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BENZODIAZEPINES REDUCE GASTRIC ULCERS INDUCED IN RATS BY STRESS

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1 The sedative and antiulcer effects of chlordiazepoxide (5 to 50 mg/kg) and lorazepam (0.25 to 2.5 mg/kg) were investigated in the rat.

2 Sedation was measured by recording locomotor activity in a holeboard. Ulceration of the glandular stomach was induced by a 2 h period of restraint at 4°C.

3 Acutely, both drugs produced significant sedation at all doses; high doses only (chlordiazepoxide 10 and 50 mg/kg; lorazepam 2.5 mg/kg) produced a significant reduction in ulcer formation.

4 With chronic treatment, after 5 and 10 days administration of chlordiazepoxide (50 mg/kg), tolerance to sedation was observed without a similar change in antiulcer action.

5 Cimetidine (20 mg/kg) and atropine (0.2 mg/kg) decreased ulcer formation without causing sedation.

6 The antisecretory profile of chlordiazepoxide (2×10^{-4} M), in the rat isolated gastric mucosa, resembled that of atropine (10^{-7} M) rather than cimetidine (10^{-5} M).

7 These observations suggest that the antiulcer effect of benzodiazepines probably results from a combination of sedative, anxiolytic and antisecretory actions.

Introduction

Clinical observations suggest that even in the absence of overt anxiety some peptic ulcer patients may benefit from combination therapy which includes a benzodiazepine (Greenblatt & Shader, 1974). This improvement may be effected by the reduction in resting gastric secretion and acid production observed after treatment with diazepam (Birnbaum, Karmeli & Tefera, 1971).

This observation is reinforced by animal studies which indicate that, even with acute treatment, diazepam and chlordiazepoxide reduce the formation of gastric lesions induced by a variety of stress situations (Haot, Djahanguiri & Richelle, 1964; Birnbaum, 1969; Bonfils & Dubrasquet, 1969; Schumpelick & Paschen, 1974; Dairman & Juhasz, 1978). However, the doses used in these studies (e.g. diazepam, 20 mg/kg, Birnbaum, 1969; chlordiazepoxide, 50 mg/kg, Haot *et al.*, 1964; Bonfils & Dubrasquet, 1969) are higher than those observed to produce marked sedation with acute administration (File, 1980).

The present experiments were designed to distinguish between general sedation and true anxiolytic or antiulcer actions of benzodiazepines. The degree of sedation produced by drug treatment was measured 30 min after injection, when the effect is greatest, by recording locomotor activity. Protection against stress ulcers, induced by a combination of cold and restraint, was tested over the 2 h period immediately following injection when plasma drug levels are highest. In pilot experiments, groups of starved non-stressed and non-starved stressed rats failed to show gastric mucosal damage. Furthermore, although some lesions could be induced in starved animals subjected to only 1 h cold-restraint, a 2 h test period was required to produce sufficient damage for a subsequent reduction by drug treatment to be demonstrable.

A preliminary account of some of this work was presented to the British Pharmacological Society (File, Mabbutt & Pearce, 1981).

Methods

Experiment 1

Male hooded Lister rats weighing 250–350 g (supplied by Olac Ltd), or females weighing 150–200 g, were group housed (6/cage) for at least 5 days before use. A controlled 11 h:13 h light/dark cycle was maintained, with the lights on at 06 h 00 min.

Sedation: The degree of sedation produced by drug

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treatment was measured by recording locomotor activity in a holeboard (File & Wardill, 1975). Rats were group housed throughout and allowed food and water *ad lib.* Thirty minutes after injection (i.p., 2 ml/kg) with drug solution or the appropriate vehicle (distilled water for chlordiazepoxide; Wyeth solvent for lorazepam; 0.15 M NaCl solution (saline) for cimetidine and atropine) rats were placed singly in the holeboard under low light conditions (13 Scotopic Lux). Locomotor activity was recorded automatically during the 10 min test period by the interruption of infra-red photobeams. Rats were tested between 07 h 00 min and 11 h 00 min in a test order randomized for drug treatment. Data were analysed by unpaired *t* tests (1 tailed; $P < 0.05$ was considered to be significant).

Ulcer formation: Stress ulcers were produced by a combination of cold and restraint. On the day before testing rats were pair housed and deprived of food, but not water, overnight. Immediately after injection (i.p., 2 ml/kg) with drug or vehicle, each rat was placed in a Bollman-type restraining cage placed on its side in a cold room at 4°C for 2 h. At the end of the test period, the rat was killed by decapitation using a guillotine. The stomach was removed and opened by cutting along the lesser curvature. The presence of food or fluid and blood or mucus was recorded before the stomach was washed and pinned out flat. The number and severity of discrete areas of damage in the glandular mucosa were scored by two independent observers who were unaware of the drug treatment (a third experimenter coded the syringes for drug administration) and the mean scores were recorded. Severity was assessed on a scale of 1 to 5; a score of 1 representing point erosions and a score of 5 representing an erosion covering approximately 4 mm^2. Areas showing local blanching only were not scored; however, such regions also demonstrating bleeding were scored as true erosions. All testing was carried out between 06 h 15 min and 18 h 15 min. Within this period, animals were randomly allocated to the time of day for testing, within the constraints of equal numbers of control and drug dose for each time.

Although the data were normally distributed, due to the unequal variance in control and drug groups, ulcer numbers and severity scores were compared by Mann-Whitney U-tests (1 tailed; $P < 0.05$ was considered to be significant). All statistical comparisons were made with test groups versus their own control group. The control scores have been combined into a single group solely for the purpose of the figures. The differences in incidence of gastric haemorrhage observed in control and drug groups, or male and female rats, were assessed by the Chi-square test (χ^2; $P < 0.05$ was considered to be significant).

Experiment 2

Immature albino rats of either sex (home bred from the Olac strain) were allowed food and water *ad lib.* Rats weighing 80 to 100 g were anaesthetized with pentobarbitone (60 mg/kg, s.c.) and the stomach exteriorized. The muscle layer overlying the non-antral glandular region was separated from the mucosa by blistering (Main & Pearce, 1978). Two 1 cm^2 pieces of mucosa from each stomach were placed in organ baths containing 35 ml of a buffered Krebs solution at 37°C (serosal solution (mM); NaCl 110.0, KCl 5.0, $CaCl_2.6H_2O$ 3.6, $MgCl_2.6H_2O$ 1.2, $NaHCO_3$ 26.0 and glucose 16.7) which bathed the serosal surface and was gassed with a 95% O_2/5% CO_2 mixture. The mucosal surface was superfused at 0.5 ml/min with an unbuffered solution (mucosal solution (mM): NaCl 136.0, KCl 5.0, $CaCl_2.6H_2O$ 3.6, $MgCl_2.6H_2O$ 1.2 and glucose 16.7) gassed with O_2. Acid output was recorded via a dual microelectrode in the mucosal solution.

Paired preparations, from a single stomach, were allocated to treatment groups. A series of three responses was obtained in each preparation with pentagastrin (1.8×10^{-8} M) added at 120 min, methacholine (2.5×10^{-7} M) at 210 min and histamine (5×10^{-5} M) at 300 min. This test order was chosen because the isolated mucosa responds better to pentagastrin and methacholine early in an experiment while histamine-induced secretion is greatest at a later stage (Main & Pearce, 1978). All secretagogues were added to the serosal solution and left in contact for 30 min. The test preparation of each pair was exposed to cimetidine (10^{-5} M), atropine (10^{-7} M) or chlordiazepoxide (2×10^{-5} M or 2×10^{-4} M) continuously from 60 min to the end of the experiment at 330 min.

The acid secretory response (μmol cm^{-2} h^{-1}) was calculated by subtraction of the basal rate from the subsequent peak response. The effect of the test compound was assessed by comparing the magnitude of the response in the treated preparation with that in the paired control. Data were analysed by paired *t* tests (1 tailed; $P < 0.05$ was considered to be significant).

Drugs

Stock solutions of the following drugs were prepared daily or stored frozen until required. Chlordiazepoxide (Roche Products Ltd) was dissolved in distilled water. Lorazepam injection (Ativan, Wyeth Laboratories) was further diluted with water. Atropine sulphate (Burroughs Wellcome & Co) was dissolved in 0.15 M NaCl solution (saline). Cimetidine (S.K. & F. Laboratories Ltd) was dissolved in 1M HCl, partially neutralized with 0.1 M

NaOH and made up to volume with water. Pentobarbitone (Nembutal, Abbott Laboratories Ltd), histamine acid phosphate (BDH Ltd), methacholine chloride (acetyl-β-methylcholine chloride, Sigma Chemical Co) and pentagastrin (Peptavlon, ICI Ltd) were all prepared in saline (0.9% w/v NaCl solution).

Results

Incidence of ulcers and bleeding in control animals

The chlordiazepoxide-control group, male rats injected with water, showed a mean (± s.e.mean) ulcer number of 16.1 ± 2.5 ($n = 37$, Figure 1) and a severity score of 25.9 ± 4.2 In the lorazepam-controls, injected with the placebo solvent mixture, the ulcer number (17.2 ± 3.5, $n = 21$) and severity scores (26.6 ± 5.4) were very similar. A total of 13 stomachs (6 chlordiazepoxide-controls, 7 lorazepam-controls, i.e. 22%) contained blood. The incidence of haemorrhage was related to the extent of mucosal damage; blood was present in 37% of stomachs with an ulcer number greater than the median (11) but in only 7% of those with a lower score ($\chi^2 = 7.96$, $P < 0.01$).

Acute chlordiazepoxide and lorezepam in male rats

Sedation: At the time of testing, 30 min after injection, all doses of chlordiazepoxide and lorazepam produced a significant reduction in locomotor activity (Figure 1). The percentages quoted indicate the mean activity level in treated animals ($8 < n < 15$) relative to that in the matched control group.

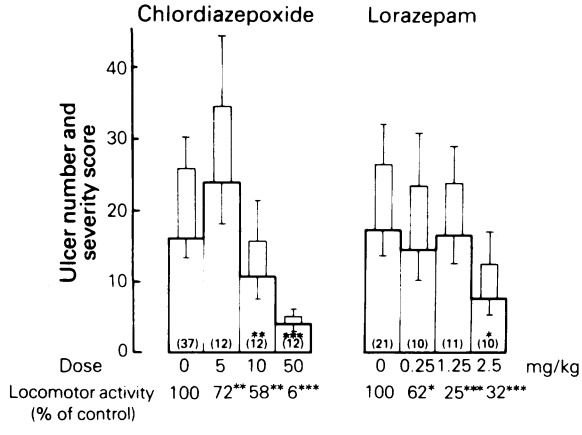

Figure 1 Acute treatment with chlordiazepoxide (5, 10 and 50 mg/kg) and lorazepam (0.25, 1.25 and 2.5 mg/kg). Results in the histogram, shown as ulcer number (lower columns) and severity score (upper columns), are the mean of (n) observations; vertical lines show s.e.mean. Locomotor activity, derived from 8 to 15 observations for each group, is shown as a percentage of the control group score. The levels of statistical significance for Mann-Whitney U (ulcer) and Student's t tests (activity) are shown by: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Ulcer formation: As shown in Figure 1, there was a slight increase in the number and severity of ulcers after chlordiazepoxide (5 mg/kg) but the higher doses produced a significant reduction in both scores ($P < 0.01$ at 10 mg/kg, $P < 0.001$ at 50 mg/kg). A similar pattern was reflected in the incidence of gastric haemorrhage which showed an increase, from 22% in controls, to 42% after 5 mg/kg and returned to 25% with the higher doses.

Also shown in Figure 1, low doses of lorazepam (0.25 and 1.25 mg/kg) had no effect against ulceration while administration of 2.5 mg/kg produced a significant reduction in both ulcer number and severity ($P < 0.05$). As observed with the low dose of chlordiazepoxide (5 mg/kg), the incidence of bleeding increased after the low doses of lorazepam (0.25 and 1.25 mg/kg), from 22%, to 50% and 45% respectively. The antiulcer effect of 2.5 mg/kg was accompanied by a decreased incidence (20%) of bleeding. However, in none of the chlordiazepoxide or lorazepam treatment groups was the incidence of bleeding significantly different from the controls.

Acute chlordiazepoxide in female rats

Ulcer formation: Following 2 h cold-restraint, the control female rats showed a greater degree of mucosal damage than did the male rats. The mean number of ulcers (23.0 ± 3.7, $n = 10$) and the severity score (33.8 ± 5.7) were both increased by 30%, although the differences were not significant (unpaired t test). However, the incidence of haemorrhage (70%) was significantly greater than in the control male rats ($\chi^2 = 10.05$, $P < 0.01$).

Chlordiazepoxide (10 mg/kg) significantly reduced both the number (7.2 ± 3.0, $n = 10$, $P < 0.01$) and severity of ulcers (9.9 ± 4.2, $P < 0.01$); the incidence of bleeding also showed a significant decrease to 20% ($\chi^2 = 5.05$, $P < 0.05$).

Chronic chlordiazepoxide treatment

Sedation: The degree of sedation existing after repeated chlordiazepoxide administration was tested on the 5th or 10th day (Figure 2). After 5 days treatment with chlordiazepoxide 10 mg/kg the reduction in activity observed after acute administration was no longer apparent. At the higher dose, 50 mg/kg, there was less sedation after 5 and 10 days treatment but there was still a significant reduction in activity.

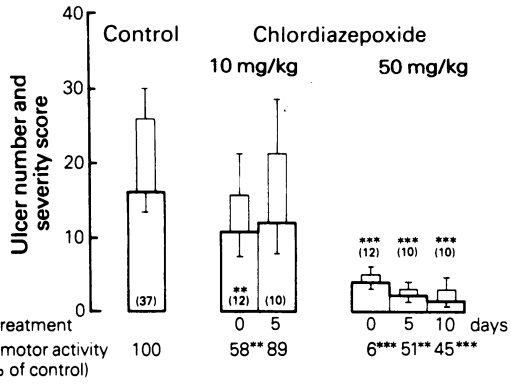

Figure 2 Chronic treatment (5 or 10 days duration) with chlordiazepoxide (10 and 50 mg/kg). Results are expressed as described for Figure 1, with activity scores from 8 or 10 observations in each group. Statistical significance: $^{**}P<0.01$, $^{***}P<0.001$.

Ulcer formation: As illustrated in Figure 2, the ulcer scores in rats injected with chlordiazepoxide 10 mg/kg were slightly higher after 5 days treatment than after a single dose, and were not significantly different from the control scores. The incidence of bleeding after repeated administration (30%) was similar to that observed after a single dose (25%).

By contrast, the antiulcer effect of the higher dose, 50 mg/kg, increased with the duration of treatment. The number and severity of ulcers decreased after 5 days treatment to 2.3 ± 0.8 ($n = 10$) and 3.1 ± 1.0 respectively, and, after 10 days, showed a further decrease to 1.5 ± 0.7 ($n = 10$, $P < 0.05$ versus acute) and 3.0 ± 1.6 ($P < 0.05$ versus acute). This effect was accompanied by a reduction in the incidence of bleeding from 25% with acute treatment to 10% after 5 days and 0% after 10 days.

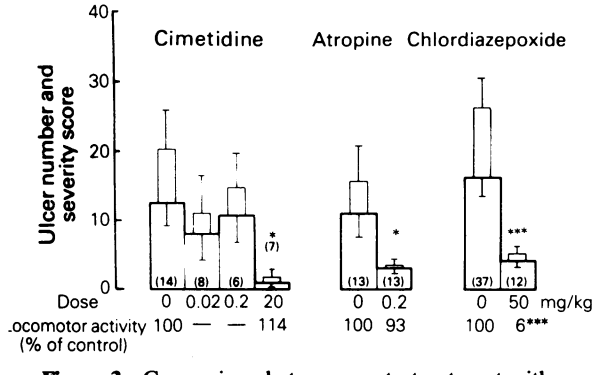

Figure 3 Comparison between acute treatment with cimetidine (0.02 to 20 mg/kg), atropine (0.2 mg/kg) and chlordiazepoxide (50 mg/kg). Results are expressed as described for Figure 1, with activity scores from 10 rats in each group. Statistical significance: $^{*}P<0.05$, $^{***}P<0.001$.

Acute cimetidine and atropine

Sedation: In contrast to the marked sedation produced by acute chlordiazepoxide and lorazepam, there was a slight increase in the level of activity measured 30 min after the administration of cimetidine, 20 mg/kg (see Figure 3). Atropine was tested only at a low dose (0.2 mg/kg) and produced a small reduction in activity; this effect was not significant.

Ulcer formation: Low doses of cimetidine (0.02 and 0.2 mg/kg) produced a small and variable reduction in ulceration (Figure 3). However, mucosal damage was almost abolished after an increase in dosage to 20 mg/kg. The number of ulcers was significantly reduced to 0.9 ± 0.6 ($n = 7$, $P < 0.05$) and the severity score to 1.7 ± 1.1 ($P < 0.05$). Blood was present in only one of the control stomachs (7%) and one of the 0.2 mg/kg treatment group (17%).

Atropine, 0.2 mg/kg, produced a significant reduction in ulcer number ($P < 0.05$) and severity score ($P < 0.05$). However, the incidence of haemorrhage was not reduced by atropine, with 23% of control and 23% of treated stomachs containing blood.

The effects of acute chlordiazepoxide, 50 mg/kg, have been included in Figure 3 to demonstrate that a reduction in ulceration, similar to that produced by cimetidine (20 mg/kg) and atropine (0.2 mg/kg), is associated with a significant degree of sedation.

Antisecretory actions of cimetidine, atropine and chlordiazepoxide in vitro

Cimetidine and atropine act directly on the gastric mucosa to decrease acid secretion and inhibit ulcer formation. The effects of these drugs and of chlordiazepoxide were investigated, on the rat isolated gastric mucosa, against secretory responses to pentagastrin (P, 1.8×10^{-8} M, Figure 4), methacholine (M, 2.5×10^{-7} M) and histamine (H, 5×10^{-5} M). The mean ($\pm$ s.e.mean) responses to these secretagogues in control preparations were: pentagastrin 0.67 ± 0.07 μmol cm^{-2} h^{-1} ($n = 22$), methacholine 3.53 ± 0.44, histamine 5.57 ± 0.44.

The first histogram in Figure 4 illustrates the antisecretory profile of cimetidine (10^{-5} M, $n = 6$) typically observed in this preparation. Cimetidine produced a small (30%) but significant ($P < 0.05$) decrease in the response to pentagastrin. The response to methacholine, in the presence of cimetidine, was only slightly reduced (10%), while that to histamine decreased by more than 80% ($P < 0.001$).

The second histogram shows that, in this prepara-

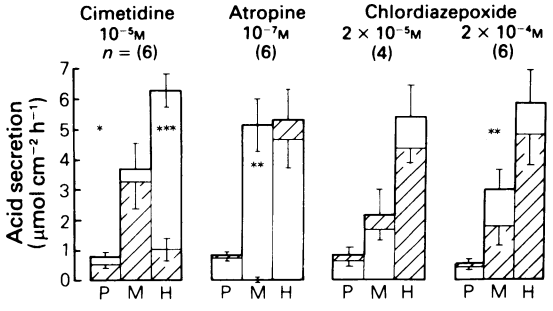

Figure 4 Stimulation of acid secretion from the rat isolated gastric mucosa by pentagastrin (P, 1.8×10^{-8} M), methacholine (M, 2.5×10^{-7} M) and histamine (H, 5×10^{-5} M). Results, shown as the increase in acid secretion over basal output (μmol cm^{-2} h^{-1}), are the mean of 4 or 6 observations; vertical lines show s.e.mean. Open columns represent responses in control preparations while hatched columns represent simultaneous responses, in paired preparations, in the presence of cimetidine (10^{-5} M), atropine (10^{-7} M) or chlordiazepoxide 2×10^{-5} M or 2×10^{-4} M). Statistical significance (paired t test) is shown by: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

tion, atropine (10^{-7} M, $n = 6$) has a selective antisecretory action. While responses to pentagastrin and histamine were slightly larger in the presence of atropine, than in paired controls, the response to methacholine was completely abolished ($P < 0.01$).

Chlordiazepoxide was tested at two concentrations, 2×10^{-5} M ($n = 4$) and 2×10^{-4} M ($n = 6$). The lower concentration was in the same ratio to cimetidine (10^{-5} M) and atropine (10^{-7} M) *in vitro* as their effective doses *in vivo* (see Figure 3) but makes no allowances for differences in drug distribution. As shown in Figure 4, chlordiazepoxide (2×10^{-5} M) had no effect on responses to any secretagogue, although inhibition was observed at 2×10^{-4} M. The antisecretory profile was similar to that described for atropine; in test preparations, the response to methacholine was significantly reduced ($P < 0.01$) while pentagastrin and histamine were not affected.

Discussion

The present experiments confirm that a period of food deprivation, in this case between 12 and 20 h, is required for the ulcerogenic effect of stress to be manifested. Although it has been suggested that such a long period of starvation favours the development of rumenal pathology (Vincent, Paré & Glavin, 1980), without exception, in our experiments, all damage was observed in the glandular mucosa.

The use of a 2 h period of cold (4°C) and restraint, in combination with food deprivation, produced a degree of damage that was consistent over the experimental period from May to December and was severe enough for a subsequent reduction by drug treatment to be demonstrable. Although restraint alone is a sufficient stimulus to induce stress ulcers, a longer period of immobilization is required (e.g. 6 h, Haot *et al.*, 1964; 22 h, Schumpelick & Paschen, 1974). More recently, the synergistic effect of cold upon restraint-induced damage has been exploited, enabling the duration of restraint to be reduced to a 3 h period at 4–10°C (Brick, Burright & Donovick, 1979; Goldstein & Wozniak, 1979; Glavin, 1980; Vincent *et al.*, 1980).

Single doses of chlordiazepoxide (5 to 50 mg/kg) produced a dose-related reduction in locomotor activity but had a variable effect on ulcer development. High doses (10 and 50 mg/kg) produced a significant degree of protection that could not be accounted for solely by the degree of sedation. Equivalent doses of lorazepam (0.25 to 2.5 mg/kg) all caused significant sedation, but ulcers were reduced only at the highest dose. An antiulcer effect following acute administration of high doses has previously been reported, in the rat, for diazepam (20 mg/kg against optic/acoustic stimuli; Birnbaum, 1969) and chlordiazepoxide (50 mg/kg against restraint; Haot *et al.*, 1964; Bonfils & Dubrasquet, 1969). In our experiments the lowest dose of chlordiazepoxide (5 mg/kg) was sedative but produced an increase in gastric ulceration and haemorrhage. This lack of protection contrasts with a previous report, that mucosal damage induced by restraint-immersion in the mouse was reduced by low doses of diazepam ($ED_{50} = 1.1$ mg/kg) and chlordiazepoxide ($ED_{50} = 4.0$ mg/kg), although much higher doses (diazepam 33.7, chlordiazepoxide 132) were required for protection against the effects of forced exertion (Dairman & Juhasz, 1978). The separation of sedative and antiulcer effects after acute administration suggests that these two actions of benzodiazepines may not be related.

Since the sedative effects of benzodiazepines demonstrate tolerance on repeated administration, the effect of 5 or 10 days treatment with chlordiazepoxide was investigated. The reduction in ulceration with 10 mg/kg was less marked after 5 days; this was associated with a reduction in sedation, suggesting that tolerance to both effects had developed. However, the antiulcer effect of 50 mg/kg increased after repeated administration while the degree of sedation was much reduced. From these observations, it is apparent that the antiulcer action of benzodiazepines is not simply related to anxiolytic effects which are observed after 5 days of treatment at the lowest doses tested here (i.e. chlordiazepoxide 5 mg/kg, File & Hyde, 1978; lorazepam 0.25 mg/kg, File, 1980). Furthermore, although sedation probably contri-

butes to the antiulcer action there is no obvious correlation between the two effects following acute treatment while, after repeated administration, sedation is markedly reduced with little or no change in the antiulcer effect. An early report of the protection afforded by 21 days repeated administration of chlordiazepoxide (50 mg/kg) to rabbits exposed to electroshock 30 min after each injection failed to mention the degree of sedation induced by such treatment (Dasgupta & Mukherjee, 1967).

In order to estimate the potential use of benzodiazepines in the treatment of stress ulcers, similar experiments were carried out with the antisecretory agents, cimetidine and atropine. In contrast to the combined antiulcer-sedative action of chlordiazepoxide, cimetidine and atropine both produced a significant reduction in mucosal damage at a dose which had no effect on locomotor activity, indicating a specific antiulcer action for each drug.

In vivo, both cimetidine and atropine act directly on the gastric mucosa to inhibit acid secretion and hence reduce ulcer formation. However, since they exhibit a degree of selectivity against induced secretion *in vitro*, the antisecretory profile and potency of chlordiazepoxide on the rat isolated gastric mucosa was compared with cimetidine and atropine. Cimetidine (10^{-5} M) greatly reduced the response to histamine but produced only a partial reduction in pentagastrin-induced secretion, while atropine (10^{-7} M) was selective against methacholine. The equivalent concentration of chlordiazepoxide (2×10^{-5} M) had no effect on any secretagogue; an increase in concentration to 2×10^{-4} M caused inhibition of methacholine only, as observed with atropine. Since this concentratiqn is higher than the level likely to be attained following acute administration of chlordiazepoxide, a direct antisecretory action is unlikely to contribute to the observed antiulcer effect.

Benzodiazepines reduce stress ulcer formation at doses that are considerably higher than required for anxiolytic activity (Cook & Sepinwall, 1975; File, 1980) or to block the pituitary-adrenal stress response (File & Peet, 1980). Their antiulcer activity therefore cannot result solely from these other actions, nor can it be explained purely by sedation since, with chronic administration, marked tolerance develops to their sedative effects while protection against ulceration does not change in this way. Although acid secretion is unlikely to be reduced by a direct action on the gastric mucosa, a central reduction due to a decrease in vagal activity may follow acute treatment. It has been proposed that the antiulcer effect of diazepam results from a direct action on centres in the hypothalamic and limbic systems producing a decrease in the vagal component of acid and pepsinogen secretion and in splanchnic nerve control of vascular tissue (Schumpelick & Paschen, 1974).

In summary, the antiulcer effect of benzodiazepines cannot be attributed to any single action of these drugs, although it probably results from a combination of sedative, anxiolytic and antisecretory actions.

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