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Effects of Manipulating Cholinergic Tone Upon the Activity-Stress Ulcer[1]

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HOUSER, V. P., R. J. CASH AND D. A. VAN HART. *Effects of manipulating cholinergic tone upon the activity-stress ulcer.* PHARMAC. BIOCHEM. BEHAV. 3(5) 825–831, 1975. –Eighty rats were housed in standard activity wheel cages and fed for only 1 hr per day. The animals were divided into 10-animal drug groups that received either 0.25, 0.50, 1.0 mg/kg of scopolamine methylbromide, or 0.06, 0.125, 0.250 mg/kg of carbachol, 3 times a day. Two separate 10-animal saline control groups accompanied each drug series. All animals died within 6 days and most demonstrated significant gastric lesions in the glandular fundus of the stomach. All dosages of scopolamine methylbromide significantly reduced the number and severity of gastric lesions and in some cases abolished all signs of stomach pathology. None of the dosages of carbachol significantly affected either the number or degree of gastric ulceration noted in the body of the stomach. These results were interpreted to suggest that the secretion of gastric acid may be an important contributing factor in the formation of gastric ulcers in animals subjected to the activity-stress procedure.

Cholinergic tone Scopolamine Carbachol Gastric ulcer Stress

RECENT reports from this laboratory [5, 10, 11] have described an experimental technique that is able to produce extensive gastric ulcers in the glandular portion of the rat stomach in a relatively brief time. In this technique animals are housed in standard activity cages and fed for only one hour each day. Animals have constant access to the activity wheel 24 hr a day. After 2 days of restricted feeding animals begin to run more and eat less. If the room temperature is kept at 67°F, all animals will die within 7 days and all will demonstrate severe lesions in the glandular portion of the stomach. Reduced food intake does not appear to be the direct cause of this pathology since animals who are food-yoked (i.e., receive the identical amount of food eaten by the experimental animals), but do not have access to the activity wheel, survive and are ulcer free [10]. Since this technique produces glandular lesions which resemble the "stress ulcer" reported by other investigators [3] and requires that animals exhibit extensive activity, the resulting pathology has been designated the "activity-stress ulcer" [11].

The above technique has been used to investigate the ulcer inhibiting properties of metiamide, an H_2 (histamine) receptor antagonist [5]. Metiamide has been reported to block gastric secretion in response to both pentagastrin and histamine [1,2]. Metiamide (50 mg/kg every 8 hr) was able to significantly reduce the number and size of the stomach lesions produced by the "activity-stress" technique [5]. This evidence suggests that a reduction in gastric secretion may, in fact, inhibit the formation of the activity-stress ulcer.

The above data with regard to metiamide are compatible with the previously proposed hypothesis that hypersecretion of hydrochloric acid is involved in the formation and maintenance of gastric ulcers [13]. Since acid secretion can be enhanced by cholinergic stimulation via the vagus nerve as well as by the administration of histamine and pentagastrin, it seemed advisable to investigate the effects of cholinergic and anticholinergic drugs upon the activity-stress ulcer. The present report is an attempt to summarize the effects of scopolamine methylbromide, a peripheral acting anticholinergic, and carbachol, a peripheral cholinergic stimulant, upon the degree of stomach pathology produced when rats were subjected to the activity-stress experimental technique.

METHOD

Animals and Apparatus

Eighty male Sprague-Dawley derived rats obtained from Hilltop Lab Animals, Inc., Scottdale, Pa., were used in the present study. They ranged in weight from 130–177 g at the beginning of the experiment. The apparatus consisted of 20 Wahmann (Model LC-34) activity wheels. Each activity wheel included a 25.4 × 15.2 × 12.7 cm adjoining

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cage. Each activity wheel was fitted with a counting device which allowed one to measure the number of revolutions every animal made during each daily period. Glass food cups were clipped to the interior of the cage and were 5 cm in dia. and 6 cm in height. A metal cover was fastened to the top of the food cups which had a hole cut in the center (3.5 cm in dia) to allow access to the granular Purina lab chow. Graduated cylinders with drinking tubes were fastened to the exterior of the cage to allow ad lib access to water for all animals throughout the experimental period.

Procedure

Animals were initially given 2 days to habituate to the activity wheels. During this period they had continuous access to the activity wheels and food cups which were filled with granular dry Purina rat chow. Water was available on an ad lib basis throughout the experimental period. The experimental room was kept at 67°F with a relative humidity of 50 percent. In addition, a reverse light-dark cycle was in force throughout the experiment with the lights switched on between 9:15 p.m. through 9:15 a.m.

After the 2 day adaptation period rats were randomly assigned to eight 10 animal weight matched groups. This was done since body weight has been reported to influence the degree of stomach pathology produced by this technique [11]. The mean body weights were as follows: Saline group – 155.6 g, Methyl Scopolamine 0.25 mg/kg group – 155.6 g, Methyl Scopolamine 0.50 mg/kg group – 158.8 g, Methyl Scopolamine 1.0 mg/kg group – 155.8 g, Saline group – 159.5 g, Carbachol 0.06 mg/kg group – 161.8 g, Carbachol 0.125 mg/kg group – 161.8 g, and finally the Carbachol 0.25 mg/kg group – 161.3 g.

Since only 20 activity cages were available at a given time, the present study was conducted by replicating the experiment on 4 separate occasions. During each replication the 20 animals were divided into 4 separate 5 animal groups; a saline, low, medium and high dosage group. The first two replications consisted of administering scopolamine methylbromide, while the final two replications dealt with carbachol. Thus, two saline control groups were included; one for each drug administered.

During the next 5 to 6 days all animals had access to the food cups for only one hour per day (i.e., 8:00 a.m. – 9:00 a.m.). Thus, the food cups were removed from the adjoining chamber at 9:00 a.m. Animals had continual free access to the wheel from the adjoining cage 24 hr a day during the entire experimental period. Body weight in grams, activity in wheel revolutions, and food consumption in grams were recorded daily for all rats. Since many animals spilled large amounts of food while eating from the jars, a metal plate was placed under the cage to collect this spillage. This spillage, in turn, was returned to the food cup before it was weighed so that an accurate measure of food actually consumed would be obtained. When the animals died the stomach was removed and inspected for ulcers. Extent of ulceration was measured by a scale in millimeters located within the eyepiece of a dissecting microscope. Ulceration was determined in measurements of: number of lesions and ulcer score (i.e., length plus width of lesions in millimeters). The criterion for gastric lesioning was an obvious erosion or absence of mucosal tissue accompanied by hemorrhaging at the site of the lesion. Gastric lesions were characterized by a deterioration of epithelial cells extending through the mucosal lining. All evaluations concerning the extent of gastric lesions were confirmed through a blind procedure in which information as to which groups were drugged was not made available to the judge.

Both scopolamine methylbromide (0.25, 0.50, 1.0 mg/kg) and carbachol (0.06, 0.125, 0.25 mg/kg) were dissolved in 0.9 percent saline and administered intraperitoneally in a volume of 1.0 ml/kg. Injections of carbachol were given 3 times daily at 8 hr intervals: 8:00 a.m., 4:00 p.m. and 12:00 midnight. Since an earlier report [4] has indicated that scopolamine methylbromide reduces food intake in deprived rats, injections of this drug were given after the 1 hr feeding period (i.e., 9:00 a.m., 4:00 p.m. and 12:00 midnight). Control animals received injections of 0.9 percent saline 3 times daily at identical time periods. The dosages for each drug given above represent the amount administered per injection. Since 3 injections were given each day, the total daily dosages were actually triple the amounts listed above.

RESULTS

Since each drug series contained a separate saline control group, the data with regard to the two drugs were treated and will be discussed separately. All 40 animals in the scopolamine methlbromide series died within 6 days from the start of the 1 hr feeding procedure. In addition, 35 of these animals demonstrated gastric lesions in the glandular fundus of the stomach. Only two animals (i.e., in the saline control group) demonstrated minor lesions in the rumenal portion of the stomach. Since the major pathology was observed in the glandular portion of the stomach, all further reference to the extent and number of lesions will refer only to those lesions found in the glandular fundus of the stomach. The remaining five animals (i.e., two each in the 0.25 and 1.0 mg/kg groups and one animal in the 0.50 mg/kg group) demonstrated no ulceration in any part of the stomach.

Figure 1 presents the mean number of ulcers and mean ulcer score (i.e., length plus width of lesions in millimeters) for the saline and various dosages of scopolamine methylbromide.

Statistical analyses consisted of performing a one-way analysis of variance based on 3 and 36 degrees of freedom on both the number of ulcers and ulcer score data. Multiple comparisons were then carried out using the Tukey a test. These comparisons were made between the control and three dosage groups. As can be seen in Fig. 1, scopolamine methylbromide administration severely inhibited both the number of lesions noted in the body of the stomach and the extent or severity of the ulceration. All doses of this drug reduced both measures of stomach pathology in a statistically significant manner ($p<0.01$). A dose-response effect was not readily apparent since the lowest dosage was as effective as the highest dosage in reducing ulceration.

Table 1 presents the mean food consumed, mean activity, mean body weight and percentage of survivors during the 6 day experimental period for those animals subjected to saline or the various doses of scopolamine methylbromide.

The data in Table 1 indicate that scopolamine methylbromide was able to reduce food intake in all three drug groups during the first two days of the experiment in agreement with earlier evidence [4]. In addition, the body weights of the three drug groups were slightly lower than

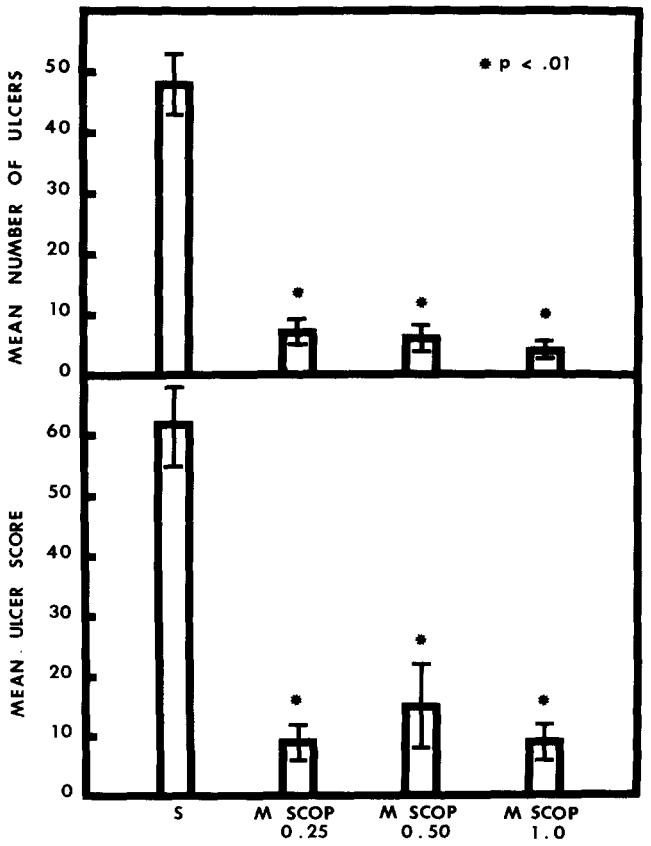

FIG. 1. Mean number of ulcers and ulcer score (i.e., length plus width in millimeters) with corresponding standard error of the means for the saline and various scopolamine methylbromide drug dosage groups. All dosages are given in mg/kg. The probability level refers to comparisons made between saline and the various dosage groups using the Tukey a test.

the control group during the initial phases of the experiment, while activity was generally greater in the drug groups. Finally, the animals in the three drug groups appeared to die slightly earlier in the experiment than the saline animals.

All 40 of the animals subjected to carbachol died within 6 days from the start of the experiment. Furthermore, all animals demonstrated gastric lesions in the glandular fundus of the stomach. Only 8 animals (i.e., 4 in the saline group and 2 each in the 0.06 and 0.25 mg/kg dosage groups) demonstrated minor lesions in the rumenal portion of the stomach. As was the case earlier, all further reference to ulceration will encompass only those lesions noted in the glandular fundus of the stomach.

Figure 2 presents the mean number of ulcers and mean ulcer score (i.e., length plus width of lesions in millimeters) for the saline and various dosages of carbachol.

Statistical analyses were identical to those described above which utilized an analysis of variance with multiple comparison using the Tukey a test. As is clear from Fig. 2, carbachol administration had little or no effect on the mean number of lesions noted in the body of the stomach. In contrast, carbachol was able to reduce the mean ulcer score in a dose-related manner. These reductions were not, however, statistically reliable.

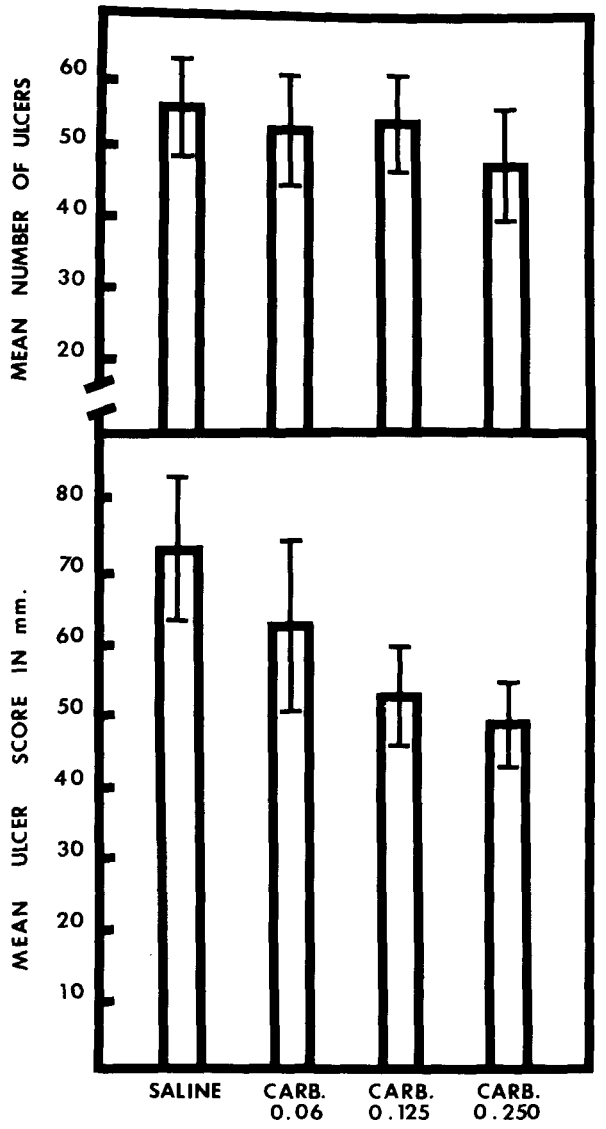

FIG. 2. Mean number of ulcers and ulcer score (i.e., length plus width in millimeters) with corresponding standard error of the means for the saline and various carbachol drug dosage groups. All doses are given in mg/kg.

Table 2 presents the mean food consumed, mean activity, mean body weight and percentage of survivors during the 5 day experimental period for those animals subjected to saline and the various dosages of carbachol.

The data in Table 2 indicate that carbachol did not appear to reliably affect the amount of food consumed, body weight, or activity rates in any of the dosages administered. Furthermore, this cholinergic stimulant did not alter the rate at which animals died under this experimental procedure.

Figure 3 presents three representative stomachs from the saline, carbachol 0.25 mg/kg and scopolamine methylbromide 1.0 mg/kg groups. As this photograph indicates, all pathology was noted in the glandular protion of the stomachs. The extent of lesions and hemorrhage was severe

TABLE 1

MEAN FOOD CONSUMED IN GRAMS, MEAN ACTIVITY IN REVOLUTIONS, MEAN BODY WEIGHT IN GRAMS, AND PERCENTAGE OF SURVIVORS DURING THE SIX DAY EXPERIMENT FOR THOSE ANIMALS ADMINISTERED SALINE OR VARIOUS DOSES OF SCOPOLAMINE METHYLBROMIDE

Days		1	2	3	4	5	6
Saline	F	5.0	4.5	4.12	4.0	2.0	–
	W	141	133	115	110	105	–
	A	2906	7799	8206	10478	8683	–
	%	100	100	80	30	20	0
Scopolamine Methylbromide	F	2.8	2.3	3.25	3.0	–	–
0.25 mg/kg	W	140	128	119	119	–	–
	A	4319	14295	7545	7222	–	–
	%	100	100	40	20	0	0
Scopolamine Methylbromide	F	2.5	2.0	1.71	4.0	1.5	–
0.50 mg/kg	W	140	126	112	121	109	–
	A	2923	7340	6736	3261	6596	–
	%	100	100	70	20	20	0
Scopolamine Methylbromide	F	1.4	1.9	4.2	3.66	2.0	–
1.0 mg/kg	W	140	127	115	119	111	–
	A	8351	8284	15866	5768	8728	–
	%	100	90	50	30	10	0

F = mean food in grams W = mean body weight in grams A = mean activity in revolutions % = percentage of survivors

in the saline treated animal. The carbachol stomach demonstrated somewhat fewer lesions, and those lesions that did develop were less extensive in total area than in the control animal. The animal treated with 1.0 mg/kg of scopolamine methylbromide demonstrated no pathology.

DISCUSSION

The present results indicate that only the peripheral acting anticholinergic, scopolamine methylbromide, was able to significantly reduce the severity of gastric glandular lesions in the rat. Scopolamine methylbromide was extremely potent in this regard and thus was actually able to inhibit the formation of lesions leading to a reduction in the number of ulcers recorded and in some instances completely abolishing all signs of stomach pathology. The peripheral cholinergic stimulant, carbachol, on the other hand, did not enhance the degree of stomach pathology and, in fact, appeared to diminish the severity of the ulcers that were noted in the highest dosage group. This effect was not recorded in all animals, however, and thus carbachol produced no statistically reliable change in stomach pathology.

Reduction of gastric secretion is known to be one of the major effects of anticholinergic drugs [6]. Gastric secretion is reduced in volume and total acid content in response to these agents. In addition, anticholinergic drugs can inhibit acid and pepsin secretion in the rat stomach from a variety of stimuli including food, hog gastrin II, and histamine [7]. The fact that anticholinergics inhibit the secretion of acid has allowed these agents to be used both experimentally [9,12] and clinically [6] to prevent the formation of peptic ulcers. The major rationale for administering these agents has been that hypersecretion of hydrochloric acid is involved in the formation and maintenance of gastric ulcers [13]. The present results with regard to scopolamine

TABLE 2

MEAN FOOD CONSUMED IN GRAMS, MEAN ACTIVITY IN REVOLUTIONS, MEAN BODY WEIGHT IN GRAMS, AND PERCENTAGE OF SURVIVORS DURING THE FIVE DAY EXPERIMENT FOR THOSE ANIMALS ADMINISTERED SALINE OR VARIOUS DOSES OF CARBACHOL

Days		1	2	3	4	5
Saline	F	3.80	4.20	3.20	0.50	–
	W	146.7	128.3	112.5	104.0	–
	A	4999	9761	8472	4833	–
	%	100	100	50	20	0
Carbachol	F	2.90	4.30	2.75	2.0	0
0.06 mg/kg	W	149.5	134.5	113.4	111.0	–
	A	3651	9606	14604	3700	–
	%	100	100	100	20	0
Carbachol	F	3.10	4.00	3.44	2.50	1.0
0.125 mg/kg	W	145.6	134.7	117.4	110.2	104.0
	A	3621	7252	8181	6431	10923
	%	100	100	90	40	20
Carbachol	F	3.20	3.44	4.00	0.33	–
0.250 mg/kg	W	143.3	131.7	111.6	103.6	–
	A	4294	9741	9974	7369	–
	%	100	90	50	30	0

F = mean food in grams
W = mean body weight in grams
A = mean activity in revolutions
% = percentage of survivors

methylbromide are in agreement with this hypothesis in that this anticholinergic which is known to reduce gastric acid secretion in the rat [12] was also able to reduce or abolish the formation of the activity-stress ulcer. The fact that a cholinergic stimulant, carbachol, was ineffective in enhancing the severity of ulceration is, however, more difficult to understand. Cholinergic stimulants such as carbachol are known to stimulate the secretion of gastric juice which is rich in acid and pepsin [8]. Thus, if hypersecretion of acid were involved in the production and maintenance of the activity-stress ulcer, one would expect greater ulceration in those animals subjected to carbachol administration. The present results demonstrate no enhancement of ulceration after carbachol administration and, in fact, suggest that high doses may slightly reduce the extent, if not number, of ulcers produced in the activity-stress technique. It may be that hypersecretion is elevated under this technique to a point where further stimulation is unable to enhance gastric secretion. Although it is always possible that these doses were not sufficient to produce enhanced secretion, it should be noted that higher doses produce severe toxic reactions that make their use inadvisable in rats.

Finally, some mention should be made concerning the possible influence of mucus secretion in producing the effects noted in this experiment. Although the anticholinergic drugs are primarily known for their inhibitory effects on gastric acid secretion, these agents also affect mucus production. Mucus is one of the major protective substances which lines the stomach preventing damage of the mucosa by acid or peptic digestion [13]. Robert and Nezamis [12] have reported that scopolamine methyl-

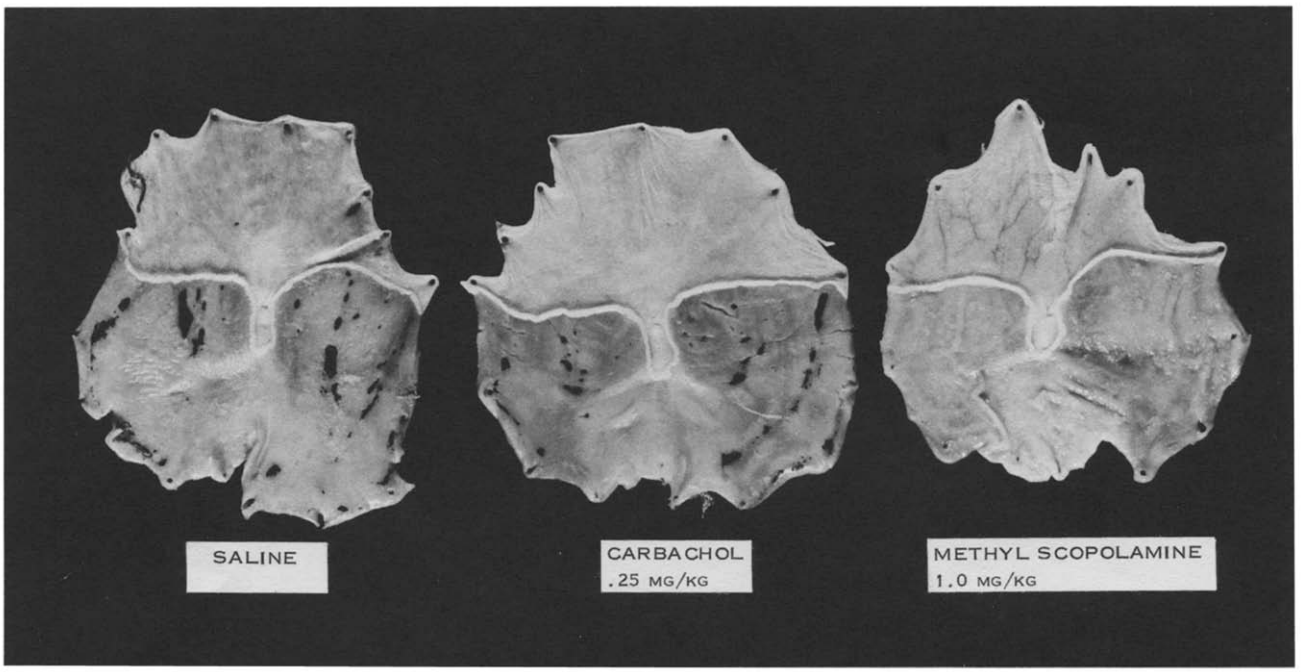

FIG. 3. Photograph of representative stomachs from the saline, carbachol 0.25 mg/kg and scopolamine methylbromide 1.0 mg/kg groups. The severe linear lesions in the glandular portion of the saline stomach are covered with hemorrhagic slough.

bromide, although able to inhibit gastric secretion and acid output, was also able to produce a 3–4 fold increase in hexosamine concentration in the stomachs of restrained rats. Hexosamine is present in most mucoproteins and can therefore be used as an index of gastric mucus content. Thus, although scopolamine methylbromide depressed secretory activity in the stomach as measured by volume, acid, and mucus output, it substantially increased the concentration of mucus in the gastric juice that was excreted. In addition, the drug was able to prevent restrain-induced gastric ulcers in the rat. Thus, these authors [12] concluded that the anti-ulcer properties of scopolamine methlybromide may be due to its effects on gastric mucus concentration, as well as its inhibitory effects on acid secretion.

If increased mucus concentration is indeed involved in inhibiting the formation of the activity-stress ulcer, it is possible that carbachol at appropriate doses could produce effects similar in direction to those noted under scopolamine methylbromide. Although carbachol is known to stimulate acid and pepsin secretion, it also stimulates the production of mucin, which is the chief constituent of mucus [8]. Thus, enhanced levels of acid secretion may not be the only factor involved in the production of the activity-stress ulcer. The concentration of mucus in the gastric juice may also play an important role in the development of this pathology. If carbachol does increase the concentration of mucus in the gastric juice, it may explain why this cholinergic stimulant was able to slightly reduce the severity of gastric lesions produced under the highest dose (i.e., 0.25 mg/kg). Further research should center on measuring hexosamine levels in rats subjected to the activity-stress procedure while under the influence of carbachol to see if the concentration of mucus is increased above that noted in control animals.

To conclude, it should be remembered that all animals died within six days of the start of the experiment, even though no ulceration was noted in some of the scopolamine methylbromide treated animals. This finding suggests that death was caused by factors other than hemorrhage from gastric lesions. Further research should be conducted to determine why animals die under this procedure when no sign of gastric pathology is evident.

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