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DECLINE OF HISTIDINE DECARBOXYLASE ACTIVITY AND HISTAMINE LEVELS IN RAT STOMACH AFTER RESERPINE

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Abstract—The previously reported reduction of rat gastric histamine following reserpine administration was investigated. It was observed that there was a rapid decrease in the histidine decarboxylase activity in the glandular portion of rat stomach after the administration of reserpine. This decrease in histidine decarboxylase activity preceded a decrease in the histamine content of the rat stomach. The effect of reserpine on histidine decarboxylase activity was seen only in the intact animal; reserpine had no action on histidine decarboxylase activity *in vitro*. The reduction of histidine decarboxylase activity by reserpine was not abolished by vagotomy or atropine treatment nor by treatment with agents producing sympathetic blockade. However, the administration of the sympatholytic agents alone reduced gastric histidine decarboxylase activity. It was concluded that the decrease in histidine decarboxylase activity in the stomach following reserpine treatment might be one factor contributing to the decrease in gastric histamine after administration of this drug.

RESERPINE is known to induce gastric acid secretion.[1–4] It has also been shown by Kim and Shore[5] that in rat the gastric secretion after reserpine was accompanied by a reduction in the histamine content of stomach. Kim and Shore[5] have suggested that the reduction in histamine content in rat stomach was due to release of histamine and that this histamine release resulted in gastric acid secretion. From studies with vagotomized rats, these workers have concluded that reserpine mobilized gastric histamine by vagal stimulation.[5,6]

Since the level of gastric histamine may also be dependent upon the level of gastric histidine decarboxylase activity, as well as upon the release of histamine from the gastric mucosa, the present study was undertaken to investigate whether reserpine altered histidine decarboxylase activity in rat stomach. It will be shown that after reserpine treatment there was a rapid decline in the histidine decarboxylase activity in the glandular portion of the rat stomach. An attempt was made to determine if the action of reserpine on histidine decarboxylase activity was a direct one or if it was mediated through the autonomic nervous system.

EXPERIMENTAL

Materials. Radioactive compounds, L-histidine-carboxyl-^{14}C, DL-3,4-dihydroxyphenylalanine-carboxyl-^{14}C (DOPA-^{14}C), and L-histidine-^{3}H (generally labeled), were obtained from New England Nuclear Corp. Pyridoxal phosphate, nonradioactive

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L-histidine and DL-3,4-dihydroxyphenylalanine (DOPA) were purchased from Calbiochem; histamine dihydrochloride from Mann Research Laboratories; and hyamine hydroxide from Packard Instrument Corporation. Reserpine (Serpasil) was purchased from CIBA, and a lyophilized reserpine phosphate preparation was kindly supplied by CIBA. Other drugs used in this study included: phentolamine HCl (Regitine, CIBA), chlorisondamine HCl (Ecolid, CIBA), guanethidine and BW 392C60 (Burroughs Welcome), pargyline HCl (Eutonyl, Abbott), and phenoxybenzamine HCl (Dibenzyline, Smith, Kline and French).

Animals. Male Sprague–Dawley rats, 200–240 g, were used for all experiments. Fasted rats were deprived of food for 24 hr, usually from 10:00 a.m. to 10:00 a.m. the following day. Reserpine solution (2 mg/ml) was prepared in a solvent consisting of 7·5% propylene glycol and 1·75% ethanol in water, and the drug solution was administered i.v. in doses of 1 or 5 mg/kg as stated. Control animals were given the same volumes of solvent. In the experiment shown in Fig. 4, atropine (10 mg/kg, i.p.) or phentolamine (15 mg/kg, i.p.) was administered 30 min before reserpine (1 mg/kg, i.v.) and the animals were killed 1 hr later. In other experiments guanethidine (10 mg/kg, i.v.), BW 392C60 (5 mg/kg, i.v.), pargyline (20 mg/kg, i.v.) or phenoxybenzamine (10 mg/kg, s.c.) was administered 30 min before reserpine (1 mg/kg, i.v.) and the animals killed 2 hr after reserpine. These various drugs were also administered to rats that received solvent instead of reserpine. Bilateral gastric vagotomy was performed according to the procedure of Lambert[7] 2 weeks prior to the experiment. Animals were killed by decapitation and the stomachs removed. Each stomach was divided into its two parts as described by Lambert;[7] the forestomach or nonglandular part was discarded and the distal or glandular part was used for the present studies.

Histamine assay. The histamine content in the glandular portion of the rat stomach was assayed according to the extraction and spectrophotofluorometric procedure of Shore *et al.*[8]

Histidine decarboxylase assay. Histidine decarboxylase activity was assayed by a modification of the procedure of Kobayashi[9] in which the $^{14}CO_2$ resulting from the enzymatic decarboxylation of histidine-carboxyl-^{14}C was collected and measured. In our procedure the glandular part of rat stomach was minced and then homogenized in 9 vol. of 0·1 M sodium phosphate buffer, pH 6·8, in an all-glass homogenizer. In most cases enzyme activity was measured in 1 ml of the whole homogenate; however, 1 ml of a 9000 × 15 *g* min supernatant fluid was also used for the assay where indicated. One ml of homogenate or supernatant was preincubated in a 20-ml glass vial for 15 min at room temperature with sodium phosphate buffer, 0·1 M, pH 6·8, and pyridoxal phosphate, 4×10^{-5} M, in a volume of 1·8 ml. To this mixture was added L-histidine-carboxyl-^{14}C, final concentration, $3{\cdot}22 \times 10^{-4}$ M, specific activity, 0·155 μc/μmole, to a total volume of 2·0 ml. Controls contained buffer or boiled tissue extracts instead of the tissue homogenate. The mixture was then incubated with shaking for 90 min at 37°. The $^{14}CO_2$ was collected in hyamine hydroxide, 1 M in methanol, in a plastic cup (Kontes Glass Co.) which was attached through a rubber stopper in the neck of the glass vial. The reaction was stopped by the injection of 0·2 ml of 2 M perchloric acid through the rubber stopper. The reaction mixture was then reincubated for 1 hr, after which time the plastic cup with the hyamine solution was transferred to 15 ml of a liquid scintillation counting solution[10] and assayed for ^{14}C in a Packard Model 4000 Tri-Carb scintillation spectrometer. With the above procedure the pro-

duction of $^{14}CO_2$ was linear with time up to 90 min and was proportional to the amount of tissue homogenate up to 1 ml. Studies with $NaH^{14}CO_3$ showed that the recovery of $^{14}CO_2$ from the incubation mixture was better than 95 per cent.

In the studies in which the properties of the histidine decarboxylase activity of stomach of reserpinized and normal rats were examined (Fig. 3), the following procedure was used: Each rat received reserpine (1 mg/kg i.v.) or solvent and was killed 1 hr after the injection. The stomach was homogenized and centrifuged for 9000 × 15 *g* min. The supernatant fraction was passed over Sephadex G-25 to remove histidine and other possible interfering substances from the supernatant fraction.[11] In later experiments the Sephadex step was omitted when its omission was found not to alter the results. The supernatant fraction was taken for assay of histidine decarboxylase activity at different concentrations of substrate. The amount of L-histidine-carboxyl-^{14}C was the same for all incubations, 0·1 μc/2·0 ml incubation, but the amount of unlabeled L-histidine was varied from incubation to incubation to give concentrations of 1·2, 2·4, 3·6, 12·0, 24·0 or 36·0 × 10^{-4} M.

Specificity of the histidine decarboxylase assay. To determine whether the amount of $^{14}CO_2$ formed was equivalent to the amount of histamine formed in the histidine decarboxylase assay, the tissue homogenate was incubated with both L-histidine-^3H and L-histidine-carboxyl-^{14}C, final concentration, 3·22 × 10^{-4} M, specific activity of both isotopes, 0·1 $\mu c/\mu$mole. The histamine-^3H was assayed, after the addition of unlabeled carrier histamine, as the dibenzene-sulfonyl derivative of histamine according to the method of Schayer *et al.*[12] The $^{14}CO_2$ was collected in hyamine as described above. Tritiated histamine and $^{14}CO_2$ were formed in equivalent amounts as found by Maudsley *et al.*[13]

DOPA decarboxylase assay. DOPA decarboxylase activity was assayed in 0·5 ml of tissue homogenate according to the procedure of Aures *et al.*[14] This assay was similar to the assay of histidine decarboxylase activity except that the incubation mixture contained DOPA-^{14}C, final concentration, 4 × 10^{-4} M, specific activity of L-isomer, 0·1 $\mu c/\mu$mole. A 30 min incubation time was used. The production of $^{14}CO_2$ was linear with time up to 30 min and was proportional to the amount of tissue homogenate up to 0·5 ml.

Protein assay. Protein was assayed according to the procedure of Lowry *et al.*[15]

RESULTS

Effect of reserpine on histidine decarboxylase activity and histamine level in rat stomach. Figure 1a depicts the changes in histidine decarboxylase activity and levels of histamine in rat stomach after the i.v. administration of reserpine to fed rats. The data show that the histidine decarboxylase activity declined immediately. In 1 hr the enzyme activity had decreased by 70 per cent and in 2 hr by almost 80 per cent. After a delay of 2–5 hr, the histamine levels then declined to about 66 per cent of the control values. The extent of decline in histamine levels was thus consistent with that found by Kim and Shore.[5]

The DOPA decarboxylase activity of the glandular part of the stomach was also measured. As seen in Table 1, reserpine treatment did not alter the DOPA decarboxylase activity. Only the histidine decarboxylase activity was affected.

Comparison of the effect of reserpine with those of feeding and fasting on histidine decarboxylase activity in rat stomach. Fasting is known to reduce activity.[16,17] Since

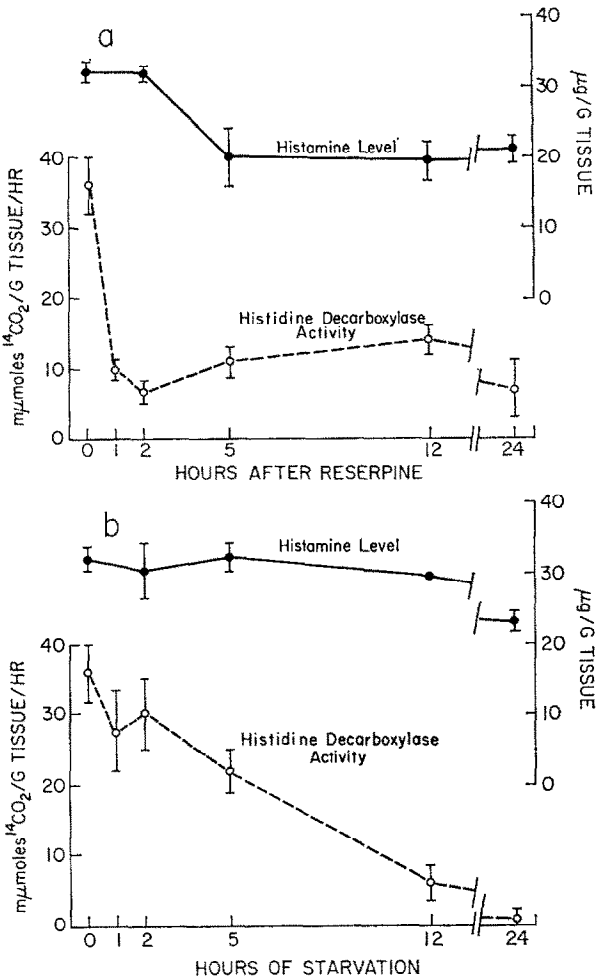

FIG. 1(a). Histamine level and histidine decarboxylase activity in rat stomach (glandular portion) at various times after administration of reserpine (5 mg/kg, i.v.).

FIG. 1(b). Histamine level and histidine decarboxylase activity in stomach (glandular portion) of untreated rats at various times after withdrawal of food. Each point represents the mean value of four to six rats; vertical lines depict S.E.M.

TABLE 1. COMPARISON OF THE EFFECT OF RESERPINE ON HISTIDINE DECARBOXYLASE AND DOPA DECARBOXYLASE ACTIVITIES IN RAT STOMACH*

Animals (*n*)	Histidine decarboxylase (mμmoles/g/hr)	DOPA decarboxylase (mμmoles/g/hr)
Control (8)	42 ± 6	866 ± 44
Reserpine treated (8)	24 ± 5†	886 ± 99

* Animals received reserpine (1 mg/kg, i.v.) or solvent and were killed 2 hr thereafter. Activities were determined as described under the Experimental section and are expressed as mμmoles $^{14}CO_2$ liberated/g stomach/hr. Each value is the mean value ± S.E.M.

† These values are significantly different from control, at $P < 0{\cdot}02$.

rats did not eat after administration of reserpine, the possibility was examined that the decline in histidine decarboxylase activity after reserpine was due to the effect of reserpine on eating. Figure 1b shows that after withdrawal of food from rats the decline in histidine decarboxylase activity was more gradual than that observed after administration of reserpine and that this decline was not readily apparent until 5 hr after removal of food. The data in Fig. 1b also show that there was no significant decline in stomach histamine levels over a 12 hr period, but the levels did decline 20–25 per cent after 24 hr of starvation. In another experiment, reserpine was shown to affect the histidine decarboxylase activity of stomach quite apart from the effect of reserpine on eating. In this experiment, fasted rats, in which the histidine decarboxylase activity was low, were fed for 20 min; food was then withdrawn and reserpine or solvent administered. In the solvent treated rats histidine decarboxylase activity in stomach increased 4-fold in 3 hr. This rise was similar to that observed by other workers[16,17] in rats fed after starvation. In reserpine treated rats, however, there was no increase in the enzyme activity (Fig. 2).

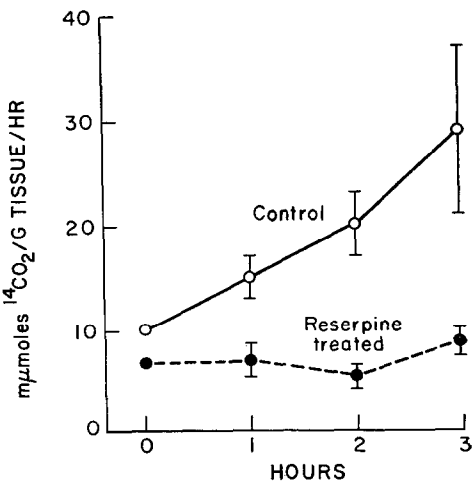

FIG. 2. Inhibition by reserpine of the increase in histidine decarboxylase activity in rat stomach (glandular portion) after feeding. Starved rats were fed for 20 min and then were given either reserpine (5 mg/kg, i.v., broken line) or solvent (solid line). Each point represents the mean value of four to six rats; vertical lines depict S.E.M.

Effects of reserpine on histidine decarboxylase activity in vivo *and* in vitro. Kinetic studies were carried out to compare the histidine decarboxylase activity in the glandular stomach of normal rats and reserpine treated rats. Figure 3 shows by Lineweaver–Burk plot that the apparent V_{max} of the enzyme was reduced by 50 per cent 1 hr after administration of reserpine. The apparent K_m was not appreciably changed. The decrease in V_{max} suggested that there was a decrease in the amount of enzyme activity but no alteration in the affinity of the enzyme for the substrate. The possibility that reserpine was an inhibitor of histidine decarboxylase *in vitro* was also examined. Incubation of the supernatant fraction of stomach homogenate with reserpine phosphate (10^{-5} or 10^{-6} M) for 15 min did not reduce the histidine decarboxylase activity. Therefore, reserpine did not appear to have a direct action on the enzyme.

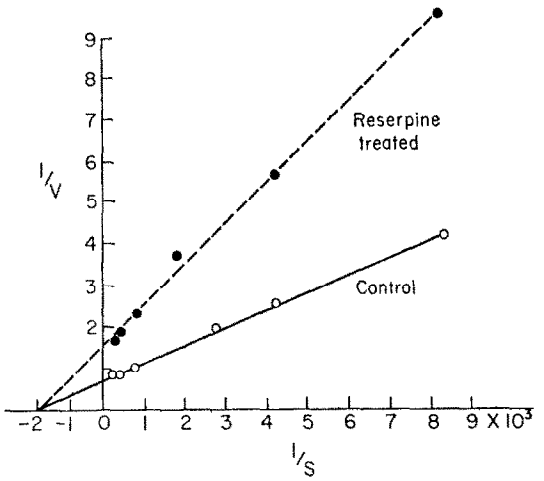

FIG. 3. A Lineweaver–Burk plot of histidine decarboxylase activity in stomach of a control rat (solid line) and of a reserpine treated rat (broken line). The activity was measured in the supernatant fraction of homogenate of a single rat stomach 1 hr after administration of reserpine (1 mg/kg, i.v.) or solvent as described in the experimental section. This experiment was one of six experiments in which similar results were obtained. V = mμmoles $^{14}CO_2$ liberated/mg stomach protein/90 min incubation. S = L-histidine concentration in moles/l.

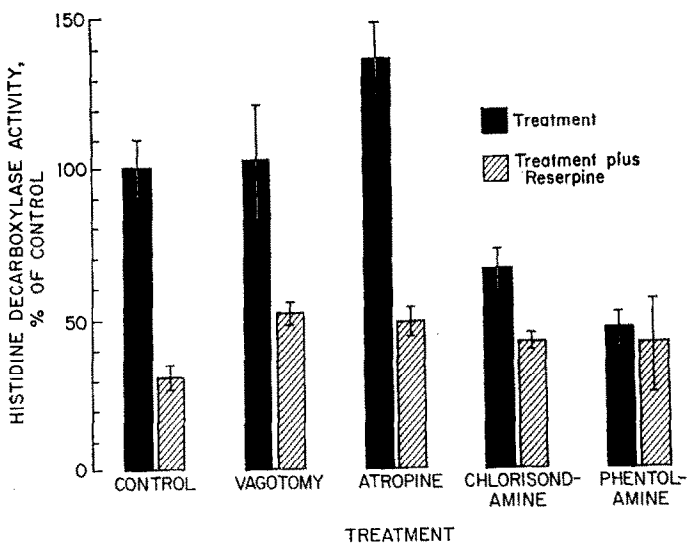

FIG. 4. The effect of various treatments producing parasympathetic and sympathetic blockade on the reserpine induced decrease in histidine decarboxylase activity of rat stomach (glandular portion). The histidine decarboxylase activity of rat stomach after the various treatments is compared with that after treatment plus reserpine as described in the Experimental section. Histidine decarboxylase activities are expressed as a per cent of the mean value of controls (no treatment, no reserpine). Each bar represents the mean value for six rats and vertical lines depict S.E.M.

Effect of parasympathetic or sympathetic blockade on the reduction of histidine decarboxylase activity by reserpine. Since reserpine did not appear to have a direct action on histidine decarboxylase, studies were undertaken to determine whether reserpine acted through the autonomic nervous system. The effects of various surgical and chemical treatments which block the parasympathetic and sympathetic nervous system are shown in Fig. 4. Bilateral gastric vagotomy did not prevent the decrease in histidine decarboxylase activity produced by reserpine. Chemical blockade of the parasympathetic nervous system with atropine did not significantly affect the activity of the enzyme nor did it alter the effect of reserpine on histidine decarboxylase. These results indicated that the effect of reserpine on histidine decarboxylase was not mediated, either centrally or peripherally, through the parasympathetic system.

The possibility that the effect of reserpine on histidine decarboxylase activity was mediated through the sympathetic nervous system was also investigated (Fig. 4). Chlorisondamine, a ganglionic blocking agent, did not prevent the reduction of histidine decarboxylase activity after reserpine. This result was ambiguous, however, since chlorisondamine itself diminished histidine decarboxylase activity. Like reserpine, chlorisondamine reduced the V_{max} of the enzyme by 50 per cent but did not affect the apparent K_m. The peripheral alpha adrenergic blocking agent, phentolamine, did not prevent the action of reserpine, but again these results were ambiguous because phentolamine alone lowered enzyme activity as effectively as reserpine. Since the actions of the ganglionic blocking agent and the alpha adrenergic blocking agent on the histidine decarboxylase activity were similar to that of reserpine, it was possible that these autonomic drugs were acting in a similar manner to reserpine. However, this does not appear to be the case since other agents, including guanethidine, BW 392C60, pargyline, and phenoxybenzamine, which affect the sympathetic nervous system, were found to have no action on the histidine decarboxylase activity in stomach of rat.

DISCUSSION

The suggestion has been made that reserpine lowers the histamine content of rat stomach by direct release of the histamine.[6] The present results raise the possibility that reserpine could also lower the gastric histamine level by reducing the amount or activity of the enzyme that synthesizes histamine.

The mechanism by which reserpine reduced histidine decarboxylase activity in stomach is not known. The inability of the drug to inhibit the enzyme *in vitro* suggested the action of reserpine on histidine decarboxylase might be an indirect one and several possible mechanisms of action were considered. The effect of reserpine on the autonomic nervous system was considered first. After treatment of rats by various means to block the parasympathetic and sympathetic systems, a decrease in histidine decarboxylase activity was still observed after reserpine. Thus, unlike the studies on histamine levels by Kim and Shore,[5] the effect of reserpine on histidine decarboxylase did not require the intact vagus. The studies with chemically sympathectomized rats were not definitive, however, since chemical sympathectomy with chlorisondamine or phentolamine by itself reduced the activity of histidine decarboxylase in stomach. Therefore, it is possible that reserpine also acted by blockade of the sympathetic system.

Since reserpine reduced the amount of enzyme without altering the affinity of the enzyme for its substrate, it seemed possible that reserpine acted by reducing the synthesis of new enzyme. The rapidity with which the histidine decarboxylase activity declined after reserpine treatment was reminiscent of the rapid disappearance of enzyme seen in rat stomach after blockade of histidine decarboxylase synthesis by administration of cyclohexamide.[18] However, an inhibition of protein synthesis would be expected to diminish the levels of DOPA decarboxylase as well and, as seen in the present studies, this was not the case. Studies in this laboratory have shown that reserpine did not alter the incorporation of leucine-^{14}C into liver ribosomal particles (unpublished observations).

Other possible mechanisms of reserpine action were that the drug released histidine decarboxylase from the stomach wall or that it reduced the histidine decarboxylase activity by reduction of the bacterial population of the stomach wall. A part of the histidine decarboxylase in rat stomach is known to be of bacterial origin.[19,20] The mechanism by which reserpine reduced the histidine decarboxylase activity of rat stomach was not completely defined. However, the present studies suggested that the reduction of histidine decarboxylase activity in stomach after reserpine might be one possible mechanism for the decrease in gastric histamine levels observed after treatment with this drug.

The present findings should be evaluated in conjunction with the findings of Rosengren and Svensson[21] whose report on the effect of reserpine and adrenaline on histamine formation in rat stomach was published while the present work was in progress. These authors found that after reserpine or adrenaline there was a transient decrease followed by a several fold increase in the histamine forming capacity of rat gastric mucosa. A large decrease in gastric histidine decarboxylase activity after reserpine injection was not observed by these authors. However, in their studies rats were starved for 16 hr before death. As seen in the present paper, histidine decarboxylase activity was reduced to low levels after 12 hr starvation (Fig. 1b) and it is possible that in stomachs of starved rats reserpine does not lower histidine decarboxylase activity to the extent seen in stomachs of fed rats (Fig. 1a). In fact, the data in Fig. 2 of the present paper showed that reserpine did not appreciably lower histidine decarboxylase activity in stomachs of rats whose histidine decarboxylase activity had been reduced previously by starvation. The present results were similar to those of Rosengren and Svensson in one respect: As seen by the data in Fig. 1a, following the initial decline in histidine decarboxylase activity after administration of reserpine there was a significant rise (2-fold) in histidine decarboxylase activity. This increase in enzyme activity was of a similar magnitude to that observed by Rosengren and Svensson[21] and may be the same phenomenon observed by these authors.

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