide concentrations in the intestines of infant mice after oral dosing with ST toxin or control*

Treatment[a]	Gut wt/body wt[b]	Concn (pmol/mg of protein)[c]	
		Cyclic AMP	Cyclic GMP
F11 control	0.050 ± 0.003	6.3 ± 0.9	3.0 ± 0.3
F11/P155 toxin	0.103 ± 0.010	5.5 ± 0.6	6.0 ± 0.4
Statistical significance (Student's *t* test)	$P < 0.001$	$P > 0.1$	$P < 0.001$

[a] Each animal was orally dosed with 0.05 ml of the stated preparation.

[b] Mean ± standard deviation for groups of seven animals killed and examined at 2 h after oral dosing.

[c] Mean ± standard deviation in five samples, each containing six intestinal biopsies obtained by freeze-clamping intestines at 40 min after oral dosing; results have been adjusted for efficiency of nucleotide extraction.

terotoxins, strongly suggests the involvement of this nucleotide in the control of intestinal fluid secretion.

LITERATURE CITED

1. **Burgess, M. N., R. J. Bywater, C. M. Cowley, N. A. Mullan, and P. M. Newsome.** 1978. Biological evaluation of a methanol-soluble, heat-stable *Escherichia coli* enterotoxin in infant mice, pigs, rabbits, and calves. Infect. Immun. **21**:526–531.
2. **Evans, D. G., D. J. Evans, Jr., and N. F. Pierce.** 1973. Differences in the response of rabbit small intestine to heat-labile and heat-stable enterotoxins of *Escherichia coli*. Infect. Immun. **7**:873–880.
3. **Evans, D. J., Jr., L. C. Chen, G. T. Curlin, and D. G. Evans.** 1972. Stimulation of adenyl cyclase by *Escherichia coli* enterotoxin. Nature (London) New Biol. **236**:137–138.
4. **Guerrant, R. L., U. Ganguly, A. G. T. Casper, E. J. Moore, N. F. Pierce, and C. C. J. Carpenter.** 1973. Effect of *Escherichia coli* on fluid transport across canine small bowel. Mechanism and time-course with enterotoxin and whole bacterial cells. J. Clin. Invest. **52**:1707–1714.
5. **Hamilton, D. L., W. E. Roe, and N. O. Nielsen.** 1977. Effect of heat stable and heat labile *Escherichia coli* enterotoxins, cholera toxin and theophylline on unidirectional sodium and chloride fluxes in the proximal and distal jejunum of weanling swine. Can. J. Comp. Med. **41**:306–317.
6. **Hughes, J. M., F. Murad, B. Chang, and R. L. Guerrant.** 1978. Role of cyclic GMP in the action of heat stable enterotoxin of *Escherichia coli*. Nature (London) **271**:755–756.
7. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
8. **Mullan, N. A., M. N. Burgess, and P. M. Newsome.** 1978. Characterization of a partially purified, methanol-soluble heat-stable *Escherichia coli* enterotoxin in infant mice. Infect. Immun. **19**:779–784.
9. **Smith, H. W., and C. L. Gyles.** 1970. The relationship between two apparently different enterotoxins produced by enteropathogenic strains of *Escherichia coli* of porcine origin. J. Med. Microbiol. **3**:387–401.
10. **Smith, H. W., and S. Halls.** 1968. The transmissible nature of the genetic factor in *Escherichia coli* that controls enterotoxin production. J. Gen. Microbiol. **52**:319–334.
11. **Steiner, A. L., C. W. Parker, and D. M. Kipnis.** 1972. Radioimmunoassay for cyclic nucleotides. J. Biol. Chem. **247**:1106–1113.
12. **Tovey, K. C., K. G. Oldham, and J. A. M. Whelan.** 1974. A simple direct assay for cyclic AMP in plasma and other biological samples using an improved competitive protein binding technique. Clin. Chim. Acta **56**:221–234.