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Protective effect of GABA and sodium valproate on stress-induced gastric lesions in guinea-pigs*

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γ-Aminobutyric acid (GABA) and sodium valproate (VPA) inhibit the formation of stress-induced gastric ulcers in guinea-pigs. The present study was conducted to evaluate the effect of these drugs on the development of cold- and restraint-induced gastric ulcers in guinea-pigs. In control saline-pretreated animals, a 3 h exposure to cold and restriction resulted in the production of gastric ulcers in 9 out of 10 animals. GABA (200 mg kg^{-1} oral, i.p.) completely prevented the development of gastric ulcers. VPA (100 and 200 mg kg^{-1} i.p.) exerted no significant effects on the development of gastric ulcers. GABA (100 mg kg^{-1} oral and i.p.) and VPA (oral) also exhibited partially protective activity. It is suggested that GABA may participate in a physiological modulation of the gastric mucosal barrier, by increasing its resistance to stress-induced lesions.

Stress produced by the technique of cold-restraint induced gastric ulceration in experimental animals, has been used as a model for acute studies on ulcerogenesis (Adami et al 1964; Djahanguiri et al 1968; Bhargava et al 1980). However, there is no unanimity regarding the mechanism of stress-induced gastric ulcers, although both central and peripheral mechanisms seem to be involved in ulceration (Brodie & Hanson 1960; Dai & Ogle 1974).

In the pathogenesis of gastric mucosal erosion it is generally accepted that gastric acid secretion (Alphin et al 1977), peripheral histamine (Hayden et al 1978), peripheral sympathetic and vagal activation (Djahanguiri et al 1968; Bhargava et al 1980), activation of the pituitary-adrenal axis leading to a discharge of corticosteroids and catecholamines from the adrenal medulla (Bhargava et al 1980), reduction of mucosal blood flow (Hase & Moss 1973) and an inhibition in the biosynthesis of prostaglandins (Whittle 1981) are factors.

GABAergic mechanisms are involved in the biological sequence that leads from an environmental stress to a state of anxiety (De Feudis 1981; 1983). Clinical (Greenblatt & Shader 1974) and experimental (Dairman & Juhasz 1978) observations suggest that benzodiazepines reduce the formation of gastric lesions induced by a variety of stress situations, probably because of a combination of sedative, anxiolytic and antisecretory actions (File & Pearce 1981).

It has been shown that GABA agonists are able to decrease both acid secretion and gastric motility mediated by the vagus nerve (Williford et al 1981). In other in-vitro experimental observations on gastrointestinal function, GABA agonists have been found to increase the production of prostaglandins (Ghirdhar et al 1981) and to decrease the production of noradrenaline, acetylcholine and histamine (Girdhar et al 1981; Giotti et al 1983).

The purpose of the present study was to compare the effects of peripherally administered GABA and sodium valproate, which increase the whole brain GABA levels but do not act postsynaptically on GABA receptors (Kerwin & Taberner 1981), on the development of stress-induced gastric ulcerations in guinea-pigs.

Materials and methods

Guinea-pigs (74), of either sex, 190–330 g, were randomly divided into groups. Since feeding has been shown to prevent the ulcerogenic activity of stress (Kelly & Robert 1969), all animals were deprived of food but had free access to water for 48 h before the experiments.

Ulcer formation. Stress ulcers were produced by a combination of cold and restraint. Immediately after drug or 0·9% NaCl (saline) administration (1 ml kg^{-1}), each animal was wrapped in a wire mesh and kept at 3–5 °C for 3 h. At the end of the test period, the animals were killed by a blow to the head, the stomach removed, placed in 0·9% NaCl and opened along the lesser curvature. The number and severity of discrete areas of damage in the glandular mucosa were scored by an ulcer index which was a slight modification of that described by Adami et al (1964). Severity was assessed on a scale of 0 to 5; a score of 0 representing normal mucosa and a score of 5 representing perforated ulcers. The scores in between were assigned as follows: 1, reddened or haemorrhagic mucosa; 2, no more than five small ulcers (largest diameter < 3 mm); 3, many small ulcers or at least one large ulcer (largest diameter > 3 mm); and 4, many large ulcers. The mucosa was examined under a strong light with a magnifying glass and a precise ruler was used when needed.

Drugs and drugs administration. Solutions of γ-aminobutyric acid (GABA, Sigma) and sodium valproate (VPA, Labaz) were prepared daily. Drugs were dissol-

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ved in saline and administered i.p. or orally at doses of 100 and 200 mg kg^{-1}. Animals treated with saline served as control.

Statistical analysis. The number of ulcers and their severity scores were compared by the Mann-Whitney *U*-test. The differences in incidence of gastric haemorrhage and the number of animals with ulcers observed in the control and treated groups, were assessed by the Chi-square test. The significance levels were set at $P < 0.05$.

Results

Effect of cold and restraint in control animals. Simultaneous exposure to cold (3–5 °C) and restraint for 3 h produced gastric ulcers in 90% of the animals, 60% developing gastric haemorrhage. Table 1 shows the mean severity score and the total number of ulcers respectively induced in control saline-pretreated animals.

Effect of oral pretreatment with GABA and VPA. Since VPA is a known gastric irritant, a control series pretreating unstressed animals with 200 mg kg^{-1} was run (n = 6). The mean score of 0 obtained by this control 3 h after VPA administration indicates that at the dose used the mucosa is not affected.

As shown in Table 1, there was a decrease in the incidence of ulcers and the total number of ulcers produced in animals pretreated with GABA and VPA, but only the higher dose of GABA produced a significant reduction of both. In none of the GABA or VPA-treated groups, was the incidence of gastric haemorrhage significantly different from the controls, although it was higher in the VPA 200 mg group and it decreased in the GABA 200 mg group compared with controls.

Table 1 also shows the severity of ulcers after oral administration of GABA and VPA. Both GABA and VPA significantly reduced the severity score of ulcers induced by cold and restraint. Only GABA 200 mg reduced significantly both the incidence and severity of ulcers; the number of ulcers also decreased significantly.

Effect of intraperitoneal administration of GABA and VPA. Table 2 shows the effect of i.p. pretreatment with GABA and VPA on gastric ulceration induced by cold and restraint. The effects on the incidence and number of ulcers and on the incidence of haemorrhage were similar to those obtained by oral administration. In contrast, the severity of ulcers decreased only after GABA treatment and was not reduced significantly after VPA injection. Once again, only the 200 mg dose of GABA produced a reduction in the incidence and in the number and severity of ulcers.

Discussion

The use of a 3 h period of cold (3–5 °C) and restraint in combination with food deprivation (48 h), produced a measurable damage on the gastric mucosa of the guinea-pig. Most of the techniques used to assess mucosal damage follow the main characteristics evaluated in the Adami test (Adami et al 1964). This is simple, gives reproducible readings, requires no special equipment and since it suits the purposes of semiquantitative assessment of lesions it was adopted with slight modifications for the work reported.

It has been shown in rats that both adrenergic and cholinergic mechanisms are operating in the CNS and at the periphery in the pathogenesis of cold restraint stress induced gastric ulceration (Bhargava et al 1980). However, other central or peripheral neuro-transmitter mechanisms may also be involved.

In this study, we have investigated whether GABA-mechanisms are implicated in the production of cold-restraint stress-induced ulcerogenic response.

We have confirmed and extended previous observations that peripherally administered GABA-agonists (γ-hydroxy-butyric acid and benzodiazepines) exert significant cytoprotection as measured by a reduction in the incidence, number and severity of cold and restraint

Table 1. Effect of oral administration of GABA and VPA on cold-restraint ulcers in guinea-pigs.

Treatment	No. of animals with ulcer / No. of animals studied	Total number of ulcers	No. of animals with haemorrhage / No. of animals studied	Mean ulcer severity score
Control				
Normal saline (0·1 ml kg^{-1})	9/10	29	6/10	3·5
Treatment (mg kg^{-1})				
GABA				
100	7/10	28	5/10	1·9*
200	4/10*	9**	2/10	1·2**
VPA				
100	5/6	21	3/6	2·1*
200	4/6	19	6/6	1·8*

The levels of statistical significance for the Mann–Whitney (number of ulcers and mean ulcer severity) and Chi-Square tests (incidence of ulcers and haemorrhage) are shown by: $*P < 0.05$, $**P < 0.01$.

Table 2. Effect of intraperitoneal injection of GABA and VPA on cold-restraint ulcers in guinea-pigs.

Treatment	No. of animals with ulcer / No. of animals studied	Total number of ulcers	No. of animals with haemorrhage / No. of animals studied	Mean ulcer severity score
Control				
Normal saline (0·1 ml kg^{-1})	9/10	29	6/10	3·5
Treatment (mg kg^{-1})				
GABA				
100	5/10	18	6/10	1·4**
200	3/10**	3***	3/10	0·9***
VPA				
100	5/6	20	5/6	2·5
200	4/6	9	4/6	2·5

The levels of statistical significance for the Mann–Whitney (number of ulcers and mean ulcer severity) and Chi-Square tests (incidence of ulcers and haemorrhage) are shown by: $**P < 0.01$, $***P < 0.001$.

stress induced gastric ulcers in rats (File & Pearce 1981; Tatevosyan et al 1982). The data indicate that oral administration of GABA and VPA (which causes significant increases in cerebral GABA concentrations) in guinea-pigs, also produces cytoprotective activity which is variable since 100 and 200 mg kg^{-1} VPA and 100 mg kg^{-1} GABA are only partially cytoprotective, they significantly reduced the severity but not the incidence or the number of gastric ulcers.

At the doses used, GABA produced substantial gastric cytoprotection whereas VPA did not, a difference that could be attributed to pharmacokinetic factors, since GABA penetrates the blood-brain barrier with difficulty (Kuriyama & Sze 1971), whilst VPA does so easily (Kerwin & Taberner 1981). Thus, it might be expected that GABA exerts its anti-ulcer action mainly by peripheral mechanisms.

However, intraperitoneal administration of VPA (unlike oral administration) did not produce a significant reduction in mucosal damage (incidence, number and severity scores). The reason for the lack of effectiveness of intraperitoneally administered VPA is not clear, but it could be explained by a lack of interaction of VPA and peripheral GABA receptors involved in the gastric actions of GABA; it may also be related to pharmacokinetic differences.

The cytoprotective action of GABA may be due partially to a stimulation of central GABA receptors. Thus, a GABAergic mechanism at the nucleus ambiguous strongly influences parasympathetic outflow to the stomach (Williford et al 1981), and there is evidence for a central suppressant effect of GABA on sympathetic activity (Bhargava et al 1964; Dhumal et al 1980).

Peripheral mechanisms, however, can also contribute to the anti-ulcer action of GABA: (a) The activation of $GABA_B$ receptors located on sympathetic and parasympathetic terminals in peripheral tissues would diminish evoked transmitter release (Bowery et al 1981, 1983; Giotti et al 1983); (b) GABA increases ATP content and oxygen consumption in the gastric wall, and increases resistance of the gastric mucosa to the action of proteolytic enzymes (Tatevosyan et al 1982); and (c) GABA may act through specific GABA receptors to inhibit or reduce ganglionic transmission (Bowery & Brown 1972). Inhibition of postganglionic responses could block the pituitary-adrenal stress response, which has been shown to be involved in cold and restraint stress-induced ulcerogenesis (Bhargava et al 1980).

The present results suggest that GABA may participate in modulating the functional gastric mucosal barrier, thereby decreasing its vulnerability to stress-induced lesions.

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