J. Pharm. Pharmacol. 1981, 33: 614–615
Communicated December 30, 1980

0022–3573/81/090614–02 $02.50/0

Effect of γ-aminobutyric acid on the rabbit isolated intestine

A. GIRDHAR, V. R. DHUMAL*†, O. D. GULATI, V. H. BHAVSAR*, G. HEMAVATHI, *Department of Pharmacology, Medical College, Baroda and Govt. Medical College, Surat†, India*

The effects of γ-aminobutyric acid (GABA) on the isolated intestine are complex, varying between the species and within any one species (Hobbiger 1958; Flory & Mclennan 1959). Both depression and stimulation has been observed (Inouye et al 1960). GABA has been shown to release prostaglandins (PGs) and noradrenaline (NA) in the central nervous system (Dhumal et al 1974). We have looked to see if putative transmitters are involved in a similar way in the action of GABA on intestinal smooth muscle of the rabbit.

A segment (2 to 3 cm) of rabbit jejunum was suspended in a 30 ml bath containing McEwen's (1956) solution of the following composition (mM): NaCl 129·9, KCl 5·6 $CaCl_2$ 1·6, $NaHCO_3$ 2·5, NaH_2PO_4 9·5, glucose 11·1, sucrose 13·1, and bubbled with oxygen. The temperature of the bath was 35 ± 1 °C. The responses of GABA were recorded isotonically on a. smoked drum with a frontal writing lever (10 fold magnification).

In another set of experiments intestinal segments were mounted according to Finkleman (1930) and bathed in McEwen's solution which was then extracted in ether (Samuelsson 1963) and assayed for prostaglandin-like substances on rat stomach strips (Vane 1957) set up in a 15 ml bath in Krebs' bicarbonate solution. A mixture of atropine, bromolysergic acid diethylamide, mepyramine, phentolamine, sotalol and indomethacin (each 1 μg ml^{-1}) was added to make the strip specifically sensitive for detection and quantitation of PG-like material (Gilmore et al 1968). To exclude the possibility of the presence of PGs of F series, the PG like substance was simultaneously assayed on rat colon (Regoli & Vane 1964) since the rat stomach strip and rat colon can differentiate between prostaglandins of the E and F series (Gryglewski & Vane 1972).

To detect the presence of GABA the intestinal segments were homogenized in 80% ethanol and extracted in 0·2 M HCl (Rai et al 1968). System GABA was separated by chromatography using n-butanol–acetic acid–water (4:1:5), and identified by comparing the R_F values of spots from test samples with those of GABA simultaneously applied. Quantitative estimation of GABA was not attempted.

Drugs used were: atropine sulphate (BDH); 2-bromolysergic acid diethylamide (BOL; Sandoz); γ-aminobutyric acid (GABA; Calbiochem); indomethacin (Merck Sharp and Dohme); mepyramine maleate (May and Baker); phentolamine methanesulphonate (Ciba); prostaglandin E_1 and E_2 (Upjohn).

*† Correspondence.

Indomethacin was dissolved in 2% sodium carbonate solution and the pH adjusted to 7·6 with HCl.

GABA in smaller doses (30–120 pg ml^{-1}) in the bath produced increase in tone of the isolated intestine which was resistant to atropine, BOL, or mepyramine, ruling out the involvement of ACh, 5-HT and histamine. The stimulant response of GABA was associated with increased PG-like material in the bathing fluid (Table 1). When preparations were exposed to indomethacin (10 μg ml^{-1}) for 1 h the stimulant action of GABA (120 pg ml^{-1}) was abolished and PG-like material in bathing fluid was markedly reduced. At doses from

Table 1. The effect of various concentration of GABA on intestinal tone and PGE-like substance in the bathing fluid. Figures in parentheses indicate number of experiment. * Indicates statistical significance compared with control.

Concentration of GABA ml^{-1} of bath solution	Intestinal response mm (mean ± s.e.)		PG content ng/90 s. (mean ± s.e.)	
Control	4·2 ± 0·05		3·3 ± 0·14	
30 pg (5)	65·2 ± 0·97	stimulant	3·4 ± 0·14	NS
60 pg (5)	86·4 ± 0·44	stimulant	4·8 ± 0·12	*
120 pg (5)	107·0 ± 3·0	stimulant	6·64 ± 0·15	*
33 μg (5)	25·0 ± 0·32	relaxant	2·3 ± 0·01	*
66 μg (5)	28·6 ± 2·23	relaxant	1·3 ± 0·22	*

150 pg-33 ng ml^{-1} there was a initial relaxation followed by increase in tone. The higher doses of GABA (33–66 μg ml^{-1}) caused relaxation associated with marked reduction in PG-like content in the bath fluid (Table 1). The relaxant response of GABA was reduced by 30% (n = 3) in presence of phentolamine (1 μg ml^{-1}) for 90 s the nerve stimulation induced response at 20 Hz was significantly (P <0·001) potentiated (control, 34·0 mm ± 0·1 s.e.); in the presence of GABA, 50·0 mm ± 1·15 s.e.). Chromatographic separation of the extract of intestinal homogenate showed the presence of GABA.

These results indicate that stimulant responses of GABA (30–130 pg ml^{-1}) may be related to the release of PG-like material from the tissue. As the stimulant response of GABA was resistant to various antagonists the involvement of acetylcholine, 5-HT and histamine is ruled out.

At higher concentrations (33–66 μg ml^{-1}) the response to GABA was inhibitory. That the response to nerve stimulation was potentiated by GABA, and that phentolamine partially blocked the relaxant response of GABA, suggest that the release of noradrenaline by GABA may contribute to the relaxant response.

REFERENCES

Dhumal, V. R., Gulati, O. D., Raghunath, P. R., Shivramkrishna, N. (1974) Br. J. Pharmacol. 50; 513–524

Finkleman, B. (1930) J. Physiol. 70: 145–157

Flory, E., Mclennan, H. (1959) J. Physiol. (London) 145: 66–75

Gilmore, N., Vane, J. R., Wyllie, J. H. (1968) Nature (London) 218: 1135–1140

Gryglewski, R., Vane, J. R. (1972) Br. J. Pharmacol. 45: 37–47

Hobbiger, F. (1958) J. Physiol 14: 147–164

Inouye, A. F., Fukuya, M., Tsuchiya, K., Tsujioka, T. (1960) Jpn. J. Physiol. 10: 169–182

McEwens, L. N. (1956) Ibid. 131; 678–679

Rai, R. M., Sabapathy, T. Rai K., Radhakrishnan, P. K. (1968) Ind. J. Biochem. 5: 75–76

Regoli, P., Vane, J. R. (1964) Br J. Pharmacol. Chemother. 23: 351–359

Samuelsson, B. (1963) J. Biol. Chem. 238: 3229–3234

Vane, J. R. (1957) Br. J. Pharmacol. 12: 344–349

J. Pharm. Pharmacol. 1981, 33: 615–616
Communicated March 30, 1981

0022–3573/81/090615–02 $02.50/0

Effect of the new H_2-receptor antagonist ranitidine on plasma prolactin levels in duodenal ulcer patients

ASHLEY H. ROBINS*, M. LYNN MCFADYEN, *Department of Clinical Pharmacology, University of Cape Town Medical School, Observatory, Cape, South Africa 7925.*

Ranitidine is a new histamine H_2-receptor antagonist which has different chemical features to cimetidine; principally the central imidazole ring of histamine has been replaced by a furan ring. Ranitidine is 4–10 times as potent as cimetidine on a molar basis in inhibiting gastric acid secretion in man (Domschke & Domschke 1980). The intravenous administration of cimetidine stimulated prolactin secretion (Carlson & Ippoliti 1977; Burland et al 1979; Macaron et al 1979) whereas the standard oral treatment did not (Van Thiel et al 1979; Nelis & Van de Meene 1980). The present study investigated the effect of oral ranitidine treatment on plasma prolactin levels in duodenal ulcer patients.

Consecutive outpatients with endoscopically proven duodenal ulcer (and free of systemic disease) were allocated to either ranitidine hydrochloride, one tablet (150 mg) twice a day, or to matching placebo, one tablet twice a day, according to a randomized double-blind design. Patients were permitted to take antacid tablets when necessary. No patients had been on neuroleptic agents and none had taken cimetidine in the previous four weeks. Patients underwent endoscopy at the beginning of the trial; they made a second visit after two weeks of therapy and a third after four weeks when endoscopy was again performed. (The clinical aspects of the trial (Marks & Wright, in preparation) will be reported elsewhere.) Blood was drawn at the first, second and third visits (between 09.00 h and 11.00 h) and the plasma was assayed for prolactin (by a radioimmunoassay kit purchased from Diagnostic Products Corporation, Los Angeles) and for ranitidine (by the method of Carey & Martin 1979). The results were analysed by non-parametric statistical tests.

There were 19 males (mean age: 33·1 years) and 6 females (mean age 31·5 years) in the ranitidine group and 20 males (mean age: 38·6 years) and 9 females (mean age: 39·0 years) in the placebo group. There were no significant changes in prolactin levels over the four weeks of treatment within either the ranitidine or the placebo groups (Table) and no significant differences emerged between the ranitidine and placebo groups at any of the three visits (Table). Spearman's rank correlation coefficient was obtained between the plasma ranitidine and the plasma prolactin concentrations in the male patients only (the female sample was too small): this was 0·28 at 2 weeks (not significant) and 0·19 at 4 weeks (not significant).

Oral ranitidine treatment (300 mg daily) therefore did not influence plasma prolactin levels in patients with duodenal ulceration. Our findings confirm the clinical trial data of Berstad et al (1980) and the intravenous ranitidine study of Nelis & Van de Meene (1980). Furthermore, in vitro experiments demonstrated that ranitidine had no dopaminergic effects and did not alter prolactin by any action on the pituitary (Yeo et al 1980). All these results refute the suggestion of Carlson & Ippoliti (1977) that the blockade of H_2-receptors produces raised prolactin levels. Sharpe et al (1980) found that another H_2-antagonist (oxmetidine HCl SK & F-92994) with similar physio-

Table 1. Effect of ranitidine and placebo on plasma prolactin concentrations.

	Plasma prolactin (ng ml^{-1})			
	Baseline*	2 weeks*	4 weeks*	P**
Ranitidine group				
Males (n = 19)	15 (5–19)	9 (2·5–15)	9 (5–15)	n.s.
Females (n = 6)	6·5 (6–10)	7 (6–7)	8·5 (6–13)	n.s.
Placebo group				
Males (n = 20)	8 (2–13·5)†	7 (1–9)†	8·5 (6–11·5)†	n.s.
Females (n = 9)	10 (4·0–14·5)†	6 (0–10)†	9 (3·0–15·5)†	n.s.

* Prolactin expressed as median (interquartile range).
** Friedman's two-way analysis of variance.
n.s. = not significant.
† n.s. when compared against corresponding values in the ranitidine group (Wilcoxon sum of ranks test).

* Correspondence.