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Drugs acting at central benzodiazepine receptors attenuate ethanol-induced gastric lesions in rats

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Abstract

The characteristics of the receptors involved in the protective action of benzodiazepines against ethanol-induced gastric lesions were investigated by studying the effect of benzodiazepine ligands on such lesions in both intact and unilaterally vagotomized rats. Clonazepam [5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepine-2-one] a specific central-type receptor agonist (0.625–2.5 mg/kg p.o. or i.p.) and CGS 9896 [2-(4-chlorophenyl)-2,5-dihydropyrazolo{4,3-c}quinoline-3(3*H*)-one] a non-sedative partial agonist with anxiolytic properties (2.5–10 mg/kg p.o.) significantly reduced the gastric damage induced by ethanol (10 ml/kg of a 50% solution v/v p.o.) in non-vagotomized rats but Ro 5-4864 [7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepine-2-one] a pure peripheral-type receptor agonist (5–20 mg/kg p.o.) failed to affect this damage. The protective action of clonazepam and CGS 9896 against ethanol-induced gastric lesions was blocked, dose dependently, by the central-type receptor antagonist, flumazenil [ethyl 8-fluoro-5,6-5-methyl-6-oxo-4*H*-imidazo{1,4}benzodiazepine-3-carboxylate] (1.25–20 mg/kg i.p.). In the unilaterally vagotomized rat, ethanol produced lesions in the right (vagotomized) and the left (non-vagotomized) halves of the gastric mucosa to nearly the same extent, while clonazepam and CGS 9896 uniformly decreased the lesions in both halves. It is concluded that central-type benzodiazepine receptors located in the stomach, specifically those mediating the anxiolytic effect of benzodiazepines, are involved in the protective action of benzodiazepines against ethanol-induced gastric lesions.

Keywords: Gastric lesion, ethanol-induced; Benzodiazepine; Unilateral vagotomy; (Rat)

1. Introduction

Since Beaumont's classical observations on his patient Alexis St. Martin in the 19th century (Beaumont, 1833), exposure of the gastric mucosa to concentrated alcoholic beverages has been known to induce damage of the acute gastritis type characterized by focal areas of marked mucosal hyperaemia, necrosis and edema with mucosal and submucosal haemorrhages. A large number of experimental studies have utilized ethanol to produce macroscopic gastric lesions in a variety of animal species. But gastric damage may also be inflicted by the application of stress, pharmacological agents and surgical procedures. Although some evidence suggests that the neuropharmacological and behavioural actions of ethanol are probably mediated through the central inhibitory synaptic neurotransmitter, GABA (γ-amino butyric acid) (Liljequist and Engel, 1982; Mendelson et al., 1985), the exact mechanism(s) of ethanol-induced gastric damage have not been clarified in spite of extensive research done so far.

A significant reduction in stress (cold restraint)-induced gastric ulceration in rats by administration of GABA and GABAergic agents was reported by Bhargava et al. (1985). Benzodiazepines were also found to protect the rat stomach against stress-induced ulceration (File and Pearce, 1981; Gupta et al., 1985). Such a protective action cannot be explained simply by the sedative effect of benzodiazepines since a non-sedative partial benzodiazepine agonist, CGS 9896, was shown to decrease gastric ulceration induced by stress in rats (Najim and Karim, 1990). Moreover drugs acting on the GABA-benzodiazepine receptor complex were also reported to prevent ethanol-induced gastric lesions. Erdö et al. (1989) found that oral pretreatment with GABAergic compounds protects the gastric mucosa against ulcers induced by acidified ethanol in both

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intact and vagotomized rats. Likewise, Najim and Karim (1991, 1993) found that clonazepam, Ro 5-3663 (atypical benzodiazepine), and two imidazobenzodiazepines, Ro 15-4513 and Ro 15-3505, decrease significantly the gastric damage caused by ethanol.

The present study was designed to investigate the characteristics of the receptors involved in the protective action of benzodiazepines against ethanol-induced gastric damage by identifying, if possible, the type or subtype of receptors involved and their location. For this purpose, the effect of drugs acting at various benzodiazepine receptors was examined in rats with gastric lesions induced by ethanol. To exclude the role of a central mechanism, both for the formation of experimental gastric lesions by ethanol and for protection by benzodiazepines against such lesions, drugs were also tested in unilaterally vagotomized rats.

2. Materials and methods

2.1. Animals

Adult male Albino-Wistar rats, weighing 180–225 g, were used in this study. The animals were housed in groups of 6 rats per cage for at least 1 week prior to the experiments, with the temperature maintained at 20–23°C, relative humidity at 50–60% and a 12 h dark-light cycle. During this period, the rats had free access to a standard laboratory pellet diet and were allowed to drink water ad libitum. Food was withheld from the animals 24 h before experimentation. During starvation, the rats were kept in cages with a wide wire-mesh floor to avoid coprophagy but were permitted free access to tap water.

2.2. Vagotomy

Right-sided vagotomy was performed under light ether anaesthesia and strict aseptic conditions. The esophagogastric junction was exposed through a midline upper abdominal incision. The right (anterior) vagus nerve was identified, isolated and severed between two ligatures. The severed ends of the vagus nerve were painted with 5% phenol solution. Thirty seconds later, excess phenol in the area was washed off with normal saline (0.9% w/v NaCl). The abdominal wall was closed in two layers. The rats were then allowed to recover from surgery; they were fed a normal diet and drank tap water during the next 7 post-operative days. Before the experiments, they were starved for 24 h as were the non-vagotomized rats.

2.3. Ethanol-induced gastric damage model

On the day of experimentation, water was withheld from the rats for 2 h before ethanol administration. Thirty minutes before ethanol administration, the animals received either the vehicle, clonazepam, CGS 9896 or Ro 5-4864, whereas flumazenil or its vehicle was given 45 min prior to ethanol. Ethanol used for the induction of gastric damage was given as a 50% aqueous solution (v/v), prepared with distilled water in a dose of 10 ml/kg. One hour after ethanol administration the animals were killed by a sharp blow on the head. The abdomen was opened longitudinally and the stomach was isolated and separated from the surrounding viscera and filled by injecting 1% formalin solution through the esophageal junction. The inflated stomach was then immersed in a 1% formalin bath for 5 min. After that, the stomach was opened along the greater curvature and placed on a petri dish for examination with a dissecting zoom stereo-microscope (Wild Heerbrugg TYP 376788) under 6-fold magnification. The number of lesions in the glandular portion of the stomach as well as their lengths (mm) and their areas (mm^2) were calculated. To avoid possible diurnal variation, all experiments were carried out between 8 a.m. and 11.30 a.m.

2.4. Compounds

The chemicals used, their source and the route of administration were as follows. Absolute ethanol (BDH): ethanol used for the induction of gastric damage was given per os via a stainless steel tube tipped with a short piece of polythene cannula (gavage). Clonazepam [5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepine-2-one], Hoffmann-LaRoche, Basel, Switzerland, a pure central-type benzodiazepine receptors agonist (Cooper and Gilbert, 1985), was administered p.o. or i.p. CGS 9896 [2-(4-chlorophenyl)-2,5-dihydropyrazolo{4,3-*c*}quinoline-3(3*H*)-one], Ciba-Geigy, Basel, Switzerland, a non-benzodiazepine compound acting as a partial agonist at the benzodiazepine receptors with anxiolytic and anticonvulsant but no sedative properties (Gee and Yamamura, 1982; Bennett and Betrack, 1984), was given p.o. by gavage. Ro 5-4864 [7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepine-2-one], Hoffmann-LaRoche, Basel, Switzerland – this 4′-chloro derivative of diazepam is a ligand which binds selectively to the peripheral-type of benzodiazepine receptors (Marangos et al., 1982) – was given p.o. by gavage. Flumazenil [ethyl 8-fluoro-5,6-5-methyl-6-oxo-4*H*-imidazo{1,4}benzodiazepine-3-carboxylate], flumazepil or Ro 15-1788, Hoffmann-La-Roche, Basel, Switzerland, is a competitive benzodiazepine receptor antagonist which binds with high affinity to the central-type but not to the peripheral-type of benzodiazepine receptor (Hunkeler et al., 1981), was given i.p.

All drugs were freshly prepared before administration on the day of experimentation. Drug solutions were prepared as suspensions in normal saline to which Tween 80 was added (2 drops of Tween 80 in 10 ml of normal saline). The concentrations were adjusted so that each rat received no more than 2 ml/kg body weight of the drug solutions.

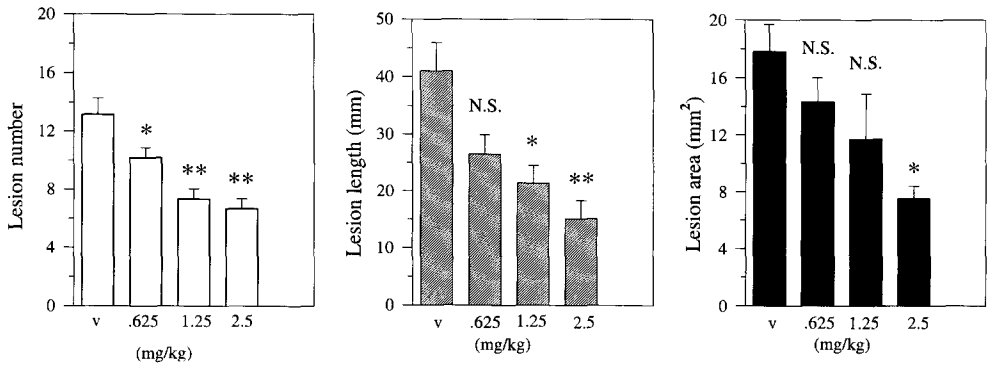

Fig. 1. Effect of oral clonazepam (0.625–2.5 mg/kg) on ethanol-induced gastric damage (lesion parameters) in rats. Clonazepam was given 30 min before ethanol administration (10 ml/kg of a 50% solution v/v p.o.). The rats were killed 1 h later. The data are expressed as means ± S.E.M. for 6 animals in each group. Lesion number is represented by open bars, lesion length by hatched bars and lesion area by filled bars. N.S not significant, * $P < 0.05$, ** $P < 0.01$ compared to vehicle-pretreated control (v).

2.5. Statistics

The data are expressed as means ± standard error of the mean (S.E.M.). Statistical evaluations of the differences between two groups was performed with an independent Student's *t*-test. A P value of less than 0.05 (two-tailed) was considered to be significant.

3. Results

Intragastric instillation of ethanol (10 ml/kg of a 50% solution v/v) produced extensive visible hemorrhagic necrosis in all rats within 1 h (100% induction). Ethanol-induced lesions were irregularly distributed in the glandular portion of the stomach, mostly in the corpus with occasional erosions in the antrum. The lesions varied in size, shape and depth. Inspection of the stomach in situ showed a distended balloon-like viscus filled with a dark-coloured hemorrhagic fluid. The over-stretched stomach wall was pale and remarkably thin so that the sites of mucosal lesions could be seen through the serosa.

3.1. The effect of clonazepam, CGS 9896 and Ro 5-4864 on ethanol-induced gastric damage in non-vagotomized rats

3.1.1. Clonazepam pretreatment

When clonazepam was given p.o. in 0.625 mg, 1.25 mg and 2.5 mg/kg doses, it produced a dose-dependent reduction in the parameters of gastric lesions caused by ethanol as compared with the vehicle-pretreated control group (Fig. 1). At a dose of 0.625 mg/kg, a significant reduction ($P < 0.05$) in lesion number only was noted. However at 1.25 mg/kg, clonazepam reduced both lesion number ($P < 0.01$) and length ($P < 0.05$) significantly, but not the lesion area. At 2.5 mg/kg, the drug produced a significant reduction in all three parameters ($P < 0.01$, $P < 0.01$, $P < 0.05$, respectively). Likewise, i.p. administration of clonazepam at doses of 1.25 mg/kg and 2.5 mg/kg produced a significant reduction in all lesion parameters (Fig. 2).

3.1.2. CGS 9896 pretreatment

When CGS 9896 was given p.o. in a dose range of 2.5–10 mg/kg, it produced a dose-dependent reduction in

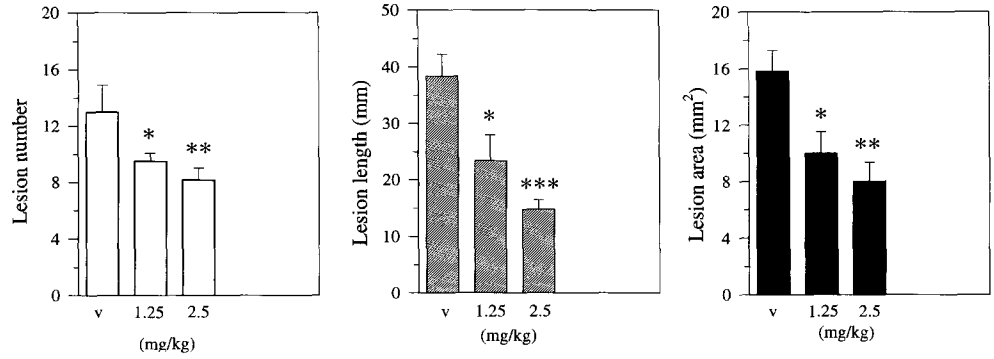

Fig. 2. Effect of intraperitoneal clonazepam (1.25, 2.5 mg/kg) on ethanol-induced gastric damage (lesion parameters) in rats. Clonazepam was given 30 min before ethanol administration (10 ml/kg of a 50% solution v/v p.o.). The rats were killed 1 h later. The data are expressed as means ± S.E.M. for 6 animals in each group. Lesion number is represented by open bars, lesion length by hatched bars and lesion area by filled bars. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle-pretreated control (v).

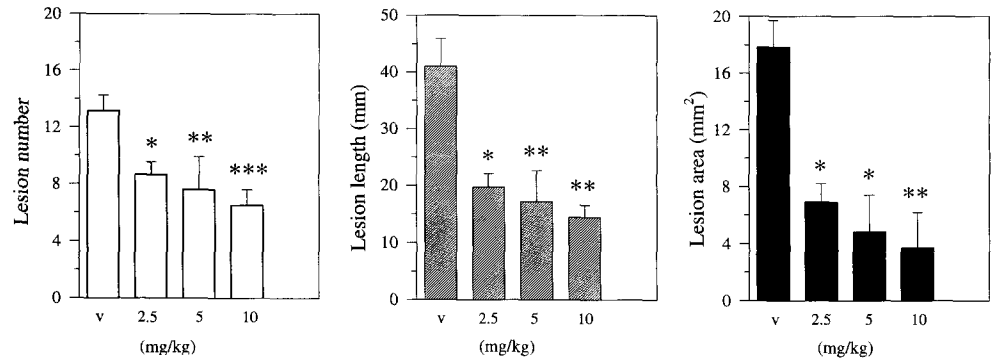

Fig. 3. Effect of CGS 9896 (2.5–10 mg/kg p.o.) on ethanol-induced gastric damage (lesion parameters) in rats. CGS 9896 was given 30 min before ethanol administration (10 ml/kg of a 50% solution v/v p.o.). The rats were killed 1 h later. The data are expressed as means ± S.E.M. for 6 animals in each group. Lesion number is represented by open bars, lesion length by hatched bars and lesion area by filled bars. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle-pretreated control (v).

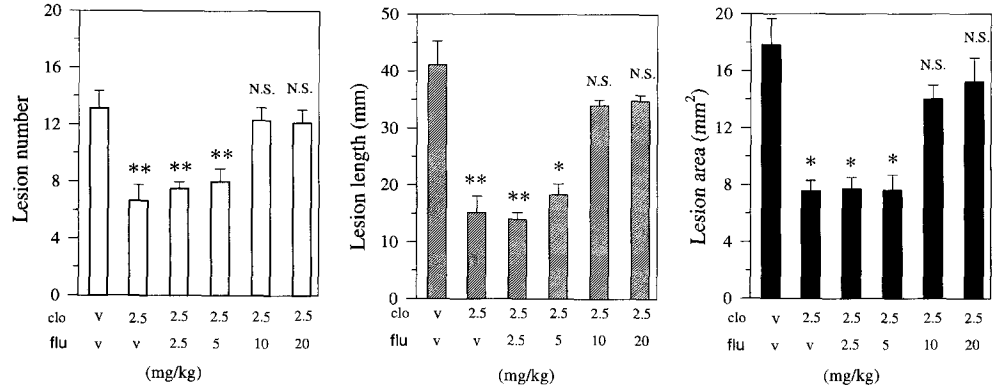

Fig. 4. Effect of flumazenil (flu) on the protective action of clonazepam (clo) against ethanol-induced gastric damage (lesion parameters) in rats. Flumazenil (2.5–20 mg/kg i.p.) was given 45 min before, while clonazepam (2.5 mg/kg p.o.) was given 30 min before ethanol administration (10 ml/kg of a 50% solution v/v p.o.). The rats were killed 1 h later. The data are expressed as means ± S.E.M. for 6 animals in each group. Lesion number is represented by open bars, lesion length by hatched bars and lesion area by filled bars. N.S not significant, * $P < 0.05$, ** $P < 0.01$ compared to vehicle-pretreated control (v).

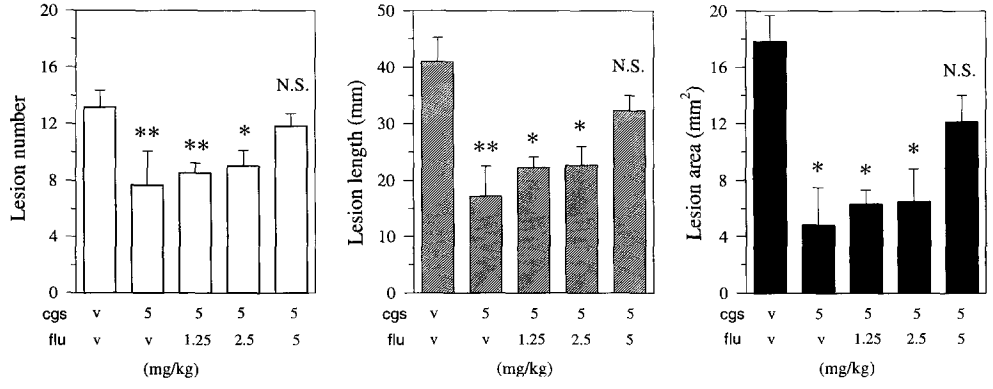

Fig. 5. Effect of flumazenil (flu) on the protective action of CGS 9896 (cgs) against ethanol-induced gastric damage (lesion parameters) in rats. Flumazenil (1.25–5 mg/kg i.p.) was given 45 min before, while CGS 9896 (5 mg/kg p.o.) was given 30 min before ethanol administration (10 ml/kg of a 50% solution v/v p.o.). The rats were killed 1 h later. the data are expressed as means ± S.E.M. for 6 animals in each group. Lesion number is represented by open bars, lesion length by hatched bars and lesion area by filled bars. N.S not significant, * $P < 0.05$, ** $P < 0.01$ compared to vehicle-pretreated control (v).

the lesion parameters of gastric damage caused by ethanol which was significant at all doses as compared with the control group (Fig. 3).

3.1.3. Ro 5-4864 pretreatment

Oral administration of Ro 5-4864 in 5, 10 and 20 mg/kg doses did not produce significant changes in the lesion parameters as compared with the control group (data not shown).

3.2. The effect of flumazenil on ethanol-induced gastric damage in non-vagotomized rats

Flumazenil in a dose range of 1.25–20 mg/kg given i.p. 45 min before ethanol administration (50% concentration) did not influence ethanol-induced gastric erosions, i.e. no significant changes in the lesion parameters were noted as compared with the control group.

3.3. The influence of flumazenil on the protective action of clonazepam and CGS 9896 against ethanol-induced gastric damage in non-vagotomized rats

When flumazenil was given i.p. at doses of 2.5, 5, 10 and 20 mg/kg, 15 min prior to clonazepam (2.5 mg/kg p.o.), it reversed dose dependently the protective action of clonazepam against ethanol-induced gastric lesions so that at 10 and 20 mg/kg flumazenil the lesion parameters were not significantly different from those of the control group (Fig. 4). Similarly, flumazenil blocked the protective action of CGS 9896 against ethanol-induced gastric lesions when administered i.p. at doses of 1.25, 2.5 and 5 mg/kg 15 min prior to CGS 9896 (5 mg /kg p.o.), whereby the lesion parameters in the group pretreated with flumazenil in a dose of 5 mg/kg followed by CGS 9896, were not significantly different from those of the control group (Fig. 5).

3.4. The effect of clonazepam and CGS 9896 on ethanol-induced gastric damage in unilaterally vagotomized rats

In unilaterally vagotomized rats, ethanol induced mucosal damage in the right (vagotomized) and the left (non-vagotomized) halves of the stomach such that no significant differences between the lesion parameters of the two halves of the gastric mucosa in the vehicle-pretreated group were noted. Moreover, the administration of clonazepam (2.5 mg/kg p.o.) or CGS 9896 (5 mg/kg p.o.) to the vagotomized animals produced a uniform reduction in the lesion parameters of both halves of the gastric mucosa as compared to the vehicle-pretreated group, i.e. the lesion parameters in the left half of the stomach were not significantly different from those of the right half (data not shown).

4. Discussion

The present results confirm previous reports of the damaging effect of ethanol on the gastric mucosa. They also demonstrated the protective action of benzodiazepines against ethanol-induced gastric damage through their action on central-type receptors but not on the peripheral-type of receptors. The concentrations and the volumes of ethanol used in previous studies were variable (Williams, 1956; Dinoso et al., 1976; Guth et al., 1984; Oates and Hakkinen, 1988; Najim and Karim, 1991, 1993). In the present study, the administration of ethanol in 50% v/v concentration successfully produced within 1 h macroscopic mucosal lesions in the stomach of rat. However, the volume of aqueous ethanol solution was given according to body weight (10 ml/kg) since the fixed small dose of ethanol (1 ml p.o.) administered in some studies did not distend the stomach and the persisting mucosal folds caused differences in ethanol contact with the mucosa (Mersereau and Hinchey, 1982).

Despite much research, the mechanism by which gastric damage is induced by ethanol remains enigmatic. Previous reports emphasize the importance of gastric acid hypersecretion in the formation of mucosal lesions following ethanol ingestion. Later, clues emerged from other studies to indicate that gastric acid is not a primary cause but plays a permissive role in gastric mucosal damage induced by ethanol (Tarnawski et al., 1983). More recent studies have focused on the effect of ethanol on factors which play a role in mucosal defense mechanisms. While a deficiency of endogenous prostaglandins is widely accepted as a major factor in the pathogenesis of gastric lesions caused by indomethacin, ethanol-induced gastric lesions are considered by some investigators to be the result of multiple factors involving prostaglandins, leukotrienes and histamine (Oates and Hakkinen, 1988; Glavin and Szabo, 1992; Lozeva et al., 1994) whereby the release of these vasoactive agents results in diminished gastric mucosal blood flow with increased vascular permeability, which produces mucosal ischemia and epithelial necrosis.

Results of recent studies also suggest that ethanol may act at the GABA-benzodiazepine receptor complex to produce gastric lesions. This assumption is based on the results obtained by Erdö et al. (1989) and Najim and Karim (1991, 1993) whereby drugs acting on this supramolecular complex (GABAergic agents and benzodiazepines) were found to protect the gastric mucosa against ethanol injury but not against indomethacin-induced mucosal damage, although the latter was proposed to share a similar etiology with ethanol-induced gastric damage (Ghanayem et al., 1987). This excludes the possibility of a non-specific local protective action by the benzodiazepine against chemically induced gastric lesions.

Our results have shown that clonazepam and CGS 9896, which bind to central-type benzodiazepine receptors,

decreased gastric mucosal damage induced by ethanol. However, Ro 5-4864, which binds to peripheral-type receptors failed to do so. In addition, clonazepam was equally effective whether administered p.o. or i.p. to decrease ethanol-induced gastric damage. This is in contrast to the results obtained by Minano et al. (1985) and Erdö et al. (1989), whereby drugs acting at the GABA binding site of the receptor complex (GABA, muscimol, sodium valproate) were found to be more effective when given p.o. than i.p. to protect the stomach against stress-induced or acidified ethanol-induced mucosal damage. Accordingly, the mucoprotective action of clonazepam is apparently not influenced by the route of administration. Although this finding is consistent with the pharmacokinetic properties of benzodiazepines, it might also be explained by the existence of local as well as central protective mechanisms operating to reduce ethanol-induced gastric damage. Therefore, clonazepam, which acts at the GABA-benzodiazepine receptor complex to increase chloride transport (Haefely, 1983), antagonized the damaging action of ethanol on the gastric mucosa. To further define the benzodiazepine receptors that might be involved in the protective action of benzodiazepines, CGS 9896 was used in this study. This drug was found to be very effective to reduce the lesion parameters of gastric mucosal damage induced by ethanol. This unique finding suggests that central-type benzodiazepine receptors which mediate the anxiolytic but not the sedative action of benzodiazepines might be involved in the protective action of benzodiazepines against ethanol-induced gastric damage and probably in the pathogenesis of such damage. In addition, the reversal of the protective action of clonazepam and CGS 9896 by the central-type receptor antagonist, flumazenil, which itself does not influence the gastric damage evoked by ethanol, confirms the fact that their effect is mediated through central-type receptors.

The failure of Ro 5-4864 to affect ethanol-induced gastric damage suggests that the peripheral-type receptors are not implicated in gastric mucosal protection or in the pathogenesis of ethanol-induced gastric damage as are the central-type receptors. In fact the presence of peripheral-type receptors in the stomach has not been reported in spite of several attempts to locate these receptors in rats.

Our findings obtained with the vagotomized animals indicate that vagal components are not involved in ethanol-induced gastric damage as was suggested by Mozsik et al. (1992) nor in the protective action of clonazepam and CGS 9896 against such damage. Thus, the possible involvement of central, vagus-mediated mechanisms in the protection by benzodiazepines against ethanol-induced gastric mucosal damage seems unlikely. Therefore, the most likely site for the protective action of benzodiazepines is a population of central-type benzodiazepine receptors situated in the stomach. The role of central mechanisms in such a protective action cannot, however, be completely ruled out since the central nervous system may affect the gastric mucosa through routes other than the parasympathetic nervous system.

The exact mechanism of the protection mediated by the benzodiazepines against ethanol-induced gastric damage is not clear. This effect might be related to increased GABA-induced production of prostaglandins since GABA has been shown to increase the prostaglandin content of the gut (Girdhar et al., 1981) and exogenous prostaglandins have been reported to prevent ethanol-induced gastric mucosal lesions (Robert et al., 1979; Szabo et al., 1985). It is unlikely, however, that benzodiazepines protect the gastric mucosa through a GABA-evoked enhancement of mucus secretion since ethanol can easily penetrate the mucus barrier (Lloyd et al., 1986; Matuz, 1992).

In summary, our results have demonstrated the protective effect of benzodiazepines against ethanol-induced gastric damage by their action on central-type receptors responsible for the anxiolytic action of benzodiazepines. The most likely site for this protection is a receptor population in the wall of the stomach. These findings indicate that ethanol-induced gastric damage may result from its local action on the GABA-benzodiazepine receptor complex in the stomach.

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