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The Effect of Cold-Restraint Stress on Gastric Emptying in Rats

M.W.L. KOO, C.W. OGLE[1] AND C. H. CHO

Department of Pharmacology, Faculty of Medicine, University of Hong Kong, 5, Sassoon Road, Hong Kong

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KOO, M. W. L., C. W. OGLE AND C. H. CHO. *The effect of cold-restraint stress on gastric emptying in rats.* PHARMACOL BIOCHEM BEHAV 23(6) 969–972, 1985.—The effects of drug treatment and of cold-restraint stress (a method used to produce experimental stomach ulcers) on gastric emptying of a resin (colestipol-phenol red complex) were investigated in rats. Gastric emptying was decreased by intraperitoneal treatment with atropine (0.3 mg/kg) or verapamil (4 mg/kg), and enhanced by bethanechol (1.2 mg/kg). Stress by restraint at 4°C for 2 hr markedly reduced gastric emptying; the pattern of effects of drug pretreatment in these stressed rats was similar to that seen in their nonstressed controls. Further experiments, with stress for 3 hr, revealed that the gastric emptying rate was triphasic; increasing in the first hr, returning to normal and then slowing in the third hr of stress. Initial increase in emptying rate was probably due to predominant vagal overactivity. Hypothermia and possibly other factors induced by cold-restraint stress could have subsequently depressed gastric motility.

Stress Gastric emptying Vagal activity Hypothermia Verapamil

GASTRIC ulcer formation in rats, produced by the method of restraint at 4°C (stress) for 2 or 3 hr, is thought to be due substantially to stomach hypermotility [30,33] evoked by vagal overactivity [2,10]. There is evidence to suggest that vagal activity and smooth muscle contraction are dependent on calcium influx into the cells [5,25].

Since it is generally accepted that gastric emptying of liquid and solid food is controlled mainly by contraction of the proximal and distal stomach respectively [14], it is, therefore, likely that stress-induced muscle hypermotility may influence gastric emptying. The purpose of this study is to examine (1) the effects of cold-restraint stress on gastric emptying of a resin (colestipol-phenol red complex), (2) the effects of cholinergic drugs and an organic calcium-channel blocker on gastric emptying, and (3) the influence of cold-induced hypothermia on gastric emptying.

METHOD

General

Female Sprague-Dawley rats (200–250 g) were used. The animals were housed in an air-conditioned room in which the temperature (22±1°C) and relative humidity (65–70%) were kept constant. They were starved for 48 hr before use, in cages with raised wire mesh floors to prevent coprophagy. Fluid, consisting of 8% sucrose in 0.2% NaCl w/v, was allowed ad lib [3] until 1 hr before experimentation. Animals subjected to stress were placed in individual close-fitting cylindrical wire mesh cages and exposed to 4°C in a cold room for 2 or 3 hr [28]. Controls remained in their starvation cages, in the air-conditioned room, for a similar period of time.

Measurement of Rectal Temperature

Rectal temperature of both the controls and stressed rats was measured with a rectal thermometer (Testoterm Medical Instant Thermometer), and recorded at 30-min intervals.

Measurement of Gastric Emptying

The modified method of Kunihara and Meshi [17] was used to measure gastric emptying of a resin and a marker (phenol red). This complex, freshly made up before use, consisted of 0.1 g colestipol (Upjohn) and 1 g phenol red (Sigma) suspended in 1 ml distilled water. This suspension (1 ml/kg body weight) was administered orally into the stomach through a stainless steel tube which was removed immediately after the resin-marker complex was deposited intragastrically.

In one experiment, rats were given intraperitoneal injections of atropine (0.3 mg/kg), verapamil (4 mg/kg), bethanechol (1.2 mg/kg) or a solution of 0.9% NaCl w/v (saline; 2 ml/kg) which was used as a vehicle for the injected drugs, 15 min before intragastric delivery of the colestipol-phenol red complex. The animals were then exposed to stress for 2 hr and killed by a sharp blow on the head at the end of that period.

The second experiment consisted of intragastric administration of colestipol-phenol red complex before stressing the animals for 3 hr. Rectal temperature was monitored during this experiment; animals acting as controls were kept at room temperature, in loose-fitting cylindrical wire mesh cages in order to prevent unnecessary movements dislodging the rectal thermometers. Groups of animals were killed, by a blow on the head, at 30-min intervals.

[1]Requests for reprints should be addressed to Professor C. W. Ogle.

TABLE 1

EFFECTS OF DRUG TREATMENT ON GASTRIC RETENTION OF COLESTIPOL-PHENOL RED COMPLEX IN RATS KILLED AFTER STRESS FOR 2 HR

	Pretreatment (IP)	Dose	Percentage gastric retention
A.	No stress (unrestrained at 22 ± 1°C for 2 hr)		
	Saline	2.0 ml/kg	15.9 ± 2.8
	Atropine	0.3 mg/kg	30.9 ± 3.9§
	Bethanechol	1.2 mg/kg	5.4 ± 1.6§
	Verapamil	4.0 mg/kg	27.4 ± 4.1†
B.	Stress (restrained at 4°C for 2 hr)		
	Saline	2.0 ml/kg	31.9 ± 4.1*
	Atropine	0.3 mg/kg	58.7 ± 2.9‡¶
	Bethanechol	1.2 mg/kg	7.2 ± 1.7¶
	Verapamil	4.0 mg/kg	52.2 ± 5.3‡§

The values are means ± S.E.M. of 15 rats.

*$p<0.01$, ‡$p<0.001$ when compared with the corresponding controls in A.

†$p<0.02$, §$p<0.01$, ¶$p<0.001$ when compared with its own saline-pretreated control.

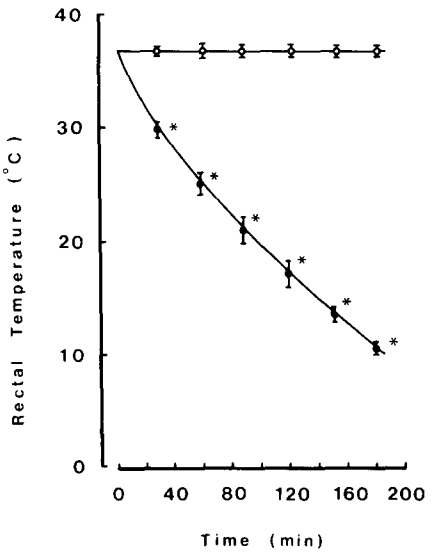

FIG. 1. Effects of cold-restraint stress on rectal temperature in rats. (○) Control, unrestrained at 22±1°C; (●) stress, restrained at 4°C. Vertical bars indicate means±S.E.M. of 5 rats, *$p<0.001$ when compared with the corresponding value in the control group.

The stomachs of all animals, immediately after killing, were clamped at the pylorus and cardiac ends before careful removal for extraction of the phenol red remaining in the organ. The amounts of dye extracted from the stomachs, including their contents, of experimental animals were expressed as a percentage of that measured in a group of reference rats killed immediately after intragastric delivery of the complex, i.e., percentage gastric retention.

Measurement of Phenol Red Concentration

The phenol red in the colestipol-phenol red complex was extracted according to the method of Kunihara and Meshi [17], with some modifications.

The excised stomach, clamped at both ends to prevent its contents escaping, was cut into small pieces before homogenisation in 10 ml HCl (1 M) with a microhomogeniser (Ultra-Turrax model D7813). The suspension was centrifuged at 6000 rpm for 5 min. One ml of the supernatant was added to a solution of 2 ml NaOH (1 M) and 3 ml chloroform, and shaken vigorously to remove lipids from the preparation; the mixture was then centrifuged at 4500 rpm for 20 min. One ml of the aqueous layer of this solution was added to 3 ml NaOH (1 M) and absorbance measured at a wavelength of 558 nm with a spectrophotometer (Varian, Cary 219 Spectrophotometer).

Drugs

Atropine sulphate (Laboratories Lacroix) 0.3 mg/kg, bethanechol chloride (MSD) 1.2 mg/kg and verapamil hydrochloride (Knoll) 4 mg/kg were dissolved in saline immediately before use and their weights expressed as salts. A comparable volume of saline, 2 ml/kg, was given to the controls.

Statistical Analysis

Student's *t*-test was used to evaluate the level of statistical significance. All values were expressed as means± S.E.M.

RESULTS

Cold-restraint stress delayed gastric emptying of the colestipol-phenol red complex in saline- or drug-pretreated animals (Table 1). Atropine pretreatment caused greater retention of the complex in both stress and nonstress conditions when compared with the saline-pretreated controls. Similar effects were seen in rats pretreated with verapamil.

Bethanechol hastened emptying of the complex from the stomachs of both stressed and nonstressed animals. Over 90% of the complex was expelled from the stomach 2 hr after administration of the cholinergic agent.

Verapamil pretreatment slowed gastric emptying of the complex. The amount of resin retention was greater in stress than in nonstress conditions. The effect of verapamil on gastric retention of the complex was not significantly different from that of the atropine-pretreated group.

The rectal temperature fell gradually and significantly when the rats were subjected to restraint stress at 4°C (Fig. 1). The rate of fall was fastest during the first 30 min. Control rats showed no significant change in rectal temperature throughout the 3-hr observation period.

Gastric emptying of saline-pretreated nonstressed rats followed first order kinetics, since a semi-log plot of percentage gastric retention against time showed a best-fit straight line passing through all the data points with a slope of −0.36 (Fig. 3). Cold-restraint stress evoked phasic changes in gastric emptying rate, being faster than the control during the first 30 min and slower during the last hr of stress (Fig. 2); three

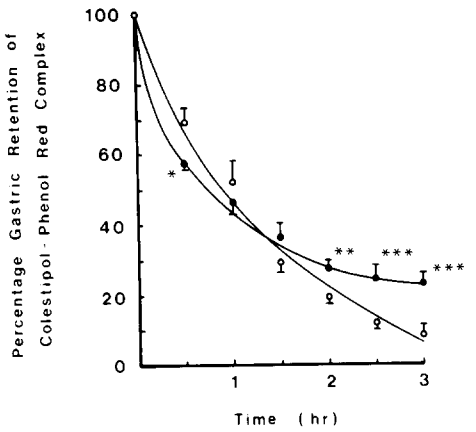

FIG. 2. Effects of cold-restraint stress on gastric emptying of colestipol-phenol red in rats. (○) Control, unrestrained at 22±1°C; (●) stress, restrained at 4°C. Vertical bars indicate means±S.E.M. of 12 rats. $*p<0.05$, $**p<0.02$, $***p<0.001$ when compared with the corresponding value in the control group.

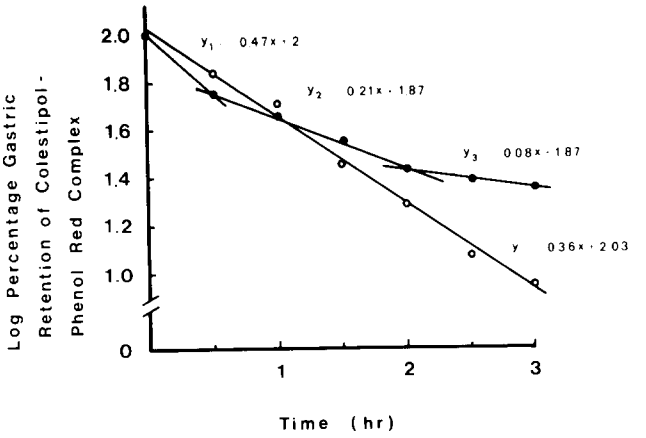

FIG. 3. Effects of cold-restraint stress on gastric emptying of colestipol-phenol red complex in rats. (○) Control, unrestrained at 22±1°C; (●) stress, restrained at 4°C. Y and Y_1–Y_3 are equations of lines of best fit for the control and stressed groups respectively.

straight lines with calculated slopes of −0.47, −0.21 and −0.08 (Fig. 3) were obtained in the semi-log plot.

DISCUSSION

In this study, colestipol-phenol red complex was chosen as a method for measuring the rate of gastric emptying because the preparation does not affect gastric motility, and is stable [17].

Atropine has been shown to inhibit [12], and carbachol to increase [18], gastric motility. In the present experiment, intraperitoneal injection of atropine 0.3 mg/kg slowed, whereas bethanechol 1.2 mg/kg increased, gastric emptying in both normal and stress conditions. These results indicate that gastric motility and emptying are indeed influenced by the cholinergic system.

Gastric emptying of colestipol-phenol red complex in nonstressed rats followed first order kinetics. This is in accord with the observations of Kunihara and Meshi [17]. These findings suggest that the rate of food emptying at any given time is proportional to the amount remaining in the stomach.

Rats subjected to cold-restraint stress at 4°C showed triphasic changes in emptying rate. The first and last phases of gastric emptying were significantly different from the controls, being faster and slower respectively, while no significant difference was observed in the intervening period.

The initial increase in gastric emptying could be the result of dominant vagal excitatory activity occurring immediately after onset of stress [2,10]. Decrease in gastric emptying rate, beginning in the second hr of stress, may be due to prolonged stimulation of the vagus nerve; this intensity of stimulation could activate the high-threshold nonadrenergic, noncholinergic vagal inhibitory efferent fibres which produce relaxation of the stomach [19,21]. The rectal temperature of the stressed animals was seen to fall continuously throughout the stress period (Fig. 1); the hypothermia could be the result of suppression of thermogenic shivering activity and lowering of metabolic rate during cold-restraint stress [29]. It is known that the catecholamines are released in increased amounts during stress [4,20]; these amines would constrict the blood vessels of the gastric tissue and, thus, interfere with blood flow [22]. This combination of hypothermia and anoxia would depress gastric electrical and mechanical activity [16], and so could suppress gastric emptying. The decrease in gastric emptying rate during the latter part of the experiment could also be due to the direct gastric smooth muscle-relaxing effects of catecholamines [32], and to the endorphins and enkephalins [6, 9, 23, 24]. These are concomitantly released when animals are stressed [4, 11, 20]. It is unlikely that restraint itself plays a major role in the observed changes in gastric emptying because immobilisation of rats in tubular close-fitting cages at room temperature for 2 hr [Koo, unpublished data], or up to 6 hr [33], does not significantly influence stomach motility measured by pressure changes in an intragastrically-placed water-filled rubber balloon.

The overall decrease in gastric emptying observed in this study is at variance with the findings of others who reported only an increase in gastric motility during stress [2,30]. This difference could be due to variations in stress intensity. The current findings indeed throw more light on past observations made in this laboratory where it was found that increased gastric emptying of amberlite pellets by stress, assumed to reflect increased stomach motility, was most evident when the experimental period lasted for 1 hr [26]; longer observation periods, however, gave conflicting results (Ogle, unpublished data) The latter findings are, therefore, likely to be due to stomach stasis occurring after the initial phase of increased contractility; during this phase of retention in the stressed animals, continued gastric emptying by the controls would reverse the relative differences seen between the two groups in the first hr. Thus, the rate of gastric emptying, when determined at the end of a given observation period, is only the sum-total of events occurring during that period; it cannot simply be taken as an absolute indicator of gastric motility because of the complexity of antral-duodenal interaction [31]. The present observations indicate that when the combination of restraint and cold is employed as a method for stressing rats to produce experimental gastric ulcers, the exposure time to immobilisation at 4°C should not exceed 2 hr because of severe generalised pathophysiological changes, due to hypothermia, occurring towards the end of this period.

It is known that calcium plays a vital role in mediating the excitation-contraction response [27] and in neurotransmitter release [5,13]. The contractions of vascular and gastric smooth muscles also depend on the availability of calcium [8,25]. Thus, verapamil, an organic calcium-channel blocker [7,15], could have decreased gastric emptying by inhibiting smooth muscle and vagal nerve calcium uptake [1]. It is possible that the inhibitory action of verapamil on stomach contractility may contribute to its ability to prevent stress-induced gastric ulceration [26]; this idea requires further study.

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