Biochemical Pharmacology, Vol. 31, No. 11, pp. 2005–2009, 1982.
Printed in Great Britain.

0006-2952/82/112005-05 $03.00/0

INHIBITION OF *ESCHERICHIA COLI* HEAT-STABLE ENTEROTOXIN EFFECTS ON INTESTINAL GUANYLATE CYCLASE AND FLUID SECRETION BY QUINACRINE

RICHARD N. GREENBERG,* RICHARD L. GUERRANT,† BING CHANG, DONALD C. ROBERTSON‡ and FERID MURAD

Divisions of Geographic Medicine, Infectious Diseases and Clinical Pharmacology, Departments of Medicine and Pharmacology, University of Virgina Medical Center, Charlottesville, VA 22908 and ‡Department of Microbiology, University of Kansas, Lawrence, KS 66045, U.S.A.

(*Received* 25 *June* 1981; *accepted* 18 *November* 1981)

Abstract—Enterotoxigenic *Escherichia coli* may produce a heat-stable enterotoxin (ST) that causes diarrheal disease in humans and in animals ST activates particulate guanylate cyclase in intestinal mucosal cells and causes intestinal fluid secretion. In this study, we examined the effects of quinacrine on ST activation of guanylate cyclase and ST-mediated intestinal fluid secretion. Quinacrine significantly reduced ST activation of particulate guanylate cyclase in rat intestinal tissue. Additionally, quinacrine reduced ST-mediated fluid secretion in a rat intestinal loop assay ($P < 0.05$). In the suckling mouse model, subcutaneous quinacrine (0.1 μmole/mouse) reduced ST-induced fluid secretion at a submaximally effective dose of the toxin, but it did not reduce ST-mediated fluid secretion at a near maximally effective dose. Quinacrine (0.1 μmole/mouse) did not significantly reduce intestinal fluid secretion induced by the analog of cyclic GMP, 8-bromo cyclic GMP. However, at a higher concentration of quinacrine (1 μmole/mouse), significant inhibition of 8-bromo cyclic GMP-induced secretion was observed. Inhibition by the antimalarial agent quinacrine of ST-induced fluid secretion, by a block prior to guanylate cyclase activation, suggests a possible role for a phospholipase early in the sequence of events of ST activation of guanylate cyclase. The results suggest that ST may activate membrane phospholipases prior to ST activation of guanylate cyclase.

Two types of enterotoxins have been associated with enterotoxigenic *Escherichia coli* diarrhea, a heat-labile toxin (LT) and a heat-stable toxin (ST). Both are important causes of diarrheal disease in humans and in animals. Their mechanisms of action, however, are quite different. LT, like cholera toxin (CT), activates adenylate cyclase in many tissues and thus induces intestinal fluid secretion [1–4]. In contrast, ST appears to cause fluid secretion through increased cyclic GMP levels [5] as a result of activation of guanylate cyclase in the particulate fraction of intestinal mucosa [6–8].

Recent studies of pharmacologic interruption of the secretory effects of CT, LT and ST have shown that phenothiazine and nonsteroidal anti-inflammatory agents inhibit effects induced by these toxins [9–14]. Indomethacin inhibits ST activation of intestinal guanylate cyclase, while chlorpromazine and promethazine inhibit ST activation of guanylate cyclase and intestinal fluid secretion induced by 8-bromo cyclic GMP [15–18].

As indomethacin is a known inhibitor of the enzyme cyclooxygenase, and hence of prostaglandin synthesis [19], the prostaglandin pathway may be involved in the mechanism of ST activation of guanylate cyclase. This hypothesis is strengthened by the observation that the free radical scavenger, butylated hydroxyanisole, blocks ST-induced fluid secretion in suckling mice and ST activation of guanylate cyclase in rate intestinal tissue [8]. Hydroxyl radicals are probably formed during prostaglandin synthesis [20] and can activate guanylate cyclase from several tissues [21].

Quinacrine HCl is an inhibitor of phospholipase A_2, an enzyme that liberates arachidonate from phospholipids of the cell membrane [22, 23]. Arachidonic acid is a precursor for prostaglandin synthesis. In this study, we examined the effects of quinacrine on (a) ST activation of guanylate cyclase, (b) ST-induced intestinal fluid secretion, and (c) 8-bromo cyclic GMP-mediated intestinal fluid secretion. If the proposed phospholipid and fatty acid pathway is involved in ST activation of guanylate cyclase, quinacrine would be expected to block both ST activation of guanylate cyclase and ST-induced intestinal fluid secretion.

METHODS

Male Sprague–Dawley rats (weighing 200–300 g) were decapitated. Rat small intestine was removed and rinsed with cold 0.25 M sucrose containing 30 mM Tris/HCl (pH = 8.0), 1 mM EDTA and 1 mM dithiothreitol. Mucosal scrapings were obtained with

* Current address: Department of Medicine, St. Louis University School of Medicine, St. Louis, MO 63104, U.S.A.

† Address correspondence to: Richard L. Guerrant, M.D., Head, Division of Geographic Medicine, Box 485 Medical Center, University of Virginia, Charlottesville, VA 22908, U.S.A.

a glass slide. Mucosa was homogenized in 0.25 M sucrose containing 30 mM Tris/HCl (pH = 8.0), 1 mM EDTA and 1 mM dithiothreitol. Homogenates were centrifuged at 105,000 *g* for 60 min for separation of supernatant and particular fractions. Particulate fractions were used the same day or stored at −70° to be used within 2 weeks.

Guanylate cyclase activity was determined as described [17]. Reaction mixtures (100 μl) contained particulate fractions (10–70 μg protein), 50 mM Tris/HCl buffer (pH = 7.6), 10 mM theophylline, 3.5 mM creatine phosphate, 7.3 μg creatine phosphokinase, 1 mM GTP and 4 mM $MgCl_2$. Assays were initiated with the addition of GTP and $MgCl_2$ and incubated at 37° for 10 min in the presence of purified ST, test drug, and/or control solutions. Incubations were terminated by the addition of 0.9 ml of cold 50 mM sodium acetate buffer (pH = 4.0) and subsequent heating for 3 min at 95°. Cyclic GMP formed was determined by radioimmunoassay [24] with acetylation of samples [25] as described previously [26]. Protein was solubilized in 1 N NaOH and was determined by the method of Lowry *et al.* [27] using bovine serum albumin as standard.

The suckling mouse assay for ST was performed using 2- to 4-day-old infant mice [28, 29]. Quinacrine and other test drugs were given intragastrically or subcutaneously either 5 min or 3 hr before an intragastric injection of either 0.1 ml of purified ST, 8-bromo cyclic GMP, or the water control. Because of the yellow color seen with quinacrine intragastrically, the marker dye was used with only some of the intragastric quinacrine injections. The purified toxin was prepared from *E. coli* strain 431 [30]. In the suckling mouse, 1.25 ng of this material is an effective dose. One unit of toxin is defined as that amount of toxin which will produce an intestinal to remaining body weight ratio in the suckling mouse of 0.09. The purified ST is active in suckling mice, and in rabbit, rat and young pig intestinal segments (unpublished data).

In a rat intestinal loop assay, Sprague–Dawley rats (150–300 g) were anesthetized with pentobarbital (<25 mg/kg). The peritoneal cavity was exposed and the small bowel was flushed with isotonic saline to remove feces through a distal ileal incision. Twelve to thirteen intestinal segments of 3 cm in length were ligated with 2–0 silk ties starting in the ileum and extending no more than 45 cm toward the stomach. Two ties were made between adjacent segments. Segments were injected with 0.3 ml of toxin or water and the abdomen was closed. Six hours later loops were isolated and measured for length and weight with and without fluid. The ratio of fluid weight (i.e. weight of loop with fluid minus weight of loop without fluid) to length of loop was calculated and reflects the amount of fluid secretion during the test period. Five milligrams of quinacrine or water was injected s.c. just prior to loop preparation and again 3 hr later. The dose of ST used was 100 units of a partially purified ST preparation.

Quinacrine HCl, quinidine HCl and 8-bromo cyclic GMP were obtained from the Sigma Chemical Co. (St. Louis, MO). Chloroquine diphosphate and primaquine diphosphate were provided by the Sterling–Winthrop Research Institute (Rensselaer, NY) and amodiaquine dihydrochloride was donated by the Parke–Davis Co. (Detroit, MI). Other reagents were obtained as described previously [5, 8, 26]. Standard errors of the means were calculated, and a two-tailed Student's *t*-test was used to test the significance of the differences.

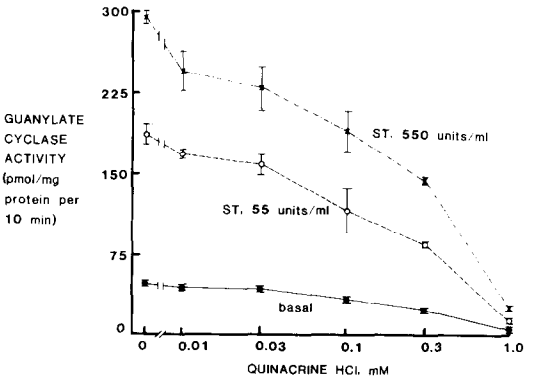

Fig. 1. Effect of quinacrine and ST on rat intestinal guanylate cyclase activity. Intestinal guanylate cyclase was incubated without (●) or with 55 units/ml (○) or 550 units/ml (×) of ST without and with variable concentrations of quinacrine, as indicated. Each point represents the mean ± S.E. of four incubations. Comparison of activity with quinacrine and without quinacrine: (1) top curve (550 units/ml), $P < 0.05$ at 0.03 mM and $P < 0.002$ for 0.1, 0.3, and 1.0 mM; (2) middle curve (55 units/ml), $P < 0.02$ at 0.1 mM, and $P < 0.002$ for 0.3 and 1.0 mM; and (3) bottom curve (0 units), $P < 0.002$ for 0.1, 0.3 and 1.0 mM.

RESULTS

Inhibition of ST activation of guanylate cyclase. Figure 1 summarizes the effects of quinacrine (0.01 to 1.0 mM) on intestinal guanylate cyclase activity without and with 55 or 550 units/ml of purified ST. These concentrations of ST result in an increasing amount of guanylate cyclase activation [8]. Quinacrine, 0.03 mM, produced an inhibition ($P < 0.05$) of guanylate cyclase that had been activated by 550 units/ml of ST. This inhibition increased to 91% with 1.0 mM quinacrine. Similar inhibition was observed when the enzyme was activated by 55 units/ml of ST. At concentrations of 0.1 mM and greater, quinacrine also significantly reduced the basal activity of guanylate cyclase. While chloroquine, primaquine or quinidine, at 0.03 to 1.0 mM, slightly reduced ST-induced activation of intestinal guanylate cyclase, the inhibitory effects of these agents were somewhat less than that of quinacrine. Another antimalarial agent, amodiaquine, at a concentration of 0.1 mM did reduce ST (550 units/ml) activation of guanylate cyclase by 44 ± 2%. At this concentration, amodiaquine also reduced basal enzyme activity by 39 ± 5%.

Inhibition of ST-induced fluid secretion in the suckling mouse

We found significant inhibition of ST-induced fluid secretion by quinacrine. As shown in Fig. 2, quinacrine (0.1 μmole/mouse), given subcutaneously

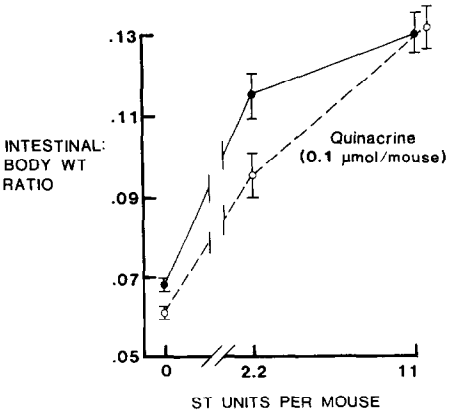

Fig. 2. Effect of quinacrine and ST on intestinal fluid secretion in the suckling mouse. Control mice (●) or those receiving 0.1 μmole of quinacrine per mouse subcutaneously (○) were then given ST intragastrically at the doses indicated. The points are the means ± S.E. of seven to thirty-seven mice. Quinacrine significantly reduced basal fluid secretion ($P < 0.01$) and the effect of 2.2 units of ST per mouse ($P < 0.02$).

5 min prior to the intragastric injection of ST, reduced the fluid secretion induced by 2.2 units of ST ($P < 0.02$). Fluid secretion was not inhibited when a larger, near maximally effective dose of ST (11 units) was tested in the mice [17]. Basal fluid secretion was also reduced by quinacrine ($P < 0.01$). A larger dose of quinacrine (1.0 μmole/mouse, s.c.) had no greater inhibitory effect on ST-induced secretion. Intragastric quinacrine (0.01 μmole/mouse to 1.0 μmole/mouse) was ineffective in reducing ST-induced secretion. The reason for the lack of an effect of intragastric quinacrine is not known; however, we observed that quinacrine (0.1 μmole/mouse), mixed with ST and given subcutaneously, still reduced fluid secretion with the submaximal ST dose.

Quinacrine (0.1 μmole/mouse) given subcutaneously but not intragastrically 3 hr before ST significantly reduced ST-induced fluid secretion in the suckling mouse by 50% ($P < 0.02$, Table 1). The inhibition was overcome by a near maximal dose of ST. The effect of subcutaneous but not intragastric quinacrine is in contrast to the effects we and others have seen with intragastric, but not subcutaneous, indomethacin [8, 17, 18]. This suggests that their sites of action are different or that their delivery to the relevant site(s) of action differs.

Other antimalarial agents, including chloroquine, primaquine, quinidine and amodiaquine, were tested at 0.1 μmole/mouse for reduction of ST-induced fluid secretion in the suckling mouse. The agents were given subcutaneously 5 min prior to ST. A significant inhibition of the secretion induced by 2.2 μmoles of ST was noted only with amodiaquine; an ST-induced fluid ratio of 0.105 ± 0.003 was reduced to 0.090 ± 0.004, N = 54 ($P < 0.005$).

Quinacrine inhibition of ST effects in rat intestinal loops. The basal ratio of fluid to length, with water as the control material, was 0.016 ± 0.004 (N = 11). This ratio was not significantly different from that of the quinacrine-treated rats, 0.015 ± 0.004 (N = 10). However, a significant increase in the ratio was seen when 100 units of ST was present in the loops; the ratio for rats with ST alone was 0.222 ± 0.033 (N = 12) versus 0.154 ± 0.020 (N = 15) for the quinacrine-treated rats with ST ($P < 0.05$). When the dose response with ST was examined in the rat, no significant secretion was seen with 10 units, and 600 units of ST produced no greater secretion than 100 units did. However, the secretory effect of 600 units overcame the inhibition with quinacrine.

Inhibition of 8-bromo cyclic GMP-induced intestinal secretion. We examined the effects of quinacrine on 8-bromo cyclic GMP-induced intestinal fluid secretion in the suckling mouse [5]. Quinacrine at doses of 0.1 μmole/mouse and 1.0 μmole/mouse given subcutaneously was tested against 0.25 and 0.5 μmole/mouse doses of 8-bromo cyclic GMP (Table 2). Quinacrine given intragastrically did not alter 8-bromo cyclic GMP-induced intestinal fluid secretion (data not shown). In mice given the lower dose of subcutaneous quinacrine (the effective dose against ST. 0.1 μmole), no significant inhibition of intestinal fluid secretion was seen with either dose of 8-bromo cyclic GMP (0.25 μmole, 0.5 μmole). The slight inhibitory effect of the higher dose of quinacrine on 8-bromo cyclic GMP-induced secretion suggests an additional effect of quinacrine beyond guanylate cyclase and cyclic GMP formation.

DISCUSSION

Since quinacrine inhibited both ST activation of guanylate cyclase in a rat small intestinal mucosal preparation and reduced ST-induced fluid secretion in the suckling mouse assay and in rat intestinal

Table 1. Effect of quinacrine (0.1 μmole/mouse) given 3 hr prior to ST on intestinal secretion in suckling mice*

Test material	Water	ST (2.2 units/mouse)	ST (11 units/mouse)
No drug	0.068 ± 0.002	0.107 ± 0.004	0.140 ± 0.003
Quinacrine, s.c.	0.067 ± 0.002	0.086 ± 0.007†	0.148 ± 0.008
Quinacrine, i.g.	0.059 ± 0.002†	0.109 ± 0.011	0.133 ± 0.005

* Mice were given quinacrine either subcutaneously or intragastrically (i.g.) 3 hr prior to the ST or water control ingastric injection. Intestinal to body weight ratio (mean ± S.E.M.) values were derived from six to eighteen mice per group.

† $P < 0.02$.

Table 2. Effect of quinacrine HCl on 8-bromo cyclic GMP-induced intestinal secretion in suckling mice*

Dose of 8-bromo cyclic GMP (μmole/mouse)	Dose of quinacrine HCl (μmole/mouse)	Intestinal to body weight ratio (mean ± S.E.M.)
0	0	0.068 ± 0.002
	0.1	0.061 ± 0.001†
	1.0	0.062 ± 0.002†
0.25	0	0.114 ± 0.002
	0.1	0.110 ± 0.004
	1.0	0.103 ± 0.003†
0.5	0	0.128 ± 0.003
	0.1	0.124 ± 0.003
	1.0	0.114 ± 0.003†

* Mice were given 0.25 or 0.5 μmole 8-bromo cyclic GMP intragastrically. Mice received quinacrine HCl subcutaneously at the indicated dose. Values were derived from nine to thirty-seven mice in each group.
† $P < 0.007$.

loops, the drug appears to have blocked prior to ST-induced formation of cyclic GMP. These results are similar to those seen with indomethacin [8, 17]. Spies *et al.* [31] found in studies with rat ductus deferens that quinacrine also decreased the effects of norepinephrine, acetylcholine and calcium, but not of nitroprusside, on cyclic GMP levels. Work by several investigators has suggested that quinacrine in the doses we have used (0.03 to 1 mm) blocks prostaglandin synthesis in a variety of tissues by inhibiting phospholipase A_2 and the release of arachidonic acid from phospholipids [22, 23, 31–33]. These findings suggest to us that arachidonate, other fatty acids, or a prostaglandin precursor may be involved in the activation of guanylate cyclase by ST, and the inhibition by both quinacrine and indomethacin may be explained by blocks at different steps in the prostaglandin synthesis pathway. In other test systems, both free fatty acids [21, 34–36] and prostaglandin endoperoxides have been shown to activate guanylate cyclase [21, 37]. Further studies of phospholipase A_2 activity, arachidonate release and prostaglandin formation are necessary to define more clearly the inhibitory mechanism of quinacrine and other antimalarial compounds (e.g. amodiaquine) and to exclude a direct effect on guanylate cyclase or a non-specific membrane effect by these agents.

The relatively high concentrations required for quinacrine effects suggest that the access to the relevant phospholipase in intestinal cells *in vivo* may be limited, or that its effect is less specific. With an even higher concentration of quinacrine (1 μmole/mouse), we also observed a decrease in the secretory effect of 8-bromo cyclic GMP. We suspect that this inhibitory effect may result from a non-specific effect of the drug when it is administered in very large doses and probably represents a different inhibitory mechanism (i.e. after cyclic GMP formation) than that seen with the smaller dose. The effect of the higher dose of quinacrine on ST-induced secretion did not exceed its effect on 8-bromo cyclic GMP-induced secretion. This observation suggests that the non-specific effect (on 8-bromo cyclic GMP-induced secretion) was not additive with the guanylate cyclase inhibiting effect seen at lower quinacrine concentrations.

A large amount of guanylate cyclase exists in the intestinal mucosal cell [38–40], predominantly in the microvillous membrane [41, 42]. Additionally, a brush border protein kinase sensitive to cyclic GMP has been reported [43, 44]. These reports, as well as our finding that quinacrine inhibits basal and ST-activated guanylate cyclase activities in rat intestinal tissue as it inhibits basal and ST-induced fluid secretion, offer further evidence that cyclic GMP is involved in regulating basal and ST-induced intestinal fluid secretion.

In summary, our findings show that quinacrine (a) reduced both basal activity and ST activation of rat intestinal mucosal particulate guanylate cyclase, (b) reduced both basal and ST-induced fluid secretion in suckling mice, (c) reduced fluid secretion due to ST in rat intestinal loops, and (d) at higher concentrations, decreased the effect of 8-bromo cyclic GMP on fluid secretion. The results suggest that the mechanism of action of ST may involve activation of a membrane phospholipase and release of arachidonate. Additional studies are needed to clarify whether specific membrane phospholipases are involved in the mechanism of action of ST.

Acknowledgements—We appreciate the technical assistance of S. E. Brown III and L. D. Halterman and the secretarial and graphics assistance of L. A. Patrick, B. S. Shannon and H. M. Kunz. This research was supported by N. I. H. Grants T32A07046, AM15316 and HL18260, and by the World Health Organization. The Division of Geographic Medicine is supported by the Rockfeller Foundation.

REFERENCES

1. L. C. Chen, J. E. Rohde and G. W. G. Sharp, *Lancet* **1**, 939 (1971).
2. D. J. Evans, Jr., L. C. Chen, G. T. Curlin and D. G. Evans, *Nature New Biol.* **236**, 137 (1972).
3. R. L. Guerrant, L. C. Chen and G. W. G. Sharp, *J. infect. Dis.* **125**, 377 (1972).

4. R. L. Guerrant, U. Ganguly, A. G. T. Casper, E. J. Moore, N. F. Pierce and C. C. J. Carpenter, *J. clin. Invest.* **52**, 1707 (1973).
5. J. M. Hughes, F. Murad, B. Chang and R. L. Guerrant, *Nature, Lond.* **271**, 755 (1978).
6. J. M. Hughes, F. Murad and R. L. Guerrant, *Clin. Res.* **26**, 524A (1978).
7. M. Field, L. H. Graf, Jr., W. J. Laird and P. L. Smith, *Proc. natn. Acad. Sci U.S.A.* **75**, 2800 (1978).
8. R. L. Guerrant, J. M. Hughes, B. Chang, D. C. Robertson and F. Murad, *J. infect. Dis.* **142**, 220 (1980).
9. R. E. Gots, S. B. Formal and R. A. Giannella, *J. infect. Dis.* **130**, 280 (1974).
10. H. I. Jacoby and C. H. Marshall, *Nature, Lond.* **235**, 163 (1972).
11. I. Lönnroth, B. Andren, S. Lange, K. Martinsson and J. Holmgren, *Infect. Immunity* **24**, 900 (1979).
12. I. Lönnroth, J. Holmgren and S. Lange, *Med. Biol.* **55**, 126 (1977).
13. G. H. Rabbani, J. Holmgren, W. B. Greenough III and I. Lönnroth, *Lancet* **1**, 410 (1979).
14. A. Wald, G. S. Gotterer, G. R. Rajendra, N. A. Turjman and T. R. Hendrix, *Gastroenterology* **72**, 106 (1977).
15. R. N. Greenberg, B. Chang, F. Murad and R. L. Guerrant, *Clin. Res.* **28**, 369A (1980).
16. D. M. Abbey and F. C. Knoop, *Infect. Immunity* **26**, 1000 (1979).
17. R. N. Greenberg, F. Murad, B. Chang, D. C. Robertson and R. L. Guerrant, *Infect. Immunity* **29**, 908 (1980).
18. G. L. Madsen and F. C. Knoop, *Infect. Immunity* **22**, 143 (1978).
19. R. Nickander, F. G. McMahon and A. S. Ridolfo, *A. Rev. Pharmac. Toxic.* **19**, 469 (1975).
20. R. W. Egan, J. Paxton and F. J. Kuehl, *J. biol. Chem.* **251**, 7329 (1976).
21. F. Murad, W. P. Arnold, C. K. Mittal and J. M. Braughler, *Adv. Cyclic Nucleotide Res.* **11**, 175 (1979).
22. R. J. Flower and G. J. Blackwell, *Biochem. Pharmac.* **25**, 285 (1976).
23. B. B. Vargaftig and N. Dao Hai, *J. Pharm. Pharmac.* **25**, 159 (1972).
24. A. L. Steiner, C. W. Parker and D. M. Kipnis, *J. biol. Chem.* **247**, 1106 (1972).
25. J. F. Harper and G. J. Brooker, *J. Cyclic Nucleotide Res.* **1**, 207 (1975).
26. S. Katsuki and F. Murad, *Molec. Pharmac.* **13**, 330 (1977).
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
28. A. G. Dean, Y-C. Ching, R. G. Williams and L. B. Harden, *J. infect. Dis.* **125**, 407 (1972).
29. R. A. Giannella, *Infect. Immunity* **14**, 95 (1976).
30. J. F. Alderete and D. C. Robertson, *Infect. Immunity* **19**, 1021 (1978).
31. C. Spies, K-D. Schultz and G. Schultz, *Naunyn-Schmiedeberg's Archs Pharmac.* **311**, 71 (1980).
32. G. J. Blackwell, W. G. Duncombe, R. J. Flower, M. F. Parsons and R. J. Vane, *Br. J. Pharmac.* **59**, 353 (1977).
33. R. M. Zusman, H. R. Keiser and J. S. Handler, *J. clin. Invest.* **60**, 1339 (1977).
34. T. Asakawa, I. Scheinbaum and R-J. Ho, *Biochem. biophys. Res. Commun.* **73**, 141 (1976).
35. D. Wallach and I. Pastan, *J. biol. Chem.* **251**, 5802 (1976).
36. T. Asakawa, M. Takenoshita, S. Uchida and S. Tanaka, *J. Neurochem.* **30**, 161 (1978).
37. G. Graff, J. H. Stephenson, D. B. Glass, M. K. Haddox and N. D. Goldberg, *J. biol. Chem.* **253**, 7662 (1978).
38. E. Ishikawa, S. Ishikawa, J. W. Davis and E. W. Sutherland, *J. biol. Chem.* **244**, 6371 (1969).
39. H. Kimura and F. Murad, *Life Sci.* **17**, 837 (1975).
40. H. Quill and M. M. Weiser, *Gastroenterology* **69**, 470 (1975).
41. H. R. DeJonge, *Fedn Eur. Biochem. Soc. Lett.* **53**, 237 (1975).
42. M. W. Walling, A. K. Mircheff, C. H. Van Os and E. M. Wright, *Am. J. Physiol.* **235**, E539 (1978).
43. H. R. DeJonge, *Nature, Lond.* **262**, 590 (1976).
44. D. V. Kimberg, L. J. Shlatz and K. A. Cattieu, in *International Colloquium in Gastroenterology: Frontiers of Knowledge in Health* (Eds. J. Janovitz and D. Sachar), p. 63. Projects in Health, Inc., Upper Montclair, NJ (1979).