Pharmacology Biochemistry & Behavior, Vol. 25, pp. 775–779, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Effects of Cold-Restraint Stress on Gastric Ulceration and Motility in Rats

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Received 2 May 1986

KOO, M. W. L., C. H. CHO AND C. W. OGLE. *Effects of cold-restraint stress on gastric ulceration and motility in rats.* PHARMACOL BIOCHEM BEHAV **25**(4) 775–779, 1986.—The effects of stress by restraint at 4°C for 2 hr and of drug treatment on gastric lesion formation and motor activities (contraction frequency, amplitude and tone) were studied in rats. Restraint at room temperature (22°C) produced a small ulcer index in the controls and did not significantly affect gastric motor activities; atropine and verapamil reduced but bethanechol increased gastric contractions under the same experimental conditions. Restraint at 4°C markedly elevated the ulcer index. The frequency of gastric contractions was significantly increased in the first hr but the amplitude was depressed during the whole 2-hr observation period. Gastric tone initially fell but rose in the second hr of cold-restraint stress. Atropine and verapamil pretreatment prevented stress-induced ulcer formation and suppressed the frequency and amplitude of gastric contraction. Bethanechol stimulated both frequency and amplitude without significantly influencing stress ulcer size. It is unlikely that gastric hypermotility plays a major role in stress ulceration; the stomach smooth muscle-relaxing action of atropine and verapamil may contribute only partly to their antiulcer effects.

Stress Gastric motility Gastric ulceration Atropine Bethanechol Verapamil

COLD-RESTRAINT stress produces triphasic responses in gastric emptying of solids [12]. These effects could be related directly to changes in gastric motility caused by stress. Stress ulceration is thought to be partly due to gastric hypermotility caused by vagal overactivation [8] and by calcium fluxes during muscular excitation-contraction coupling [17]. However, the relationship between gastric motility and ulcer formation is still unclear. This communication reports the results of a study on the influence of cholinergic drugs and a calcium channel blocker on stress-induced gastric ulceration and motility.

METHOD

General

It has been reported that there is no sex difference in the production of stress ulceration in rat stomachs [14]; thus, only female Sprague-Dawley rats (weighing 200–250 g) were used in the present study. They were housed in a room with controlled temperature (22±1°C) and humidity (65–70%); the animals were fed a standard laboratory diet (Ralston Purina Company) and drank tap water. All rats were starved, for 48 hr before use, in cages with raised wire mesh floors to prevent coprophagy; drinking fluid, comprising 8% sucrose in 0.2% NaCl w/v, was allowed ad lib but was withheld 1 hr before experimentation [4]. The animals were initially restrained at room temperature (22°C) in individual cylindrical wire mesh cages for 4 hr; this was the stability period preceding measurement of gastric contractile activities. One group, acting as controls, continued to be restrained in the same cylindrical cage at 22°C for 2 hr; the other batch was similarly restrained but was exposed to 4°C for the same period of time [19]. Gastric motor activities were measured during this 2-hr period. Both groups of animals were then killed and their ulcer indices determined. Drugs were administered intraperitoneally (IP) 30 min before stress.

Measurement of Gastric Mucosal Lesions

The animals were killed by a sharp blow on the head at the end of the experiments. Their stomachs were removed, opened along the greater curvature and examined under an illuminated magnifier (×3). Each lesion was measured along its greatest length; in the case of petechiae, five of these were taken as the equivalent of a 1 mm ulcer. The sum of the lesion lengths in each group of animals was divided by its number and expressed as the mean ulcer index [3].

Evaluation of Gastric Motility

The rats were lightly anaesthetised with diethylether and laparotomy performed. The stomach was exposed and a small incision made in the forestomach, through which a deflated air balloon attached to a polyethylene tube was inserted into the organ. The free end of the tube was then exteriorised through a stab incision in the left flank and connected to a water mannometer which was attached to a

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TABLE 1

EFFECTS OF DRUG PRETREATMENT (GIVEN IP 30 MIN BEFOREHAND) ON GASTRIC ULCERATION IN COLD-RESTRAINT STRESSED RATS

Pretreatment	Dose	Glandular mucosal ulcer index (mm)
A. Restrained at 22° C for 2 hr (after 4 hr of restraint at 22° C)		
Saline	2.0 ml/kg	0.02 ± 0.01
Atropine	0.3 mg/kg	0.04 ± 0.03
Verapamil	4.0 mg/kg	0.03 ± 0.02
Bethanechol	1.2 mg/kg	0.02 ± 0.01
B. Restrained at 4° C for 2 hr (after 4 hr of restraint at 22° C)		
Saline	2.0 ml/kg	5.86 ± 0.65*
Atropine	0.3 mg/kg	0.38 ± 0.33††
Verapamil	4.0 mg/kg	3.01 ± 0.42*†
Bethanechol	1.2 mg/kg	6.31 ± 0.79*

The values are the means ± S.E.M. of 10 rats. *$p<0.001$ when compared with the corresponding group in A. †$p<0.01$, ††$p<0.001$ when compared with its own saline-pretreated group.

Statham pressure transducer (Statham Model P23 BB). The laparotomy incision was closed and the rat immediately put into a tubular wire mesh restraint cage. The gastric balloon was then inflated with air to a pressure of 5 cm water and stomach motility recorded on a physiograph (E & M Instrument Co.). The contraction frequency, amplitude (the height of contraction in cm H_2O) and tone (intragastric pressure in cm H_2O) were measured for 5 min at a fixed time during the 2-hr observation period (i.e., at 0, 0.5, 1, 1.5 and 2 hr subsequent to the 4-hr stability period).

Drugs

Atropine sulfate (Laboratories Lacroix) 0.3 mg/kg, verapamil hydrochloride (Knoll) 4.0 mg/kg and bethanechol chloride (Merck Sharp and Dohme) 1.2 mg/kg (all doses expressed as their salts) were dissolved in 0.9% w/v NaCl solution (saline) immediately before IP injection. A similar volume of saline (2 ml/kg) was given by the same route to the controls.

Statistical Analysis

All values were expressed as means±S.E.M. Differences were analysed for statistical significance, using the unpaired two-tailed Student *t*-test.

RESULTS

Restraint at 22°C for the whole experimental period of 6 hr produced a small ulcer index in both the saline- and drug-treated groups (Table 1A). Restraint at 22°C for 4 hr and then stress at 4°C for a further 2 hr produced severe haemorrhagic ulceration in the gastric glandular mucosa. Both atropine and verapamil, but not bethanechol, pretreatment prevented stress-induced ulcer formation (Table 1B).

Table 2 shows the gastric motor activities in rats restrained at 22°C for 6 hr. Gastric motility was increased 30 min after balloon insertion, but it was stable by the fourth hr after operation (Table 2). The experimental period between the fourth and sixth hr was, therefore, used to evaluate the influence of cold-restraint stress and of drug treatment on gastric motor activities. In the saline-pretreated group exposed to 22°C, the frequency and amplitude of gastric contractions were stable during this 2-hr experimental period (Tables 3A, 4A). Atropine and verapamil lowered, but bethanechol increased, gastric motility (Tables 3A, 4A). Stress significantly raised the frequency of gastric contraction in the first hr. This increased frequency then subsided, returning to a basal level at 1.5 hr, but activity continued to fall and was below normal at the end of the 2 hr stress period (Table 3B). The contraction amplitude was markedly depressed during the 2-hr observation period (Table 4B). Both atropine and verapamil decreased the frequency and amplitude of gastric contractions, but bethanechol significantly stimulated these two parameters (Tables 3B, 4B). The gastric tone was stable when the rats were restrained at 22°C; drug treatment also showed a similar effect (Table 5A). When the rats were exposed to cold-restraint stress, gastric tone immediately fell to a significantly low level (data not shown), but it returned to a basal level a few minutes after commencing stress so that a normal value was obtained at 30 min. The gastric tone at the second hr after stress was found to be significantly increased. Drug treatment did not significantly alter this pattern of changes in the gastric tone of stressed rats (Table 5B).

DISCUSSION

Cold-restraint stress stimulated stomach contraction frequency in the first hr, this returned to a basal level 1.5 hr later but became depressed in the second hr. The pattern of motility changes closely paralleled the triphasic responses found with gastric emptying of solids in stressed rats [12]. Thus, the present experiment provides further evidence that emptying of solids from the stomach is indeed controlled by its contraction frequency. It has been reported that restraint at normal room temperature for as long as 6 hr does not produce a significant increase in ulceration [20]. Restraint for 6 hr evoked a low ulcer index (Table 1), and this finding is in accord with that of others [18,20].

The cholinergic control of gastric motility was demonstrated by the suppressive action of atropine and the stimulating effect of bethanechol on stomach motor activity. As cholinergic stimulation facilitates calcium influx through slow channels to trigger off muscular contraction [6,11], the ability of verapamil, a calcium channel blocker, to depress gastric motility, therefore, adds further support to the deductions from atropine and bethanechol.

Cold-restraint stress lowered the amplitude of gastric contractions. The mechanism for this action remains unclear, but it could be due to the hypothermia occurring during cold-restraint stress [12]. The gastric tone was also affected by the same experimental conditions. Cold-restraint stress caused an immediate fall in intragastric pressure, but it returned to a basal value by 30 min. It is likely that the vago-vagal reflex could have contributed partly to this effect [10]. The stimulation of nonadrenergic and noncholinergic vagal fibers could result in decreased intragastric pressure through the actions of ATP [1], bradykinin [7] or 5-HT [2]. Reduced pressure may also be due to adrenaline and/or noradrenaline [5] relaxing the gastric musculature by β-adrenoceptor activation [9,16].

TABLE 2

EFFECTS OF ACUTE OPERATION ON GASTRIC MOTOR ACTIVITIES (RESTRAINED AT 22° C)

Motor activities	Time after operation (hr) 0.5	1	2	3	4	5	6
Frequency (contractions/hr)	161.3 ± 8.7	136.3 ± 8.6	83.8 ± 7.2***	63.8 ± 3.5*	57.5 ± 4.1	61.3 ± 3.3	56.3 ± 3.5
Amplitude (cm H_2O)	2.1 ± 0.4	1.5 ± 0.3	1.6 ± 0.3	2.2 ± 0.1	1.9 ± 0.1	2.0 ± 0.2	1.7 ± 0.3
Tone (cm H_2O)	6.4 ± 0.2	5.6 ± 0.1***	5.0 ± 0.2**	4.8 ± 0.3	5.2 ± 0.2	4.9 ± 0.1	4.7 ± 0.2

The values are the means ± S.E.M. of 6 rats. *$p<0.05$, **$p<0.02$, ***$p<0.01$ when compared with the preceding value of the same motor activity.

TABLE 3

EFFECTS OF DRUG PRETREATMENT (GIVEN IP 30 MIN BEFOREHAND) ON GASTRIC CONTRACTION FREQUENCY (NUMBER OF CONTRACTIONS/HR) IN COLD-RESTRAINT STRESSED RATS

A. Restrained at 22° C for 2 hr (after 4 hr of restraint at 22° C)						
Saline	2.0 ml/kg	56.6 ± 2.9	58.8 ± 5.4	55.0 ± 6.6	63.8 ± 5.1	64.5 ± 3.0
Atropine	0.3 mg/kg	25.7 ± 2.1††††	29.1 ± 3.9†††	24.8 ± 2.5†††	25.0 ± 1.7††††	23.5 ± 2.1††††
Verapamil	4.0 mg/kg	45.7 ± 5.9	37.8 ± 5.3††	37.5 ± 4.2†	38.3 ± 3.5†††	46.3 ± 2.5††††
Bethanechol	1.2 mg/kg	196.2 ± 7.5††††	206.3 ± 9.2††††	135.0 ± 8.9††††	118.8 ± 9.3††††	88.8 ± 5.8††††
B. Restrained at 4° C for 2 hr (after 4 hr of restraint at 22° C)						
Saline	2.0 ml/kg	62.3 ± 1.6	112.5 ± 8.8****	82.6 ± 8.2*	73.8 ± 4.9	45.7 ± 7.9*
Atropine	0.3 mg/kg	25.6 ± 2.7††††	13.8 ± 5.8††††	0****††††	0****††††	0****††††
Verapamil	4.0 mg/kg	41.3 ± 6.5†††	53.8 ± 5.8††††	46.3 ± 6.5†††	0****††††	0****††††
Bethanechol	1.2 mg/kg	182.5 ± 12.1††††	218.8 ± 4.9††††	138.8 ± 8.2††††	74.9 ± 6.0***	67.5 ± 4.2**†

The values are the means ± S.E.M. of 6 rats.
*$p<0.05$, **$p<0.02$, ***$p<0.01$, ****$p<0.001$ when compared with the corresponding group in A.
†$p<0.05$, ††$p<0.02$, †††$p<0.01$, ††††$p<0.001$ when compared with its own saline-pretreated group.

TABLE 4

EFFECTS OF DRUG PRETREATMENT (GIVEN IP 30 MIN BEFOREHAND) ON GASTRIC CONTRACTION AMPLITUDE (CM H_2O) IN COLD-RESTRAINT STRESSED RATS

Pretreatment	Dose	Time (hr) 0	0.5	1	1.5	2
A. Restrained at 22° C for 2 hr (after 4 hr of restraint at 22° C)						
Saline	2.0 ml/kg	2.4 ± 0.3	1.9 ± 0.2	1.9 ± 0.2	2.1 ± 0.2	2.1 ± 0.3
Atropine	0.3 mg/kg	1.0 ± 0.1†††	0.7 ± 0.1†††	0.7 ± 0.1†††	0.8 ± 0.1†††	0.7 ± 0.1††
Verapamil	4.0 mg/kg	0.7 ± 0.1†††	0.6 ± 0.1††	0.5 ± 0.1†††	0.6 ± 0.1†††	0.5 ± 0.1††††
Bethanechol	1.2 mg/kg	2.4 ± 0.2	1.8 ± 0.3††††	2.9 ± 0.4†	2.0 ± 0.5	2.5 ± 0.3
B. Restrained at 4° C for 2 hr (after 4 hr of restraint at 22 ° C)						
Saline	2.0 ml/kg	2.0 ± 0.2	0.9 ± 0.1*	0.8 ± 0.1**	0.5 ± 0.1**	0.5 ± 0.1**
Atropine	0.3 mg/kg	0.9 ± 0.1†††	0.5 ± 0.1††	0****††††	0****††††	0****††††
Verapamil	4.0 mg/kg	0.8 ± 0.2††	0.5 ± 1.0††	0.5 ± 0.1†	0****††††	0****††††
Bethanechol	1.2 mg/kg	2.1 ± 0.4	1.7 ± 0.3†	1.8 ± 0.3††	1.1 ± 0.2†	0.6 ± 0.1**

The values are the means ± S.E.M. of 6 rats. *$p<0.01$, **$p<0.001$ when compared with the corresponding group in A.
†p0.05, ††$p<0.01$, †††$p<0.001$ when compared with its own saline-pretreated group.

TABLE 5

EFFECTS OF DRUG PRETREATMENT (GIVEN IP 30 MIN BEFOREHAND) ON GASTRIC TONE (CM H_2O) IN COLD-RESTRAINT STRESSED RATS

Pretreatment	Dose	Time (hr) 0	0.5	1	1.5	2
A. Restrained at 22° C for 2 hr (after 4 hr of restraint at 22° C)						
Saline	2.0 ml/kg	4.8 ± 0.2	4.8 ± 0.3	5.3 ± 0.2	4.7 ± 0.3	4.8 ± 0.2
Atropine	0.3 mg/kg	5.2 ± 0.4	4.9 ± 0.2	4.6 ± 0.3	5.2 ± 0.2	5.1 ± 0.3
Verapamil	4.0 mg/kg	4.7 ± 0.1	5.1 ± 0.3	5.2 ± 0.2	4.7 ± 0.2	4.8 ± 0.3
Bethanechol	1.2 mg/kg	4.5 ± 0.2	5.3 ± 0.4	4.7 ± 0.4	5.6 ± 0.4	5.2 ± 0.1
B. Restrained at 4° C for 2 hr (after 4 hr of restraint at 22° C)						
Saline	2.0 ml/kg	5.0 ± 0.2	4.8 ± 0.3	5.4 ± 0.4	5.0 ± 0.4	5.9 ± 0.2**
Atropine	0.3 mg/kg	4.8 ± 0.4	4.8 ± 0.2	5.2 ± 0.2	5.3 ± 0.2	6.3 ± 0.3*
Verapamil	4.0 mg/kg	5.5 ± 0.3	5.2 ± 0.3	4.9 ± 0.1	4.8 ± 0.2	5.8 ± 0.1*
Bethanechol	1.2 mg/kg	4.7 ± 0.3	4.6 ± 0.3	5.5 ± 0.3	4.6 ± 0.1	6.2 ± 0.1***

The values are the means ± S.E.M. of 6 rats.
*$p<0.02$, **$p<0.01$, ***$p<0.001$ when compared with the corresponding group in A.

The relationship between gastric motility and ulceration is still unclear. Although cold-restraint stress markedly elevated the contraction frequency in the first hr, contractility was depressed in the second. A pilot study (unpublished findings) has already shown that the ulcerogenic potency of cold-restraint stress in the first hr (mean ulcer index = 2.84 mm) is nearly half that of 2-hr stress (mean ulcer index = 5.65 mm), thus, indicating that increased contraction frequency may contribute only partly to ulceration produced by 2 hr of cold-restraint stress. Atropine and verapamil lowered gastric motility and decreased ulceration; however, bethanechol treatment, which increased stomach contractions, did not aggrevate stress-induced ulcer formation. It was also found that the amplitude of gastric contractions was reduced throughout the 2 hr of stress. On the other hand, gastric tone, which was initially lowered, remained normal during most of this 2-hr experimental period. These findings reinforce the idea that gastric hypermotility is unlikely to contribute substantially to stress ulceration. Thus, the antiulcer effect of both atropine and verapamil could mainly be due to other actions, e.g., prevention of mast cell degranulation and preservation of mucus [13,15], in addition to their smooth muscle-relaxing property.

ACKNOWLEDGEMENT

The authors thank Dyechem Trading Company (Hong Kong) for the generous gift of verapamil.

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