J. Pharm. Pharmacol. 1981, 33: 348–352
Received November 19, 1980

0022–3573/81/060348–05 $02.50/0

The effect of stress and indomethacin, carbenoxolone, salbutamol, metiamide, zolimidine and CF19415 on rat gastric mucus

A. P. GREEN*, J. E. LANDER AND D. H. TURNER

Beecham Pharmaceuticals Research Division, Medicinal Research Centre, The Pinnacles, Harlow, Essex CM19 5AD, U.K.

A method for measuring mucus adherent to the gastric mucosa using the dye alcian blue has been evaluated. Mucus was reduced by cold-restraint stress, reserpine and pyloric ligation, but not by 18 h fasting or indomethacin. The fasting mucus level was increased by carbenoxalone, but not by salbutamol or metiamide, and zolimidine only prevented the reduction in mucus induced by stress. A novel compound, 2-α-methoxyiminopyrazine acetonitrile (CF 19415), at 100 mg kg^{-1} orally increased alcian blue binding in fasted rats and in a cold restraint test, 100 mg kg^{-1} orally inhibited gastric damage and stimulated mucus secretion.

The gastric mucosa is coated by a layer of mucus which may have a part to play in protecting the mucosa from the damaging effects of acid and pepsin (Parke et al 1975). Anti-ulcer drugs such as carbenoxolone (Domschke et al 1972) and zolimidine [2-(*p*-methyl-sulphonyl)-imidazo(1,2a)pyridine] (Murmann et al 1974a) are believed to act, at least in part, by stimulating mucus production. The study of the mucus lining of the stomach has proved difficult and has relied mainly on either histological techniques, or scraping off the mucus before biochemical determinations (Wise & Ballinger 1970). Corne et al (1974) reported a technique for the quantitation of mucus in situ using the dye alcian blue, a cationic dye that binds to acidic mucopolysaccharides (Pearse 1968). We have used this technique to look at the effects of starvation, stress and various drugs on the mucus lining of the rat stomach.

The technique has been used to further evaluate the mucostimulant properties of a novel compound, 2-α-methoxyiminopyrazine acetonitrile (CF 19415) which has previously been reported to inhibit indomethacin or cold/restraint stress, inhibit gastric secretion and have some effect on gastric mucus (Van Zorge et al 1980).

METHODS

Animals

Male, Wistar rats, 200 to 250 g, except where indicated, were fasted overnight in grid-bottomed cages, but allowed free access to water. They were used to evaluate the effects of starving and of ulcerogenic procedures on gastric mucus and tissue.

Mucus determination (adapted from Corne et al 1974)

Rats were stunned and decapitated. The stomachs were dissected, opened along the greater curvature, rinsed in ice-cold 0·25 M sucrose and the rumen discarded. After they had been weighed, the stomachs were incubated in 10 ml of alcian blue (1 mg ml^{-1}) in 0·15 M sucrose, 0·05 M sodium acetate, pH 5·8, for 1·5 h at room temperature (20 °C). The dye solution was freshly made up and filtered before use. The stomachs were transferred to 10 ml 0·25 M sucrose for 15 min and then this sucrose wash was repeated. Finally, the stomachs were placed in 15 ml 0·5 M magnesium chloride solution for 2 h, and shaken occasionally, removed and the magnesium chloride solution shaken briefly with 10 ml diethyl ether. The optical density of the aqueous layer was read at 605 nm. Results are expressed as optical density units g^{-1} tissue.

Damage

To avoid artifacts arising from handling, no assessment of gastric damage (other than that noted at the time of dissection) was attempted. In the cold-restraint stress system it was found possible to assess damage microscopically after destaining the stomachs in magnesium chloride. The stomachs were pinned on cork boards and damage assessed on a 0–9 scoring system when viewed under a binocular microscope at ×10 magnification.

* Correspondence.

Table 1. The effect of ulcerogenic procedures on gastric mucus. Rats were treated as detailed below and in Methods section. Except where indicated rats were fasted overnight, but allowed water. Alcian blue binding on the glandular portion of the stomach was determined as described in the text. Pyloric ligation was carried out under halothane anaesthesia. Sham-operated rats were laparotomized, but the pylorus was not ligated.

Treatment (no. in group)	Duration of treatment (h)	Alcian blue binding (OD units g^{-1} tissue)	% change	Damage present (+) absent (—)
Free fed (12)		0·47 ± 0·02		—
Fasted (12)	18	0·46 ± 0·04	—2	—
Fasted (12)		0·71 ± 0·05		—
Cold-restraint stress (12)	2·5	0·57 ± 0·03	—22*	+
Saline, i.p. (6)		0·63 ± 0·05		—
Reserpine 5 mg kg^{-1}, i.p. (6)	4	0·51 ± 0·03	—20*	+
Sham-operated (7)		0·82 ± 0·04		—
Pyloric ligated (7)	4	0·66 ± 0·04	—20*	—
Saline, s.c. (7)		0·40 ± 0·06		—
Indomethacin 15 mg kg^{-1} s.c. (7)	3	0·34 ± 0·03	—16	+
Indomethacin 15 mg kg^{-1} s.c. (6)	4	0·40 ± 0·04	0	+

* $P < 0·05$ (Student's t-test).

Chemicals

Alcian blue was purchased from Hopkins and Williams. Zolimidine was a gift from Selvi Co. Ltd., Milan, and salbutamol was a gift from Allen and Hanbury Ltd., Ware, Herts. Metiamide and carbenoxolone sodium were prepared by Beecham Pharmaceuticals Research Division. Other chemicals were purchased from British Drug Houses or Sigma Ltd. in the purest grades available.

The synthesis of CF 19415 was carried out by ACF Chemiefarma, nv, and has been described by Van Zorge et al (1980).

RESULTS

There was a large variation in control values from day to day, but within each experiment the standard error of the mean usually fell within 4–10% of the mean.

Distribution of mucus

Five rat stomachs were dissected into rumen, fundus and antrum, and alcian blue binding in each area was 0·36 ± 0·03, 0·59 ± 0·08 and 0·87 ± 0·24 OD units g^{-1} tissue respectively. Routinely the rumen was dissected away before measurement of binding.

Effect of fasting

Table 1 shows that 18 h fasting, a procedure which did not produce gastric damage, did not alter the dye binding.

Effects of ulcerogenic procedures

We examined changes in mucus in various animal models of gastric damage and the results are summarized in Table 1. The damage in these systems, consists of erosions of the mucosa.

2·5 h cold-restraint stress (rats were placed vertically with their heads up in metal Bollman-type restraining cages in a room at 4 °C) produced consistent gastric damage and lowered gastric mucus by 22%. A similar lowering of mucus was produced by reserpine (5 mg kg^{-1} i.p.) where this produced gastric damage.

Prolonged pyloric ligation has been reported to produce gastric damage (Shay et al 1945). We routinely use a short, 4 h, test to study gastric secretion and no damage is seen under these conditions. However, 4 h pyloric ligation did reduce gastric mucus.

Indomethacin (15 mg kg^{-1} s.c.) produced consistent and severe gastric damage, but there was a non-significant reduction in mucus at 3 h, and no change at 4 h. Indomethacin has been reported to reduce the rate of synthesis of mucus (Parke et al 1975).

Action of drugs on gastric mucus in fasted rats

As some drugs were administered suspended in 0·5% methyl cellulose, the vehicle itself was tested and found not to affect gastric mucus (Table 2), although among the procedures used marked gastric damage was apparent in control animals that received this vehicle.

Table 2. Effect of drugs on alcian blue binding in fasted rats. Except for salbutamol, drugs were administered orally suspended in 0·5% methyl cellulose. Salbutamol was dissolved in 0·9% NaCl and given subcutaneously. Metiamide was given 30 min before the stress test. Other conditions are as given in the text and legend to Table 1.

Treatment (no. in group)	Duration of treatment (h)	Alcian blue binding (OD units g^{-1} tissue)	% change
Undosed (6)		0·43 ± 0·08	
Methyl cellulose 1 ml/100 g oral (6)	2	0·47 ± 0·03	+8
Methyl cellulose oral (6)		0·63 ± 0·06	
Zolimidine 200 mg kg^{-1} oral (6)	3	0·57 ± 0·02	—9
Methyl cellulose oral (6)		0·50 ± 0·03	
Zolimidine 200 mg kg^{-1} oral (6)	17	0·56 ± 0·03	+12
Methyl cellulose oral (6)		0·52 ± 0·02	
Carbenoxolone 50 mg kg^{-1} oral (6)	18	0·64 ± 0·05	+23*
Saline, s.c. (8)		0·49 ± 0·05	
Salbutamol 2 mg kg^{-1} s.c. (8)	2	0·43 ± 0·02	—12
Salbutamol 20 mg kg^{-1} s.c. (8)	2	0·50 ± 0·01	+3
Stress Control (8)		0·29 ± 0·02	
Stress + metiamide 50 mg kg^{-1} s.c. (8)	2	0·33 ± 0·04	+13

* $P < 0·05$, *t*-test for mucus.

Carbenoxolone sodium was given at the start of an 18 h fasting period; at the end of fasting there was a significant increase in mucus. Zolimidine on the same dose schedule did not significantly increase mucus, nor did it have an effect when given 3 h before the rats were killed.

Salbutamol has been reported to prevent indomethacin-induced gastric damage and to inhibit gastric acid secretion (McCloy et al 1976; Fielding et al 1977) but it was without significant effect on gastric mucus.

Metiamide, an H_2 receptor antagonist, inhibited gastric damage in the stress test by 74% ($P < 0·05$), but was without effect on mucus secretion in the same test.

Further studies with zolimidine (Table 3)

Zolimidine was inactive on mucus in starved rats, but it has been reported to stimulate mucus production (Murmann et al 1974a). In the cold-restraint stress test at 200 mg kg^{-1} orally, it inhibited damage by 87% and significantly elevated mucus. Neither of these effects was lost after 5 days dosing at 100 mg kg^{-1} day^{-1}. There was a small, but not significant, elevation of mucus by zolimidine in the presence of reserpine and a similar effect in pyloric ligated rats.

Effects of CF 19415

CF 19415, 100 mg kg^{-1} orally, increased alcian blue binding in starved rats with a maximum effect at 1–2 h and a duration of action greater than 3 h (Fig. 1). The compound was effective at 50 and 100, but not at 25 mg kg^{-1} orally.

In the cold-restraint stress test, CF 19415, 100 mg kg^{-1}, either s.c. or orally inhibited gastric damage and stimulated mucus secretion (Table 4). Although some tolerance appeared to develop to the mucostimulant effects with twice a day dosing at 100 mg kg^{-1} orally for 5 days, CF 19415 still significantly increased mucus levels above the control value at the end of this period.

Table 3. Effect of zolimidine on mucus secretion. Zolimidine was given at a dose of 200 mg kg^{-1} orally except in the chronic dose experiment where it was given at 100 mg kg^{-1} day^{-1}, orally for 5 days, the last dose being given 30 min before the stress test. In the reserpine test, zolimidine was given 1 h before reserpine and the rats killed 4 h after the reserpine. In the pyloric ligated rat experiment the drug was given intraduodenally at the time of ligation and the test lasted 3 h. In the stress test the drug was given 30 min before the start of the stress.

Treatment (no. in group)	Alcian blue binding (OD g^{-1} tissue) Control	+ Zolimidine
Reserpine 5 mg kg^{-1} i.p. (6)	0·54 ± 0·04	0·64 ± 0·03 +18%
Pyloric ligation (12)	0·55 ± 0·03	0·63 ± 0·08 +15%
Cold-restraint stress (8)	0·57 ± 0·06	0·90 ± 0·05 +59%*
Stress [chronic dosed] (8)	0·45 ± 0·02	0·61 ± 0·04 +37%*

* $P < 0·05$, *t*-test for mucus.

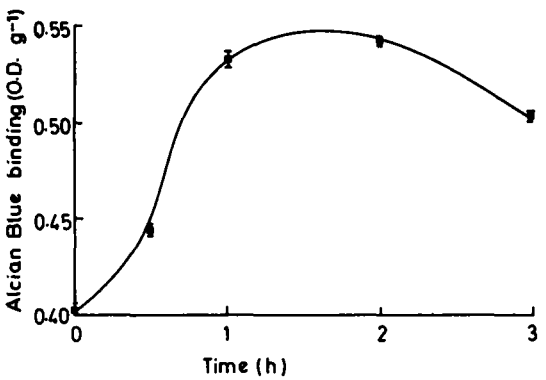

FIG. 1. Time course of the effects of CF 19415 (100 mg kg^{-1} oral) on alcian blue binding in fasted rats. Experimental details are given in the text.

Table 4. Effect of CF 19415 on gastric mucus levels in the cold-restraint stress test. CF 19415 was given 30 min before the start of the 2 ½ h stress period. In the chronic dosing experiment the final dose was given at this time. Other experimental details are given in the text.

Treatment	Alcian blue (OD g^{-1})	Gastric damage (% change)
Methyl cellulose (oral)	0·380 ± 0·018	
CF 19415 (50 mg kg^{-1} oral)	0·469 ± 0·026 (+23%*)	−65*
70% propylene glycol (0·1 ml s.c.)	0·321 ± 0·021	
CF 19415 (100 mg kg^{-1} s.c.)	0·494 ± 0·035 (+54%*)	−88*
Methyl cellulose × 2 daily 4 days and once before the test.	0·320 ± 0·029	
Methyl cellulose × 2 daily 4 days and CF 19415 (100 mg kg^{-1} oral) once before test.	0·531 ± 0·031 (+66%*)	−100*
CF 19415 100 mg kg^{-1} oral × 2 daily 4 days and once before test.	0·415 ± 0·027 (+30%*)	−88*

DISCUSSION

The alcian blue technique showed an uneven distribution of mucus in the rat stomach with least in the rumen and most in the antrum. The high level in the antrum agrees with Menguy (1969).

Dekanski et al (1975) have shown that starvation lowers the rate of synthesis of mucus and that this is further lowered by stress. In our experiments the mucus lining of the stomach was not reduced by starvation, but it was by the additional stress factor. It may be that the duration of fasting in our experiments was too short for the effect on the rate of mucus synthesis to be seen in the quantity of extracellular mucus lining the stomach. Mucus was also lowered in two other models of gastric damage, the reserpine test and the pyloric ligated rat, although in the latter test the change in mucus was seen at a time too short for actual erosions to occur. These results may indicate a role of mucus in preventing gastric damage. However, indomethacin, despite causing gastric damage, had, at most, a slight effect on mucus—a result that conflicts with the reported reduction in mucus synthesis produced by indomethacin (Parke et al 1975). Factors such as back diffusion of hydrogen ions (Cooke 1976) and blood flow effects (Main & Whittle 1975) are reported to be important in damage produced by anti-inflammatory agents and mucus changes may have a lesser role to play.

Salbutamol is reported to inhibit gastric damage (Fielding et al 1977) and β-agonists have been shown to stimulate mucus secretion in the respiratory system of the cat (Gallagher et al 1975). It was therefore possible that salbutamol could stimulate gastric mucus secretion in the rat and this might contribute to its anti-ulcer effects. However, no effects on gastric mucus secretion were observed. This agrees with the reported lack of effect of adrenaline on gastric mucus secretion (Menguy & Thompson 1967). Gastric mucus was, however, increased by the anti-ulcer drug carbenoxolone which is in agreement with its reported effects on mucus synthesis (Dekanski et al 1975).

Zolimidine has been reported to inhibit gastric ulceration and to stimulate mucus production (Murmann et al 1974a,b). However, we found that mucus was increased by zolimidine only where it had been lowered by procedures such as cold-restraint stress. Thus it would seem that zolimidine's main action on mucus is to protect the mucus layer from depletion by a challenge. A similar normalizing action on the gastric mucosa has been described for carbenoxolone (Croft et al 1977), though in our studies carbenoxolone was active in starved rats where mucus had not been depleted.

CF 19415 is one of a novel series of compounds which inhibit gastric ulceration in animal models (Van Zorge et al 1980). As well as the mucostimulant properties of CF 19415 described here, the compound has also been shown to inhibit gastric acid secretion in rats and dogs (Van Zorge et al 1980) and as such has a novel and potentially useful spectrum of activity. The mechanism whereby CF19415 stimulates mucus secretion is unknown but it is unlikely to be due to a local effect on the gastric mucosa as the compound was as active when given subcutaneously as it was when given orally. It is also unlikely that the compound merely releases preformed mucus from the goblet cells as it retains significant activity after 5 days of dosing. However, it is possible that 5 days treatment is not long enough to fully deplete mucus stored in the goblet cells and glands.

The stimulation of mucus associated with inhibition of damage, as found with compounds like zolimidine and CF19415, was not a consequence of damage inhibition because metiamide, which acts by inhibiting acid secretion, inhibited damage in the stress test, but had no effect on mucus.

The quantitative determination of mucus adherent to the gastric mucosa is difficult. The dye binding technique of Corne et al (1974) as described here, provides a way of overcoming this problem and has potential use in elucidating mechanisms of action of drugs that inhibit gastric damage.

The help of Miss L. Ball is gratefully acknowledged.

REFERENCES

Cooke, A. R. (1976) Dig. Dis. 21: 155–164

Corne, S. J., Morrissey, S. M., Woods, R. J. (1974) J. Physiol. (London) 242: 116p–117p

Croft, D. N., Domschke, W., Domschke, S., Hagel, J., Demling, L. (1977) in: Elstein, M., Parke, D. V. (eds) Mucus in Health and Diseases. Plenum Press, New York, pp 39–50

Dekanski, J. B., MacDonald, A., Sacra, P., Parke, D. V. (1975) Br. J. Pharmacol. 55: 387–392

Domschke, W., Domschke, S., Classen, M., Demling, L. (1972) Scand. J. Gastroenterol. 7: 647–651

Fielding, L. P., Raute, M., Curwain, B. P. (1977) Eur. Surg. Res. 9: 253–257

Gallagher, J. P., Kent, P. W., Passatore, M., Phipps, R. J., Richardson, P. S. (1975) Proc. Roy. Soc. London B. 192: 49–76

Main, I. H. M., Whittle, B. J. R. (1975) Br. J. Pharmacol. 47: 666p

McCloy, R. F., Dawson, V. A., Baron, J. H. (1976) Gut 17: 830

Menguy, R. (1969) Am. J. Surg. 117: 806–812

Menguy, R., Thompson, A. E. (1967) Ann. N.Y. Acad. Sci. 140: 797–803

Murmann, W., Carminati, G. M., Cattaneo, R. (1974a) Panminerva Med. 16: 347–359

Murmann, W., Carminati, G. M., Cattaneo, R. (1974b) Ibid. 16: 301–320

Parke, D. V., Lindup, W. E., Shillingford, J. S. (1975) Gut 16: 396

Pearse, A. G. C. (1968) Histochemistry Theoretical and Applied, Vol. 1, J. A. Churchill, London

Shay, H., Komarov, S. A., Meranze, D., Siplet, H. A. (1945) Gastroenterology 5: 43–61

Van Zorge, J. A., Welle, H. B. A., Turner, D. H., Green, A. P., Terrey, J. P. (1980) presented at VII Int. Symp. Med. Chem., Torremolinos, Spain, Sept. 1980

Wise, L., Ballinger, W. F. (1970) Ann. Surg. 171: 229–235