Br. J. Pharmac. (1985), **84**, 619–623

Central GABA-ergic mechanism in stress-induced gastric ulceration

K.P. Bhargava, G.P. Gupta[1] & M.B. Gupta

Neuropharmacology Unit (CSIR), Department of Pharmacology & Therapeutics, King George's Medical College, Lucknow—226003, India

1 The effect of exogenous administration of central amino acid neurotransmitters γ-aminobutyric acid (GABA), glycine, glutamic acid and aspartic acid) into the cerebroventricular system was studied on gastric ulceration induced in albino rats either by 2 h restraint at 4°C or by 6 h restraint at room temperature (30 ± 2°C).

2 GABA (5, 10, 20 and 50 μg) injected intracerebroventricularly (i.c.v.) showed a dose-dependent reduction of gastric ulceration induced by 2 h restraint at 4°C (CRU), whereas glycine (5, 10 and 20 μg i.c.v.) failed to alter this response.

3 Muscimol (5 and 10 μg i.c.v.), a GABA agonist, and sodium valproate (400 mg kg^{-1} p.o.), which increases the concentration of GABA in the CNS, significantly reduced CRU.

4 Pretreatment with the GABA antagonists, bicuculline (40 μg i.c.v.) or picrotoxin (5 μg i.c.v.) reversed the anti-ulcerogenic effects of GABA (50 μg i.c.v.) and sodium valproate (400 mg kg^{-1} p.o.).

5 Bicuculline (20 and 40 μg i.c.v.) and picrotoxin (5 and 10 μg i.c.v.) *per se* did not induce gastric ulceration in normal rats but significantly enhanced the minimal ulcerogenic response induced by 6 h restraint at room temperature.

6 Pretreatment with GABA (i.c.v.) significantly reduced the gastric ulceration induced by i.c.v. administration of aceylcholine and adrenaline or pylorus ligation.

7 Glutamic acid (20 μg i.c.v.) and aspartic acid (20 μg i.c.v.) did not significantly enhance the minimal ulcerogenic response induced by 6 h restraint at room temperature.

8 These observations show that GABA in the CNS exerts an inhibitory effect on stress-induced ulcerogenesis.

Introduction

It is well known that the central nervous system is intimately concerned in the genesis of stress-induced gastric ulceration. Bhargava *et al.*, (1980) have reported that central adrenergic as well as cholinergic mechanisms are responsible for the activation of peripheral sympathetic and parasympathetic outflow in ulcers caused by cold restraint (CRU). Furthermore, adrenaline, noradrenaline and acetylcholine administered into the lateral cerebral ventricle of the normal rat induced gastric ulceration similar to CRU, but dopamine, histamine and 5-hydroxytryptamine (5-HT) were ineffective. However, the incidence of stress-induced gastric ulceration is enhanced by central administration of 5-HT but not dopamine or histamine (Gupta *et al.*, 1983). There is convincing evidence that the amino acids viz. γ-aminobutyric acid (GABA), glycine, glutamic acid and aspartic acid serve as central neurotransmitters (De Feudis, 1975; Johnston, 1978; Watkins & Evans, 1981). However, the role of central amino acid neurotransmitters in the pathogenesis of stress-induced gastric ulceration is not known. Hence, the present study was carried out to elucidate the central involvement of GABA and other amino acid transmitters in stress-induced gastric ulceration in rats.

Methods

Adult albino rats of either sex weighing 100–120 g were divided into groups of ten. Pregnancy was excluded and the animals were deprived of food for 24 h before the commencement of experiments. The animals were allowed water *ad libitum*.

[1] Author for correspondence.

Stress-induced gastric ulceration

The rats were restrained by tying the fore and hind limbs together either for 2 h at 4°C (Senay & Levine, 1967) or for 6 h at room temperature (30 ± 2°C; Seyle, 1936). The former procedure induced gastric ulcers in 90% of rats and was employed to detect any inhibitory effect of the exogenously administered agents while the latter procedure induced ulceration only in 30% of rats and was employed to study any augmentative effect. The drugs were administered either into the lateral ventricle (i.c.v.) through an indwelling polyethylene cannulla or orally 2 h before restraining the rats. The rats were killed immediately after the restraint period. The stomach was removed and examined for evidence of gastric ulceration. The presence of shedding of epithelium, petichial and frank haemorrhages, one or two ulcers, multiple ulcers and perforated ulcers was considered to be a positive ulcerogenic response and the results were statistically analysed using the χ^2 test. To determine the severity of the ulcerative lesion, an arbitrary scoring system was used to determine the 'ulcer index', as described by Bhargava *et al.* (1980).

Gastric ulceration induced by central administration of adrenaline and acetylcholine in rats

Adrenaline and acetylcholine in doses of 10 and 20 μg, respectively, were injected into the lateral cerebral ventricle through an indwelling polyethylene cannula implanted two days earlier (Bhargava *et al.*, 1980). The rats were killed 24 h after drug administration and the stomach was examined for the incidence and severity of ulceration, as described above.

Shay preparation

Pylorus ligation was carried out according to the technique of Shay *et al.* (1945). GABA was administered (i.c.v.) before pylorus ligation. The animals were killed 4 h after pylorus ligation for observation of gastric lesions. The volume of gastric secretion was measured and its pH determined using a Philips pH meter. The results were analysed using Student's *t* test.

The drugs used in the present study were: γ-aminobutyric acid, glycine, L-glutamic acid, DL-aspartic acid, muscimol hydrochloride, bicuculline, picrotoxin, adrenaline bitartrate, acetylcholine chloride and sodium dipropylacetate (sodium valproate). All the drugs were dissolved in 0.9% w/v NaCl solution (saline).

Results

Intracerebroventricular (i.c.v.) administration of GABA (5, 10, 20 and 50 μg), glycine (5, 10 and 20 μg), glutamic acid (20 μg) and aspartic acid (20 μg) did not induce gastric ulceration in normal rats.

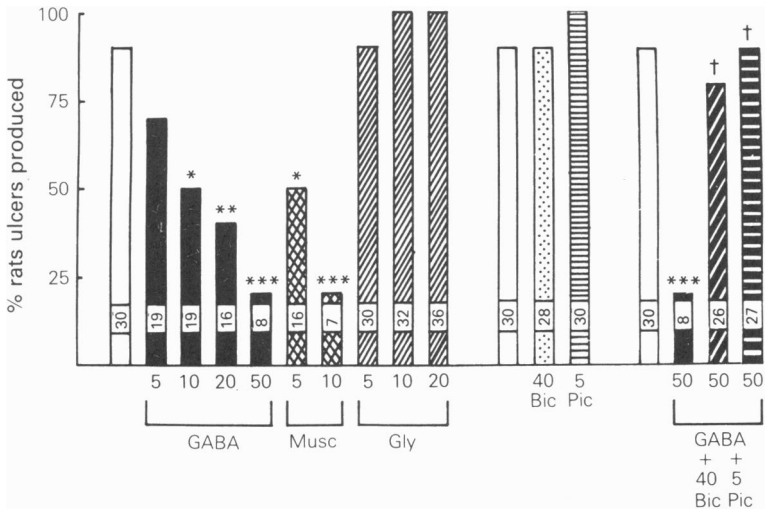

Figure 1 Effect of intracerebroventricularly administered GABA, muscimol (Musc) and glycine (Gly) on ulcers induced by cold restraint (2 h at 4°C) in rats, and reversal of the inhibitory effect of GABA by i.c.v. bicuculline (Bic) and picrotoxin (Pic). Each column represents the occurrence of gastric lesions (10 animals = 100%). Number within the column indicates ulcer index. Open columns represent effects of saline 10 μl (controls). Analysis done using χ^2 test: Doses in μg, administered i.c.v. $^*P<0.05$, $^{**}P<0.02$ and $^{***}P<0.001$ vs. saline (control) treated rats. †Significant ($P<0.001$) reversal of anti-ulcerogenic effect of GABA by bicuculline and picrotoxin.

Table 1 Effect of sodium valproate on cold restraint ulcers in rats and the reversal by bicuculline and picrotoxin

Drug treatment 2 h before restraint (2 h at 4°C)	*Dose* (p.o.)	*No. of rats with ulcer/ No. of rats studied*	*% with an ulcer*	*Ulcer index*
Saline (control)	0.5 ml	9/10	90	30
Sodium valproate	400 mg kg^{-1}	3/10	30*	10
Bicuculline + Sodium valproate	40† + 400 mg kg^{-1}	9/10	90§	31
Picrotoxin + Sodium valproate	5† + 400 mg kg^{-1}	8/10	80§§	24

† μg administered into lateral ventricle of rat brain.
* $P<0.001$ vs. saline (control).
§ $P<0.001$ vs. sodium valproate.
§§ $P<0.02$ vs. sodium valproate.

Gastric ulceration induced by restraining rats for 2 h at 4°C

GABA (5, 10, 20 and 50 μg) and muscimol (5–10 μg) administered i.c.v. significantly reduced the ulcerogenic response induced by the cold restraint technique (CRU) in a dose-dependent manner. Pretreatment with bicuculline (40 μg i.c.v.) and picrotoxin (5 μg i.c.v.) did not significantly alter the incidence of CRU but reversed the inhibitory effect on the ulcerogenic response induced by 50 μg of GABA (i.c.v.). Glycine up to a dose of 20 μg i.c.v. did not alter the incidence of CRU (Figure 1).

Table 1 shows that restraining rats for 2 h at 4°C induced gastric ulceration in 90% of animals. Sodium valproate (400 mg kg^{-1} p.o.) significantly reduced the incidence of ulcerogenic response from 90% to 30%. Pretreatment with bicuculline (40 μg i.c.v.) or

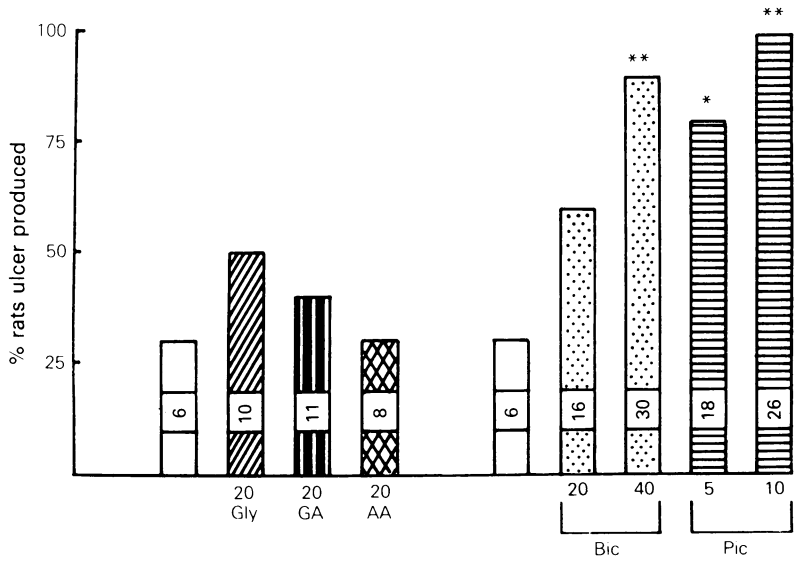

Figure 2 Effect of intracerebroventricularly administered glycine (Gly), glutamic acid (GA), aspartic acid (AA), bicuculline (Bic) and picrotoxin (Pic) on gastric ulceration induced in rats by restraint for 6 h at room temperature (30 ± 2°C). Each column represents the occurrence of gastric lesions (10 animals = 100%). Open columns represent effects of saline 10 μl (controls). Number within the column indicates ulcer index. Doses in μg, administered i.c.v. * $P<0.02$ and ** $P<0.001$ vs. saline (control) treated rats.

Table 2 Effect of i.c.v. pretreatment with GABA on gastric ulceration induced by i.c.v. acetylcholine and adrenaline in rats

Pretreatment before ulcerogenic agent (μg i.c.v.)	*Ulcerogenic drugs (μg i.c.v.)*	*No. of rats with ulcer/ No. of rats studied*	*% with an ulcer*	*Ulcer index*
Saline (10 μl)	Adrenaline (10)	9/10	90	28
GABA (50)	Adrenaline (10)	3/10	30*	9
Saline (10 μl)	Acetylcholine (20)	9/10	90	32
GABA (50)	Acetylcholine (20)	4/10	40**	10

* $P<0.01$ and ** $P<0.05$

picrotoxin (5 μg i.c.v.) significantly reversed this antiulcerogenic effect of sodium valproate.

Gastric ulceration induced by restraining rats for 6 h at room temperature

The results in Figure 2 show that glycine, glutamic acid and aspartic acid administered in a dose of 20 μg i.c.v. did not significantly alter the incidence of gastric ulceration induced by restraining rats for 6 h at room temperature.

Bicuculline (20 and 40 μg i.c.v.) and picrotoxin (5 and 10 μg i.c.v.) did not induce gastric ulceration in normal rats but significantly enhanced the incidence of ulcerogenic response induced by restraining rats for 6 h at room temperature (Figure 2).

Gastric ulceration induced by i.c.v. adrenaline and acetylcholine

The results in Table 2 show that i.c.v. pretreatment with GABA significantly reduced the incidence of gastric ulceration induced in rats by i.c.v. adrenaline and acetylcholine.

Gastric ulceration in pylorus ligated (Shay) rats

Intracerebroventricular administration of GABA significantly decreased the gastric secretory volume, raised the pH and lowered the incidence of gastric ulceration in Shay rats (Table 3).

Discussion

Central administration of GABA and muscimol, a GABA agonist, reduced the incidence of gastric ulceration induced by the cold restraint technique in a dose-dependent manner (Figure 1). Sodium valproate (400 mg kg^{-1} p.o.) which is known to increase the concentration of GABA in the CNS (Pinder *et al.*, 1977), significantly reduced the incidence of cold restraint ulcers (CRU).

In view of the above findings we explored the augmentary effect of GABA antagonists, bicuculline and picrotoxin (Johnston, 1978) on stress-induced ulcerogenesis. Bicuculline (20 and 40 μg i.c.v.) and picrotoxin (5 and 10 μg i.c.v.) *per se* did not induce gastric ulceration in normal rats but they significantly

Table 3 Effect of i.c.v. GABA on gastric secretion, pH and ulceration in pylorus ligated rats

Drug pretreatment before pylorus ligation (μg i.c.v.)	*Mean gastric secretory volume in ml (±s.e.)*	*Mean pH of gastric secretion (±s.e.)*	*No. of rats with ulcer/ No. of rats studied*	*% with an ulcer*	*Ulcer index*
Saline (10 μl)	2.5 ± 0.16	3.45 ± 0.13	10/10	100	32
GABA (50)	1.6 ± 0.25**	4.5 ± 0.45*	5/10	50*	14

* $P<0.05$ and ** $P<0.01$.

enhanced the ulcerogenic response induced by restraint for 6 h at room temperature (Figure 2). Furthermore, pretreatment with i.c.v. bicuculline and picrotoxin blocked the inhibitory effect of GABA and sodium valproate on gastric ulceration (Figure 1 and Table 1). These findings support the view that GABA-ergic transmission is inhibitory to stress-induced ulcerogenesis.

The possibility that the anti-ulcerogenic effect of GABA, muscimol and sodium valproate is due to sedation, and the reversal of this effect by bicuculline and picrotoxin is due to excitation produced by these agents, seems unlikely since File & Pearce (1981) have clearly demonstrated a separation between sedative and antiulcerogenic effects of benzodiazepines. A quantitative comparison of the anti-ulcerogenic and sedative activities of several tranquillo-sedatives of diverse chemical groups has also revealed a lack of correlation between these two activities (Gupta *et al.*, 1985).

Scatton & Bartholini (1982) and Scatton *et al.* (1982) have shown that the central administration of GABA has a strong influence on adrenergic, cholinergic and 5-hydroxytryptaminergic neurotransmitter functions in the CNS. They found that GABA and related agonists (muscimol or progabide) inhibit acetylcholine and 5-HT transmission and their turnover rate in brain areas of the rats. However, i.c.v. GABA and muscimol activate noradrenergic neurones, enhance the synthesis, utilization and turnover rate of noradrenaline in rat brain. Since the cholinergic and adrenergic systems appear to be involved in stress-induced ulcerogenesis (Bhargava *et al.*, 1980), we studied the effect of GABA on gastric ulceration induced by i.c.v. administration of acetylcholine and adrenaline in rats. Pretreatment with i.c.v. GABA significantly reduced the gastric ulceration induced by i.c.v. acetylcholine and adrenaline (Table 2). Bhargava *et al.* (1980) have reported that there is a central cholinergic link distal to adrenergic activation in i.c.v. adrenaline-induced gastric ulceration, whereas only a cholinergic link is involved in i.c.v. acetylcholine-induced gastric ulceration. Thus, it appears that reduction, by i.c.v. GABA, of gastric ulceration induced by i.c.v. acetylcholine and adrenaline results from an inhibition of the central cholinergic mechanism. This was supported by the findings that i.c.v. GABA significantly inhibited gastric secretion, raised the pH and reduced the incidence of gastric ulceration in pylorus ligated rats (Table 3) where mainly central cholinergic mechanisms are involved (Sharma *et al.*, 1963).

Administration of glycine (5–20 μg i.c.v.), an inhibitory amino acid neurotransmitter (De Feudis, 1975), neither altered the incidence of CRU significantly (Figure 1) nor enhanced the incidence of gastric ulceration induced by restraining rats for 6 h at room temperature. Similarly, the excitatory amino acid neurotransmitters, glutamic acid and aspartic acid (Watkins & Evans, 1981) did not significantly enhance the ulceration (Figure 2). Thus, it may be concluded that central GABA-ergic mechanisms are inhibitory to stress-induced ulcerogenesis, whereas glycine, glutamate and aspartate do not appear to be involved.

References

BHARGAVA, K.P., DAAS, M., GUPTA, G.P. & GUPTA, M.B. (1980). Study of central neurotransmitters in stress-induced gastric ulceration in albino rats. *Br. J. Pharmac.*, **68,** 765–772.

DE FEUDIS, F.V. (1975). Amino acids as central neurotransmitters. *Ann. Rev. Pharmac.* **15,** 105–130.

FILE, S.E. & PEARCE, J.B. (1981). Benzodiazepines reduced gastric ulcers induced in rats by stress. *Br. J. Pharmac.*, **74,** 593–599.

GUPTA, M.B., GUPTA, G.P. & BHARGAVA, K.P. (1983). Effect of central dopamine, histamine and 5-hydroxytryptamine on stress-induced gastric ulceration in rats. *Ind. J. med. Res.*, **78,** 281–283.

GUPTA, M.B., NATH, R., GUPTA, G.P. & BHARGAVA, K.P. (1985). A study of the antiulcer activity of diazepam and other tranquillo-sedatives in albino rats. *Clin. exp. Pharmac. Physiol.*, (in press).

JOHNSTON, G.A.R. (1978). Neuropharmacology of amino acid inhibitory transmitters. *Rev. Pharmac. Tox.*, **18,** 269–289.

PINDER, R.M., BROGDEN, R.N., SPEIGHT, T.M. & AVERY, G.S. (1977). Sodium valproate: A review of its pharmacological properties and therapeutic efficacy in epilepsy. *Drug*, **13,** 81–123.

SCATTON, B. & BARTHOLINI, G.(1982). γ-Aminobutyric acid (GABA) receptor stimulation IV. Effect of progabide (SL-76002) and other GABA-ergic agents on acetylcholine turnover in rat brain areas. *J. Pharmac. exp. Ther.*, **220,** 689–695.

SCATTON, B., ZIVKOVIC, B., DEDEK, J., LLOYD, K.G., CONSTANTINIDIS, J., TISSOT, R. & BARTHOLINI, G. (1982). γ-Aminobutyric acid (GABA) receptor stimulation III. Effect of progabide (SL 76002) on norepinephrine, dopamine and 5-hydroxytryptamine turnover in rat brain areas. *J. Pharmac. exp. Ther.*, **220,** 678–688.

SELYE, H. (1936). Methods for stress induced ulcers. *Nature*, **138,** 32–33.

SENAY, E.C. & LEVINE, R.J. (1967). Synergism between cold and restraint for rapid production of stress ulcers in rats. *Proc. Soc. exp. Biol. Med.*, **45,** 1221–1223.

SHARMA, K.N., DUA, S. & ANAND, B.K. (1963). Effect of hypothalamic lesion on experimental gastric ulceration in rats. *Ind. J. med. Res.*, **51,** 705–715.

SHAY, H., KAMAROW, S.A., FELE, S.S., MERANZE, D., GRUENSTEIN, H. & SIPLET, H. (1945). A simple method for uniform production of gastric ulceration in rats. *Gastroenterology*, **5,** 43.

WATKINS, J.C. & EVANS, R.H. (1981). Excitatory amino acid transmitters. *Rev. Pharmac. Tox.*, **21,** 165–204.

(Received May 21, 1984.
Revised September 21, 1984.
Accepted October 21, 1984).