J. Pharm. Pharmac., 1977, 29, 748–751 Received July 4, 1977

Effect of drugs on rats exposed to cold–restraint stress

DOREEN BEATTIE

Institute of Medical Research, Wyeth Laboratories, Huntercombe Lane South, Taplow, Maidenhead, Berkshire SL6 0PH U.K.

The extent of protection afforded by over 40 drugs of various pharmacological groups against the development of gastric erosions induced in rats by a combination of cold and restraint has been investigated. Some drugs offered good protection, others had no effect on, or exacerbated, the lesions. Attempts are made to explain the mode of action of the protective drugs.

To identify drugs potentially useful in peptic ulcer therapy it is necessary to measure their protecting effect in a suitable model. The stress-induced gastric erosion test in rats is such a system. This paper describes the effect of drugs on the production of acute gastric mucosal erosions in rats by a combination of cold and restraint, two stresses that have been shown to act synergistically (Senay & Levine, 1967).

The 3 h cold–restraint model has not featured in the literature as widely as has the 24 h restraint model (Brodie & Hanson, 1960) and the etiology of the induced erosions is not fully understood. In the course of using this model for routine screening, it became apparent that whereas all compounds that inhibited gastric acid secretion protected against the development of stress erosions, some compounds having no effect on gastric secretion were also able to reduce or abolish the incidence of gastric mucosal erosions. This suggests there is more than one mechanism whereby drugs can prevent the development of cold–restraint-induced gastric mucosal damage.

METHODS

The method was based on that described by Senay & Levine (1967). Male Charles River rats, 80–100 g, fasted overnight, but having free access to water, were dosed orally, either with 0·3% carboxymethylcellulose (CMC) in 0·9% saline, or with the test drug in CMC–saline vehicle, in a volume of 10 ml kg^{-1} body weight. Where it was known that the drug would not be absorbed orally, the drug was administered by a more appropriate route. Each drug was tested at a dose equal to, or above, that which would be expected to produce the predominant pharmacological effect characteristic of its classification.

Immediately after dosing, the rats were inserted in aluminium open wire restraint cages, 4·2 cm diameter, 12·7 cm length, and placed in a cold room ($4° \pm 1°$). After 3 h the rats were killed, their stomachs removed and opened along the greater curvature and the severity of mucosal erosions was assessed on a 0–6 scale*.

The possible inaccuracy inherent in this subjective assessment was minimized by ensuring that the lesions were always scored by the same person. The results were analysed using Student's *t*-test and the percentage inhibition of erosions was calculated:

$$\frac{\text{Mean score control—Mean score test}}{\text{Mean score control}} \times 100$$

RESULTS

More than 40 drugs were tested for their ability to protect against cold–restraint-induced gastric erosions. The results are shown in Table 1. Where a drug was tested at more than one dose, the lowest dose at which it showed statistically significant protective activity is given.

Clearly, some classes of drugs, notably cholinolytics, α-adrenoceptor blockers, antidepressants and some 'mucosal protectants' afforded good protection, while other classes, including adrenergic neuron blockers, β-adrenoceptor blockers, major and minor tranquillizers, local anaesthetics and antihistamines of both H_1- and H_2-blocking types were ineffective. Certain drugs, such as non-steroidal anti-inflammatory compounds, bile salts and urea, significantly increased the severity of mucosal erosions.

DISCUSSION

It proved difficult to determine conclusively the mode of action of drugs that protect in the model used, since many have peripheral pharmacological properties that could contribute to their overall protective

* Score 0, no visible erosions or blood; 1, blood in stomach, or a single pinpoint erosion; 2, several pinpoint erosions, or one intermediate erosion; 3, several intermediate, or one large erosion; 4, 5, 6, progressively greater mucosal erosion severity.

Table 1. *Effect of different classes of drugs on the development of cold-restraint-induced gastric erosions.*

Class and compounds		Dose (mg kg^{-1}, orally)	% inhibition
Adrenergic neuron blockers	Bethanidine	100	53% N.S.
	Guanethidine	100	23% N.S.
α-Adrenoceptor blockers	Indoramin	30	67%*
	Phenoxybenzamine	30	60% N.S.
Anabolic steroids	Ethylestrenol	10	80%*
Antacids	Magnesium trisilicate	2 g kg^{-1}	65%*
	Sodium bicarbonate	2 g kg^{-1}	91%**
Cholinolytics	Ambutonium bromide	10	67%*
	Atropine sulphate	0·3	68%**
Antidepressants	Imipramine	3	72%**
	Amphetamine	10	58%*
	Iproniazid	100	−29% N.S.
	Caffeine	100	−100%*
Antihistamines H_1-receptor blockers	Mepyramine	30	19% N.S.
H_2 receptor blockers	Burimamide	100	53% N.S.
	Cimetidine	100	36% N.S.
	Metiamide	100	50% N.S.
Anti-5-HT agents	Cyproheptadine	30 s.c.	67%**
	Methysergide	100	−75%*
Barbiturates	Amylobarbitone	50	19% N.S.
	Hexobarbitone	100	13% N.S.
	Pentobarbitone	30 i.p.	33% N.S.
	Phenobarbitone	50	25% N.S.
β-Adrenoceptor blockers	Practolol	10	−73%*
	Propranolol	10	20% N.S.
Ganglion blockers	Hexamethonium	30 i.m.	57%*
	Mecamylamine	5	88%*
Local anaesthetics	Lignocaine	10	38% N.S.
	Procainamide	30	29% N.S.
Major tranquillizers	Reserpine	2·5 s.c.	16% N.S.
	Chlorpromazine	10	33% N.S.
Minor tranquillizers	Meprobamate	150	20% N.S.
	Lorazepam	3	18% N.S.
	Oxazepam	30	32% N.S.
'Mucosal barrier breakers'	Sodium taurocholate	500	−47%*
	Urea	60 i.v.	−32% N.S.
'Mucosal protectants'	Carbenoxolone	50	47% N.S.
	Castor oil	10 ml kg^{-1} 10%	52% N.S.
	Olive oil	10 ml kg^{-1} 10%	80%*
Narcotic analgesic	Morphine	3	91%**
Non-steroidal anti-inflammatories	Aspirin	100	−22% N.S.
	Indomethacin	10	−75%*
	Phenylbutazone	100	−50%*

* $P < 0.05$, ** $P < 0.01$.
N.S. not statistically significant, $P > 0.05$.

effect and some pharmacological features may be common to protective and non-protective drugs.

If the presence of gastric acid is considered to be an essential contributory factor in the development of stress erosions, then the protective effect of cholinolytic compounds is predictable since they abolish vagal activity. Basal gastric acid secretion, which is produced at high concentrations in the rat, is under a high degree of vagal control, and in stress this is more pronounced (Francois & Sines, 1961) so that the protective activity of cholinolytics is to be expected. Other drugs, including antihistamines, some α-adrenoceptor blocking drugs and tricyclic antidepressants, have a measure of cholinolytic activity so their degree of effectiveness in the model used may reflect the relative potency of their cholinolytic activity.

Antihistamines, of the H_1-receptor blocking type, were ineffective against the development of stress erosions. H_1-receptor blockers, such as mepyramine, oppose the effects of histamine on smooth muscle and blood vessels, but do not prevent the gastric secretory response, and only partially antagonize the effects of histamine released from mast cells. This, coupled with the fact that mepyramine has only weak cholinolytic activity, probably explains its lack of a protective effect. A second type of histamine receptor, H_2, not mepyramine-sensitive, controls gastric secretion (Black, Duncan & others, 1972), therefore the H_2-blocking compounds, burimamide, metiamide and cimetidine might be expected to protect against cold-restraint-induced erosions by inhibiting the production of acid secretion. However, they are not very effective in inhibiting basal acid secretion in rats, which probably limits their protective activity against the development of cold-restraint-induced erosions in this species. The relative inactivity of burimamide when given to rats by mouth is a feature of the drug (Black, Duncan & others, 1973).

Local anaesthetics, given by mouth, showed no protective activity. They may not be well-absorbed orally, or may not act on the superficial mucosa.

α-Adrenoceptors predominate in the blood vessels of the stomach wall, and α-adrenoceptor blocking drugs inhibit the vasoconstrictor actions and other excitatory responses of smooth muscle to catecholamines. The protective action is therefore in accordance with the theory that cold-restraint-induced erosions are largely due to a vascular disturbance in the gastric mucosa (Guth & Kozbur, 1969). If cold-restraint causes a localized ischaemia (Guth & Hall, 1966) then a drug that causes vasodilation might be expected to prevent the ischaemia and subsequent gastric damage. β-Adrenoceptor blocking drugs showed no significant protective activity against cold-restraint-induced erosions.

The protective activity of cyproheptadine, a 5-HT antagonist, suggests the involvement of 5-HT in the development of cold-restraint-induced erosions. In the rat there is a high mast cell population in the gastric mucosa and when mast cell degranulation occurs, as in conditions of restraint (Guth & Hall, 1966), 5-HT is released. 5-HT may play a part in the mechanism of ulcerogenesis, since it is ulcerogenic in its own right (Blackman, Campion & Fastier, 1959). However, the marked ulcerogenic effect of methysergide in this test sheds doubt on the definitive role of

5-HT antagonists in the formation of stress-induced erosions.

Adrenergic neuron blocking drugs act at the sympathetic neuron, therefore their blocking action does not discriminate between nerve-endings at α- and β-receptor sites. Their lack of protective activity against cold–restraint-induced erosions supports the proposal of Francois & Sines (1961) that a predominance of vagal activity occurs in cold–restraint. However, the fact that α-adrenoceptor blocking drugs afford protection indicates that sympathetic activity does have a subtle involvement in the model used.

Ganglion blocking drugs which cause both sympathetic and parasympathetic blockade showed good protective activity. In unpublished observations, mecamylamine, 5 mg kg^{-1} intraduodenally, caused a 77% inhibition of gastric secretion in the pylorus-ligated rat. Since the involvement of the parasympathetic nervous system would seem to be more important than that of the sympathetic nervous system in the etiology of stress-induced erosions, it may be valid to attribute the protective activity of ganglion blockers to their action on the cholinergic system.

Several drugs act locally on the gastric mucosa. So-called 'mucosal protectants', such as olive oil and castor oil, showed good protective activity. These presumably act by providing a 'coating' to the stomach, protecting it from acid. Although, acid is not thought to be the prime factor in the development of stress erosions, gastric lesions apparently cannot form in its absence, since sodium bicarbonate and magnesium trisilicate, which neutralize free acid, showed good activity.

Bile salts, and urea, caused a marked increase in the severity of the erosions. This agrees with the findings of Davenport (1968) who showed that both bile salts and urea cause a breakdown of the gastric mucosal 'barrier'. Non-steroidal anti-inflammatory agents, such as phenylbutazone and indomethacin, caused a significantly higher incidence of stress erosions, and these drugs may be grouped with bile salts and urea as being 'mucosal irritants' which challenge the integrity of the mucosal barrier. Phenylbutazone is though to act by causing direct damage to the parietal cell (Segal, 1960) and indomethacin by altering mucus secretion (Menguy & Desbaillets, 1967) but in both cases the normal protective action of the mucosa is impaired, and becomes more susceptible to the erosive effect of acid and pepsin.

Of the centrally acting drugs, barbiturates, given at sedative doses, offered no protection. In unpublished studies, it was found that barbiturates given at general anaesthetic doses, and other general anaesthetic agents (fluothane and urethane) prevented the development of cold-restraint-induced erosions. Their protective activity can probably be attributed to the fact that they cause a marked depression of gastric acid secretion (unpublished observation in pylorus-ligated rats).

The lack of protective activity shown by benzodiazepines in this test may be seen as evidence against the existence of an 'anxiety' component in the development of stress erosions in rats. Similarly chlorpromazine, a powerful tranquillizer with anxiolytic properties, was not active in this test situation, although Takagi, Kasuya & Watanabe (1964) found it protected against 20 h water-immersion stress-induced erosions. Presumably, the individual peripheral features of the drug are not sufficiently powerful to afford protection against cold–restraint-induced erosions. Chlorpromazine and reserpine both cause hypothermia, but there is no correlation between fall in body temperature and severity of gastric erosions (Beattie, 1975).

Imipramine and amphetamine showed good activity against cold–restraint-induced erosions. Imipramine has various peripheral pharmacological features, including cholinolytic activity, which may contribute to its protective action and it also prevents peripheral uptake of catecholamines. This may be taken as further evidence that the development of cold–restraint-induced erosions is predominantly a peripheral phenomenon. Amphetamine, a powerful cns stimulant, protects against gastric erosions and like imipramine, is an uptake inhibitor affecting noradrenaline release. It is possible that, by a central mechanism, amphetamine affects sympathetic discharge to a degree sufficient to over-ride excessive vagal drive.

The lack of protective activity seen with sedatives and tranquillizers, and the predominantly peripheral activity seen with tricyclic antidepressants, are strong challenges to the concept that central effects may be important in the development of stress erosions. However, morphine sulphate showed very good activity in protecting against cold–restraint-induced erosions. It has widespread central and peripheral actions; it causes cns depression and in man it reduces fear and anxiety. It reduces noradrenaline release, and blocks the action of 5-HT at some peripheral receptors; it also inhibits acetylcholine hydrolysis and reduces acetylcholine release from some nerve endings.

Thus while many drugs tested have peripheral features such as α-blocking, cholinolytic and anti-histamine activity, it is probable that there is more than one mechanism of protection.

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