However, this retarded effect is in agreement with the fact that in mice pharmacological activity (anticonvulsant effect) and receptor occupancy still occur 48 h after a single i.p. injection of diazepam when total brain concentrations of drug and metabolites are no longer detectable (Garattini et al 1973).

In 1971, Birnhaum et al have shown that intravenous diazepam decreases nocturnal basal (unstimulated) gastric secretion in man. This result has been partially reproduced by Stacher & Stärker (1974) using bromazepam and more recently it has been shown that diazepam inhibits pentagastrin-stimulated acid secretion during nocturnal tests, suggesting that it acts by inhibition of the secretory stimuli arising centrally during sleep (Roberts & Oldrey 1975).

These changes in gastric acid secretion do not appear as a possible factor mediating the diazepam or GABA-induced intestinal hyperactivity, since blockade of gastric acid secretion by cimetidine, a H_2 antagonist, does not affect the MMC pattern in the fasted dog (Buéno & Garcia-Villar 1979).

Recently it has been shown that some neuropeptides such as somatostatin and cholecystokinin octapeptide (Buéno & Ferré 1982) or calcitonin (Buéno et al 1983) act at picomolar level within the brain to affect the intestinal motility, consequently it may be suggested that the release of these substances during sleep (NREM or REM sleep) is emphazised under diazepam, the GABA-ergic neuronal system being implicated in these effects.

Benzodiazepines are largely employed in the treatment of digestive disorders such as irritable bowel syndrome (Haubrich 1976) and sometimes in anxious patients with peptic ulceration (Roberts et al 1975), this work shows that they affect the intestinal motility probably in relation to the sleep-stages but it does not explain the mechanism involved.

REFERENCES

Birnhaum, D., Karmeli, F., Tefera, M. (1971) Gut 12: 616–618

Buéno, L., Fioramonti, J., Ruckebusch, Y. (1975) J. Physiol. (London) 249: 69–85

Buéno, L., Ferré, J. (1982) Science 216: 1427–1429

Buéno, L., Ferré, J., Fioramonti, J. (1983) Peptides in the press

Buéno, L, Garcia-Villar, R. (1979) Vet. Sci. Com. 3: 249–256

Costa, E., Guidotti, A. (1979) Ann. Rev. Pharmacol. Toxicol. 19: 531–545

Garattini, S., Mussini, E., Marcucci, F., Guaitani, A. (1973) in: Garattini, S., Mussini, E., Randall, L. O. (eds) The benzodiazepine. pp 75–87, Raven Press, New York

Hall, A. W., Moossa, A. R., Clark, J., Cooley, G. R., Skinner, D. B. (1975) Gut 16: 347–352

Haubrich, W. S. (1976) in: Bockus, H. L. (ed.) Gastroenterology. Sennders Company, Philadelphia, vol. II, 3rd ed. pp 894–917

Inouye, A., Fukuya, M., Tsuchiya, K., Tsujioka, T. (1960) 10: 167–182

Klotz, U., Antonin, K. H., Bieck, P. R. (1976) J. Pharm. Exp. Ther. 199: 67–73

Krantis, A., Costa, M., Furness, J. B., Orbach, J. (1980) Eur. J. Pharmacol. 67: 461–468

Löscher, W., Frey, H. H. (1981) Arch. Int. Pharm. Ther. 254: 180–195

Pascaud, X. B., Genton, M. J., Bass, P. (1978) Am. J. Physiol. 235: E532–537

Roberts, D. M., Oldrey, T. B. N. (1975) Am. J. Gastroenterol. 63: 396–399

Stacher, G., Stärker, D. (1974) Gut 15: 116–120

Szurszewski, J. H. (1969) Am. J. Physiol. 217: 1757–1763

Tallman, J. F., Thomas, J. W., Gallager, D. W. (1978) Nature (London) 274: 383

Tallman, J. F., Paul, S. M., Skolnick, P., Gallager, G. W. (1980) Science 207: 274–281

Wastek, G. J., Speth, R. C., Reisine, T. D., Yamamura, H. I. (1978) Eur. J. Pharmacol. 50: 445–449

Weihrauch, T. R., Förster, Ch. F., Köhler, H., Ewe, K., Krieglstein, J. (1979) Gut 20: 64–67

J. Pharm. Pharmacol. 1984, 36: 132–133
Communicated July 4, 1983

Suppression of laxative action of phenolphthalein by orally-administered indomethacin or aspirin

F. Capasso, N. Mascolo, G. Autore, M. R. Duraccio, *Institute of Experimental Pharmacology, School of Pharmacy, University of Naples, via L. Rodinò 22, 80138 Naples, Italy*

Oral administration of phenolphthalein produced a dose-dependent increase of wet faeces excreted by mice. The response to phenolphthalein was reduced by pretreatment with indomethacin, aspirin or polyphloretin phosphate (PPP), but not with benoxaprofen. These findings support the view that the effect of phenolphthalein may be suppressed by PG synthetase inhibitors (indomethacin, aspirin) and by PPP.

The mechanism by which phenolphthalein and other contact laxatives exert their cathartic effect has long been a matter of controversy but, there is now a substantial literature that argues that these drugs stimulate PG biosynthesis and release PGE_2-like material in the gut (Beubler & Juan 1979; Cohen 1982). So it has been suggested that the PG may mediate the cathartic action of phenolphthalein and other laxatives. However, the contribution of these mechanisms to the cathartic effect in-vivo still remains to be established. The present paper demonstrates that treatment with indomethacin or aspirin reduces the incidence of the laxative effect of phenolpthalein in-vivo.

* Correspondence.

Table 1. Laxative effect of phenolphthalein in normal mice and mice treated with indomethacin, aspirin, benoxaprofen and polyphloretin phosphate (PPP). Experimental details are as described in the text. Each value represents the number of unformed faeces produced by groups of 6 mice. $^{**}P < 0.01$.

Treatment and dose mg kg^{-1} orally		No of unformed faeces excreted at: 3 h	12 h	24 h	Total response
Phenolphthalein	5	10	15	25	50
Phenolphthalein	10	15	25	30	70
Phenolphthalein	20	20	30	40	90
Phenolphthalein	10				
+indomethacin	0·5	10	20	30	60
+indomethacin	1·5	15	10	15	40**
+indomethacin	5	10	10	5	25**
+aspirin	30	15	20	30	65
+aspirin	60	10	15	15	40**
+aspirin	120	10	10	15	35**
+benoxaprofen	30	15	20	30	65
+PPP	100	10	15	20	45**

A modification of the method of Lou (1949) was used. Male albino mice (20–23 g) were first conditioned by being caged and subjected to weekly training periods with food deprivation and treatment with laxative. After this initial training (2 weeks) the mice were randomized in groups of 6 and treated orally over 3 days with indomethacin, aspirin or benoxaprofen. Four hours after the last treatment, food, but not water, was withheld and the mice were isolated in Perspex cages placed on wire grids over blotting paper. Any animal that showed evidence of diarrhoea was excluded from the test. Phenolphthalein, 5–20 mg kg^{-1} was then given orally. Food was placed in the cages 6 h after the test began, and the blotting paper was changed at intervals during the ensuing 24 h. The total unformed faeces excreted in 24 h within each dose group was used to evaluate drug activity. Some animals were also treated with polyphloretin phosphate (100 mg kg^{-1}) 1 h before phenolphthalein administration.

The results are summarized in Table 1. Oral administration of phenolphthalein (5 to 20 mg kg^{-1}) produced a dose-dependent increase in the number of wet faeces excreted by mice, for the subsequent 24 h. Initial experiments had indicated that the total number of wet faeces during 24 h excreted by mice after phenolphthalein administration provided a satisfactory response.

Pretreatment with indomethacin (0·5, 1·5 and 5 mg kg^{-1}) or aspirin (30, 60 and 120 mg kg^{-1}) reduced the number of unformed faeces excreted after administration of phenolphthalein, 10 mg kg^{-1}. This reduction was observed throughout the 24 h.

The results also show that the inhibitory effect of either indomethacin or aspirin on phenolphthalein-induced wet faeces was dose-dependent. But the laxative effect of phenolphthalein (10 mg kg^{-1}) was only partially inhibited by benoxaprofen (30 mg kg^{-1}) pretreatment while polyphloretin phosphate (100 mg kg^{-1}) given 1 h before the laxative prevented its effect.

These results demonstrate the ability of indomethacin and aspirin given by the oral route, to suppress the laxative effect of phenolphthalein. There is now evidence suggesting that phenolphthalein increases PG release into the lumen of gut (Beubler & Juan 1978; Cohen 1982). Our findings imply that the PG are of some importance in the development of the laxative effect of phenolphthalein, since this effect was reduced by inhibition of PG synthesis with indomethacin or aspirin.

These results were also supported by experiments with polyphloretin phosphate, a drug that prevents PGE_2-induced diarrhoea in mice and selectively blocks the stimulant effect of prostaglandins on the gastrointestinal smooth muscle (Eakins et al 1970; Karim 1974), since it effectively prevented the laxative effect of phenolphthalein.

If this view were correct, the failure of benoxaprofen to depress the laxative effect may be explained by its mild ability to inhibit PG biosynthesis (Cashin et al 1977).

Our results suggest that inhibition of PG biosynthesis may have a useful role in treating conditions such as diarrhoea as originally advocated by Collier (1974), who also provided evidence that aspirin ameliorates radiation-induced diarrhoea.

REFERENCES

Beubler, E., Juan, H. (1978) Experientia 34: 386–387

Beubler, E., Juan, H. (1979) J. Pharm. Pharmacol. 31: 681–685

Cashin, C. H., Dawson, W., Kitchen, E. A. (1977) Ibid. 29: 330–336

Cohen, M. M. (1982) Prost. Med. 8: 389–397

Collier, H. O. J. (1974) in: Robinson, H. J., Vane, J. R. (eds) Prostaglandin Synthetase Inhibitors. Raven Press, New York, pp 121–133

Eakins, K. E., Karim, S. M. M., Miller, J. D. (1970) Br. J. Pharmacol. 39: 556–563

Karim, S. M. M. (1974) Ann. Acad. Med. 3: 201–206

Lou, T. C. (1949) J. Pharm. Pharmacol. 1: 673–682