Pharmacology Biochemistry & Behavior, Vol. 33, pp. 563–566. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Does Acidosis Contribute to Stress-Induced Ulceration in Rat Stomachs?

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Received 19 December 1988

KOO, M. W. L., C. H. CHO AND C. W. OGLE. *Does acidosis contribute to stress-induced ulceration in rat stomachs?* PHARMACOL BIOCHEM BEHAV **33**(3) 563–566, 1989.—The present study examines the involvement of acidosis in stress ulceration in rat stomachs. Cold restraint stress for 2 hr did not affect the blood lactate level; however, it produced respiratory acidosis, as reflected by the depressed respiratory rate which was associated with increased CO_2 tension and a lowered blood pH. Severe hemorrhagic ulceration was found in the glandular mucosa. The effects of stress on blood pH and the stomach were reversed by IV infusion of $NaHCO_3$. Infusion of HCl IV decreased the blood pH and HCO_3^- level and produced gastric ulceration. It is concluded that respiratory acidosis could be involved in stress ulceration. The metabolic acidosis evoked by HCl also induced gastric damage, but the effect was much less.

Cold-restraint stress Gastric ulcers Acidosis $NaHCO_3$ HCl

COLD-RESTRAINT stress has profound effects on the stomach. It produces mucosal blood vessel engorgement (3,9), mast cell degranulation (4, 5, 15, 16) and hemorrhagic ulceration (6, 16, 18). However, the effects of cold-restraint stress on blood acid-base balance, possibly resulting in acidosis, have not been studied. These pathologic changes, if any, could be involved in stress-induced gastric ulceration because gastric acid secretion has been found to be affected by acidosis (1,11). Furthermore, administration of ammonium chloride, an acidifying agent, induces gastric ulceration in rats (13). This communication reports the results of a study on the adverse effects of cold-restraint stress on blood acid-base balance, whether the latter is metabolic or respiratory in origin and its relationship to ulcer formation in rat stomachs.

METHOD

General

Female Sprague-Dawley rats (200–220 g) were housed in an air-conditioned room in which the temperature (22 ± 1°C) and relative humidity (65–70%) were controlled. Solid food was removed 48 hr before experiments, but fluids, consisting of 8% sucrose (Sigma) in 0.2% NaCl (Sigma) w/v, were allowed ad lib until 1 hr before the animals were used. Rats subjected to cold-restraint stress (stress) were placed in individual close-fitting cylindrical wire mesh cages and exposed to 4°C for 2 hr (17). Control animals were put into individual restraint cages but left in the room where they were normally housed.

Blood Vessel Cannulation and Blood Acid Base Determination

Animals were operated upon 1 day before experimentation. A small mid-line incision was made on the ventral side of the neck of ether-anesthetised rats. The right carotid artery and the left jugular vein were identified. A polyethylene tube (0.7 mm i.d., 1 mm o.d.), prefilled with a heparinized (50 I.U./ml) solution of 0.9% NaCl w/v (saline), was used to cannulate the right carotid artery; the free end was made to emerge dorsally on the left side of the neck. Cannulation of the left jugular vein was done in the same manner, with the free end of the cannula drawn out through the right side. The open end of the cannula was sealed and the neck incision closed with a suture. The cannulae were then tied in place onto the skin at the back of the neck. Infusions of drug or saline were given IV, arterial cannulae were used to collect blood samples.

During experiments, the free end of the cannula was cut and flushed with heparinized saline. The escape of blood from the patent end was stopped by inserting a pin into the opening of the cannula. The rats were allowed a stabilization period of 1 hr inside their restraint cages before blood samples were taken for blood acid-base analyses. Samples were collected into heparinized capillary tubes and then immediately introduced into a gas analyzer (Gas check 938 AVL). Blood acid and base were measured at 0, 30, 60, 90 and 120 min during cold restraint.

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TABLE 1

EFFECT OF COLD-RESTRAINT STRESS ON RESPIRATORY RATE (NUMBER OF INHALATIONS/MIN)

	Time (hr) After Stress			
0	0.5	1	1.5	2
A. Restrained at 22°C for 2 hr				
161.3 ± 5.3	148.5 ± 6.4	139.3 ± 6.8	141.5 ± 8.4	146.7 ± 9.3
B. Restrained at 4°C for 2 hr				
165.2 ± 4.5	153.3 ± 7.1	117.4 ± 7.3*§	108.7 ± 7.7†§	102.7 ± 8.5‡§

Values are the means ± SEM of 9 rats in each group.
*$p<0.05$, †$p<0.02$, ‡$p<0.01$ when compared with the corresponding control in A.
§$p<0.001$ when compared with 0 hr in B.

$NaHCO_3$ (BDH) solution 8.4% w/v was infused IV with a Harvard infusion pump at a rate of 2.2 ml/hr during the 2-hr period of stress; HCl (BDH) 0.5 M was infused at the same rate to rats which were restrained at 22°C for 2 hr. Control animals were infused with saline at the same rate and period of time. Arterial blood samples were taken for blood acid-base analyses. All animals were killed 2 hr after infusions. The gastric luminal content was collected and the titratable acid determined by titration with NaOH (BDH) 0.01 M to pH 7.4, using an autotitration system (Radiometer, Model TTT80). Stomachs were examined for lesions, using an illuminated magnifying lens (3×). Ulcer size was determined by measuring each lesion along its greatest length. In the case of petechiae, five such lesions were taken as the equivalent of a 1-mm ulcer. The total lesion lengths divided by the number of rats in each group was expressed as the mean ulcer index (3,4).

Determination of Respiratory Rate and Blood Lactate Concentration

Each rat was restrained in a tubular wire-mesh cage which was painted with insulating white paint. Two wire leads, each attached to a safety pin, were pinned to the left and right lateral sides of the abdomen, just beneath the diaphragm. The free ends of these wires were then connected to the positive and negative poles of a transducer which measured the impedance across the two safety pins. The changes in impedance induced by breathing was then electrically recorded on a physiograph; the respiratory rate per min calculated by counting the number of deflections recorded in a specific time period.

The method of Varley (19) was used to determine the blood lactate level. At the end of experiments, rats were killed by stunning and decapitation. One ml of whole blood was immediately collected and mixed with ice-cold trichloroacetic acid (E. Merck). The supernatant was collected and added to p-hydroxydiphenyl (Sigma). The final concentration of blood lactate was determined by measuring the absorbance at 560 nm by a spectrophotometer (Cary 219).

The results were expressed as means ± SEM. Data were analyzed for statistical significance by the two-tailed Student *t*-test.

RESULTS

In cold-restrained animals, the respiratory rate, when compared with its own value at 0 hr and with the control restrained at 22°C (Table 1), was depressed 1 hr after exposure to stress and this persisted until the end of the 2-hr experiment.

Cold-restraint stress for 2 hr did not significantly induce any changes in blood lactate concentration. There was no significant difference in the blood lactate levels between nonstressed (0.65 ± 0.17 mmol/l) and stressed (0.51 ± 0.07 mmol/l) rats.

Stress for 1.5 or 2 hr depressed the CO_2 tension in the blood (Table 2); the pH was decreased starting from 0.5 hr after onset of stress and remained lowered up to the end of the 2-hr stress period. The blood HCO_3^- was unaffected by cold-restraint stress. Sodium bicarbonate infusion (8.4% w/v) significantly prevented stress-induced decrease in blood pH, the value of HCO_3^- was elevated when compared to the stressed saline-treated control. The in-

TABLE 2

EFFECTS OF $NaHCO_3$ TREATMENT (GIVEN AS AN IV INFUSION OF 2.2 ml/HR AT 0 HR) ON COLD-RESTRAINT STRESS-INDUCED CHANGES IN ARTERIAL PCO_2, pH AND HCO_3^- CONCENTRATION

Time (hr) After IV Infusion	PCO_2 (mmHg)	pH	HCO_3^- (mmol/l)
A. Saline 0.9% w/v (restrained at 4°C for 2 hr)			
0	25.5 ± 1.9	7.423 ± 0.013	16.5 ± 0.5
0.5	27.3 ± 1.8	7.307 ± 0.015¶	15.8 ± 0.7
1.0	30.8 ± 2.2	7.258 ± 0.012¶	15.2 ± 0.8
1.5	37.6 ± 4.7†	7.155 ± 0.024¶	14.8 ± 1.5
2.0	44.5 ± 6.5§	7.119 ± 0.034¶	16.3 ± 0.9
B. $NaHCO_3$ 8.4% w/v (restrained at 4°C for 2 hr)			
0	24.9 ± 1.2	7.446 ± 0.011	17.4 ± 0.5
0.5	25.8 ± 1.3	7.482 ± 0.018†	19.4 ± 1.3*
1.0	26.5 ± 3.6	7.496 ± 0.022†	25.0 ± 1.2†§
1.5	34.4 ± 6.6	7.486 ± 0.030†	35.2 ± 2.6†¶
2.0	41.5 ± 6.6‡	7.494 ± 0.032†	45.5 ± 6.3†¶

Values are the means ± SEM of 12 rats in each group.
*$p<0.05$, †$p<0.001$ when compared with the corresponding control in A.
‡$p<0.05$, §$p<0.02$, ¶$p<0.001$ when compared with the corresponding group at 0 hr.

TABLE 3

EFFECTS OF HCl TREATMENT (GIVEN AS AN IV INFUSION OF 2.2 ml/HR AT 0 HR) ON ARTERIAL PCO_2, pH AND HCO_3^- CONCENTRATION

Time (hr) After IV Infusion	PCO_2 (mmHg)	pH	HCO_3^- (mmol/l)
A. Saline 0.9% w/v (restrained at 22°C for 2 hr)			
0	24.9 ± 1.2	7.437 ± 0.007	17.6 ± 0.5
0.5	27.6 ± 1.7	7.433 ± 0.006	17.5 ± 0.9
1.0	26.8 ± 1.8	7.427 ± 0.019	17.1 ± 1.1
1.5	27.6 ± 2.7	7.411 ± 0.025	18.9 ± 0.9
2.0	24.2 ± 2.7	7.417 ± 0.016	17.8 ± 1.0
B. HCl 0.5 M (restrained at 22°C for 2 hr)			
0	25.4 ± 0.6	7.449 ± 0.007	17.4 ± 0.3
0.5	25.8 ± 0.6	7.334 ± 0.014†‡	13.6 ± 0.6*‡
1.0	25.7 ± 0.8	7.234 ± 0.018†‡	10.6 ± 0.5†‡
1.5	23.6 ± 1.5	7.087 ± 0.052†‡	7.7 ± 0.9†‡
2.0	23.4 ± 1.1	7.072 ± 0.033†‡	6.6 ± 0.7†‡

Values are the means ± SEM of 12 rats in each group.
*$p<0.01$, †$p<0.001$ when compared with the corresponding control in A.
‡$p<0.001$ when compared with the corresponding group at 0 hr in B.

creased PCO_2 induced by stress was unaffected by $NaHCO_3$ infusion.

Saline infusion in rats restrained at 22°C for 2 hr did not affect the PCO_2, pH level and HCO_3^- concentration in the blood (Table 3). HCl infusion IV did not alter the PCO_2 level, however, it decreased the pH and HCO_3^- concentration.

Cold-restraint for 2 hr induced severe hemorrhagic ulcers in the gastric glandular mucosa (Table 4). The severity of stress ulceration was markedly decreased by $NaHCO_3$ treatment. HCl IV induced gastric ulcers, with an ulcer index which was significantly higher than the saline-infused controls. The titratable acid in the gastric lumen was not significantly different among the saline-, $NaHCO_3$- and HCl-treated groups.

TABLE 4

EFFECTS OF HCl OR $NaHCO_3$ TREATMENT ON GASTRIC GLANDULAR ULCERATION AND LUMINAL ACIDITY IN RATS

Treatment Groups (IV infusion, 2.2 ml/hr for 2 hr)	Luminal Titratable Acid (μEq/100 g body weight)	Glandular Ulcer Index (mm)
A. Restrained at 22°C for 2 hr		
Saline	4.45 ± 0.56	0.08 ± 0.05
0.5 M HCl	5.22 ± 0.98	1.18 ± 0.35*
B. Restrained at 4°C for 2 hr		
Saline	4.84 ± 0.49	5.74 ± 1.82
8.4% w/v $NaHCO_3$	4.13 ± 0.58	0.29 ± 0.06*

Values are the means ± SEM of 8 rats in each group.
*$p<0.01$ when compared with the corresponding saline-treated group.

DISCUSSION

The present study shows that cold-restraint stress increases the blood PCO_2. This effect is probably due to disturbance of the respiratory and vascular systems, as cold exposure has been shown to increase pulmonary vascular resistance (8) and blood viscosity (14). The rate and the volume of blood flow through the lung could, therefore, have been lowered. Such effects, coupled with a decreased respiratory rate, could lead to CO_2 accumulation in the blood. In addition, there was a decrease in blood pH, indicating that acidosis occurred during cold-restraint stress. This acidosis could be either respiratory or metabolic in origin. The present study shows that neither the blood HCO_3^- nor lactate was affected, implying that the acidosis may not be metabolic but be the result of respiratory depression. The elevation of PCO_2 during cold-restraint substantiates this idea.

Intravenous infusion of saline at room temperature (22°C) did not affect the blood pH throughout the 2-hr experimental period, however, the pH was markedly decreased by cold-restraint stress (Tables 2, 3). These findings indicate that IV infusion alone does not impose any significant stress on the animals.

The observation that maintenance of the arterial pH within the normal range, by IV infusion of $NaHCO_3$, prevented ulcer formation in cold-restraint rats confirms the findings of Cheung and Porterfield (2). Neutralization of blood acidity, by $NaHCO_3$, during cold-restraint stress is due to the increased blood HCO_3^- levels. HCl infused IV decreased the blood pH and HCO_3^- levels, without affecting the PCO_2, thus indicating that the acidosis produced by HCl is a metabolic one. This IV infusion of acid induced gastric ulceration. Although both cold-restraint stress and IV infusion of HCl produce acidosis, the type of acidosis is different, as shown in the present study. It has been found that for a similar depression in extracellular pH, the decrease in intracellar pH is greater in respiratory than in metabolic acidosis (12). This could be due to differences in permeability between CO_2 and ionic substances like hydrogen ions; carbon dioxide can easily diffuse into cells to lower their intracellular pH. Cold-restraint stress induces mainly respiratory acidosis and, thus, could result in an intracellular pH which is lower than that obtained by IV infusion of acid. Lowering of intracellular pH, through decreased extracellular pH, could disrupt cellular metabolism and enzymatic reactions, to result in greater degeneration of the gastric mucosal cells.

As cold-restraint ulcers have been shown to be prevented by mast cell stabilizers (4,15), histamine blockers (3,5) and cholinergic antagonists (5, 7, 10, 20), it is likely that a change in acid-base balance is not the immediate cause of stress-induced ulceration. It could be an initiator for the subsequent pathological changes which happen at the later stage of 2-hr cold-restraint stress, since acidosis is already present 30 min after onset of stress (Table 2). However, no observable lesions are found in the stomach at this particular point in time (unpublished finding). Although it has been reported that gastric acid secretion is affected by acidosis (1,11), the present study did not demonstrate such an effect, either in respiratory or metabolic acidosis (Table 4).

The occurrence of respiratory acidosis is probably not unique for stress caused by cold and restraint. A similar effect has been observed in salicylate intoxication, respiratory disorders and sepsis in newborns where gastrointestinal bleeding has occurred (6,18). However, the exact role of respiratory acidosis in the aetiology of gastrointestinal dysfunction is still unclear. The present study attempts to clarify the mechanism of cold-restraint stress ulceration, and the findings do not negate the usefulness of this ulcer-producing model as an investigative tool.

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