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# Effects *in vivo* and *in vitro* of nonsteroidal anti-inflammatory drugs on (rat stomach) histidine decarboxylase*

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Several acidic nonsteroidal drugs, e.g. indomethacin, phenylbutazone, acetylsalicylic acid and fluphenamic acid have been successfully employed in the treatment of inflammatory diseases. Several investigators[1, 2] have shown that these compounds, as well as other salicylates,[3] inhibit *in vitro* the enzyme, histidine decarboxylase (HD), which catalyzes the decarboxylation of histidine to form histamine. The available data[1, 2] suggest that the anti-inflammatory action *in vivo* of there drugs parallels their HD inhibitory activity *in vitro*.

The present study confirms the inhibition *in vitro* by these drugs of specific HD from rat glandular stomach (RS)[4–6] and mouse mastocytoma (MMCT),[7, 8] but demonstrates that anti-inflammatory acids increase HD activity *in vivo*.

## METHODS AND MATERIALS

Fundic stomach from CD male rats (250–300 g) and mastocytomas from $LAF_1$ female mice (25–30 g) were employed in experiments *in vitro*, whereas only the former tissue was used in obtaining data *in vivo*. *In vivo* and *in vitro* in this paper refer to the method of evaluation of drug action. In experiments *in vivo*, the compounds were administered to rats for various periods of time before tissues were removed and assayed for HD activity. With testing *in vitro*, drugs were dissolved in the buffer of the enzyme assay system. MMCT were harvested 1 month after subcutaneous implantations of tumor tissue. The original solid mastocytomas were taken from $LAF_1$ female mice provided by Dr. J. P. Green of the Department of Pharmacology, Mount Sinai School of Medicine, New York, N.Y.

All tissue was removed after the animals were killed by cervical dislocation. The MMCT's were pooled and frozen until homogenized, but RS homogenates were prepared from fresh tissue and immediately assayed for HD activity.

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Tissue homogenates were prepared in cold distilled water (9 ml/stomach or as a 10% MMCT solution) with a VirTis "45" homogenizer. RS homogenates were prepared individually for studies *in vivo* and as a pooled sample for testing *in vitro*.

Homogenates were centrifuged at 55,000 *g* for 30 min in the Spinco ultracentrifuge. Two-ml aliquots of the stomach supernatants were used in the assay of RSHD. The supernatant from the MMCT preparation was lyophilyzed and stored as a dry powder. When reconstituted with $H_2O$ it was diluted to contain 20 mg per ml of dry powder and 1 ml was used.

HD activity was assayed by a modification of Kobayashi's[9] radioactive method. The reaction mixture contained D,L-histidine-1-$^{14}C$ (12·8 $\mu$g or 0·1 $\mu$c/0·1 ml), pyridoxal-5-phosphate (50 $\mu$g/0·1 ml), aminoguanidine (75 $\mu$g/0·1 ml) and 1·7 ml of 0·2 M potassium phosphate buffer (pH 7·2). The final volumes after adding the RS or the MMCT enzyme preparations were 4 ml and 3 ml respectively.

$^{14}CO_2$ evolved from the reaction was trapped by hyamine 10x (Packard) in the center well of a 50-ml Erlenmeyer flask. An aliquot of the hyamine was dissolved in an alcohol/toluene fluor mixture (Packard's PPO-POPOP) and analyzed for radioactivity in a Packard Tri-Carb liquid scintillation counter. Activity *in vivo* is expressed as cpm of $^{14}CO_2$ produced per gram of tissue, wet weight. Inhibition *in vitro* is expressed as the negative logarithm of the concentration of inhibitor which gives 50 per cent inhibition or the $pI_{50}$. All figures represent values above substrate blank.

The drugs used in the experiments were indomethacin (INDO) from Merck, Sharpe & Dohme; phenylbutazone (PB) from Geigy; acetylsalicylic acid (ASA); fluphenamic acid (FLA) from Parke-Davis; and NSD 1055 (4-bromo-3-hydroxylbenzyloxyamine) from Smith & Nephew, Ltd. When tested *in vitro*, the compounds were dissolved in the phosphate buffer. When tested *in vivo*, INDO (20 mg/kg), ASA (800 mg/kg), PB (450 mg/kg) and FLA (150 mg/kg) were suspended in carboxymethylcellulose (CMC) and administered orally. NSD 1055 (150 mg/kg) was given i.p. in a saline solution. Control animals were given the appropriate test vehicle.

## RESULTS AND DISCUSSION

Table 1 shows the relative potency of the compounds in the system *in vitro*. The four acidic nonsteroidal anti-inflammatory drugs all showed weak inhibitory activity at final concentrations between $10^{-2}$ and $10^{-4}$M. However, NSD 1055 proved to be a potent inhibitor of HD confirming previous findings of others.[5, 10] Rat stomach HD could be more easily inhibited than MMCT HD, as seen by the higher $pI_{50}$'s.

TABLE 1. INHIBITION *IN VITRO* OF HISTIDINE DECARBOXYLASE

| | $pI_{50}$* | |
|---|---|---|
| | Mouse mast cell tumour | Rat stomach |
| Indomethacin | 3·0 | 3·9 |
| Acetylsalicylic acid | 2·2 | 2·5 |
| Phenylbutazone | 2·9 | 3·1 |
| Fluphenamic acid | 3·5 | 3·7 |
| NSD1055 | 6·3–7·0 | 8·0 |

* $pI_{50}$ is defined as the negative logarithm of the concentration of inhibitor which gives 50 per cent inhibition.

Initial studies with nonfasted rats showed that 20 mg per kg of INDO decreased gastric HD activity 16–72 hr postdrug administration (Fig. 1). It was noted, however, that the stomachs of the drug-treated animals did not contain food. Since Kahlson[6] has already shown a suppression of histamine-forming capacity (HFC) in the fasted animal, it was suspected that the results obtained with indomethacin might reflect a drug-induced inanition. When fasted animals were compared to nonfasted ones, it was seen that gastric HD activity of the former was lower at 16 hr and remained low for the duration of the 72-hr fast (Fig. 1). Further, the INDO-treated animals displayed significantly higher levels of HD activity than those observed in the fasted animals at all times tested.

A second nonsteroidal anti-inflammatory drug, ASA (800 mg/kg administered 3 hr prior to autopsy), elicited a 3-fold increase in gastric HD in nonfasted rats (Table 2). The 3-hr period was

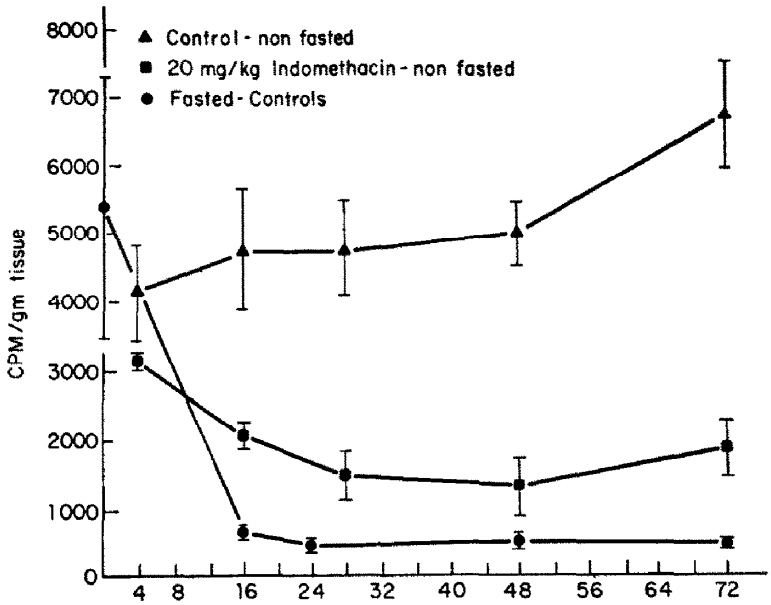


FIG. 1. Effects of indomethacin and fasting on rat stomach HD *in vivo*. Mean value ± the standard error is shown. See text for explanation.

chosen because it has been shown[11] that ASA administration yields serum levels which peak and plateau over a 45-min to 5-hr period in 24-hr fasted rats.

In contrast to the results obtained with ASA, NSD 1055 (150 mg/kg) given to nonfasted rats attenuated gastric HD activity 3 hr postinjection (Table 2). Consistent with this finding, others have shown that NSD 1055 decreased gastric histamine levels[10] and hypersecretion of gastric acids in[12] rats and lowered the increased urinary histamine observed in humans with systemic mastocytosis.[13]

The inhibition of HD by NSD 1055 was not a reflection of drug-induced inanition since the amount

TABLE 2. EFFECTS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND NSD 1055 ON RAT STOMACH HD ACTIVITY *IN VIVO* IN NONFASTED AND FASTED ANIMALS

| Drug | mg/kg | Route | Duration of drug $R_x$ (hr)* | Hours fast | RSHD activity† | Significance‡ |
|---|---|---|---|---|---|---|
| Control | | po | 3 | | 12,700 ± 970 | |
| ASA | 800 | po | 3 | | 38,100 ± 4800 | $P < 0.05$ |
| Control | | ip | 3 | | 24,000 ± 3800 | |
| NSD 1055 | 150 | ip | 3 | | 5900 ± 1600 | $P < 0.01$ |
| Control | | po | 48 | 48§ | 666 ± 101 | |
| INDO | 20 | po | 48 | 48 | 1600 ± 270 | $P < 0.01$ |
| Control | | po | 3 | 24‖ | 375 ± 74 | $P < 0.01$ |
| ASA | 800 | po | 3 | 24 | 2100 ± 89 | |
| Control | | po | 3 | 24 | 396 ± 188 | |
| PB | 450 | po | 3 | 24 | 1700 ± 110 | $P < 0.01$ |
| Control | | po | 3 | 24 | 373 ± 84 | |
| FLA | 150 | po | 3 | 24 | 669 ± 73 | $P < 0.05$ |

* Length of time between drug administration and sacrifice.

† RSHD is expressed as CPM$^{14}CO_2$ produced per g tissue (wet weight) ± standard error of the mean for 5 rats per group.

‡ P values derived from Student's *t*-test.

§ Drug given at onset of fast.

‖ Drug given after 24-hr fast.

of food present in the stomachs of the NSD-1055-treated animals and their controls was similar. Indeed, no apparent differences were observed in the degree of gastric distention produced by NSD 1055 and ASA treatment.

To avoid the problem of drug-induced inanition, INDO and ASA, as well as PB and FLA, were tested in fasted rats. INDO was given at the onset of a 48-hr fast and HD activity measured at the end of that period. The other 3 anti-inflammatory acids were given to 24-hr fasted rats, and the enzyme activity measured 3 hr later. Table 2 shows that all acids tested (INDO, ASA, PB, FLA) significantly increase RSHD activity when compared with their respective controls. The finding with phenylbutazone is in agreement with the work of Schwartz *et al.*[14]

In examining the possible mechanisms of action for the increased response in enzyme activity, the role of toxicity was considered. Acetylsalicylic acid was given orally at 800, 400 or 100 mg/kg to 24-hr fasted rats. The increase in HD activity noted 3 hr later was the same with the near toxic dose of 800 mg/kg and the lower nontoxic dose of 400 mg/kg. The lowest dose of 100 mg/kg did not influence HD activity. These results appear to exclude the role of drug toxicity.

Since it has been demonstrated that corticoids[12, 15] as well as stressful stimuli[16] will increase HD, it was important to determine whether the increased response in HD was due to an indirect effect of the drugs mediated by adrenal stimulation. Consequently, male rats were adrenalectomized and maintained on saline and food *ad libitum* for 16 days before being treated with drugs. On the morning of day 16, half the number of adrenalectomized rats were given 800 mg/kg acetylsalicylic acid orally. The remaining half of the operated rats and normal intact rats were given vehicle only. Gastric HD measured 3 hr later showed that the adrenalectomized animal retained its ability to respond to the drug-induced elevation in enzyme activity (Fig. 2). The ASA-induced increase observed in adrenalectomized rats (2·5-fold) was only slightly different from the 3-fold increase seen in the intact animal treated with ASA (Table 2).

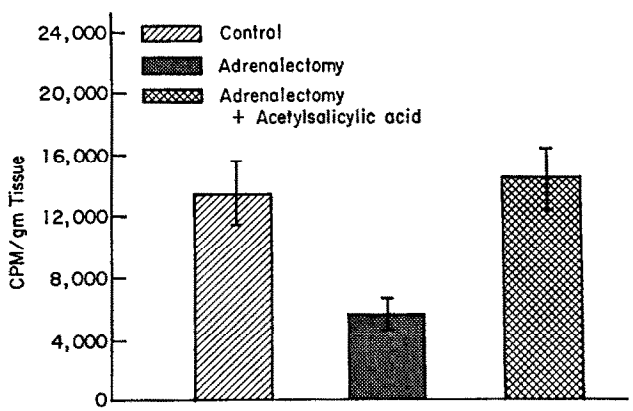


FIG. 2. Effects of adrenalectomy on rat stomach HD response to acetylsalicylic acid treatment *in vivo*. Mean value ± the standard error is shown. See text for experimental details.

It is interesting to note that adrenalectomy, itself, significantly lowers gastric HD activity. Whether this decrease is a direct effect of the lack of adrenal hormones on the enzyme or a reflection of the metabolic changes that occur in a 16-day adrenalectomized animal remains to be clarified.

It has been shown that several nonsteroidal anti-inflammatory agents increase stomach HD *in vivo* and decrease activity *in vitro*. Further, this effect *in vivo* is observed in fasting animals using INDO, ASA, FLA, PB and in fed rats employing ASA. It is difficult, therefore, to continue to explain the anti-inflammatory actions of those drugs on the basis of inhibition of HD *in vitro* unless the action of the nonsteroidal anti-inflammatory drug on HD is inhibitory at sites other than stomach. Indeed, Schayer[17] has shown that rat pyloric stomach is different from other tissues in that corticoids increase histamine formation in the former while having an opposite effect in the latter. In any case, the adrenal hormones are probably not the primary mediators of the ASA-induced increase in gastric HD since the effect was observed in adrenalectomized rats.

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# Methionyl-lysyl-bradykinin release from plasma kininogen by plasmin

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EVIDENCE that incubation of plasmin with plasma globulins results in the release of vasoactive substance was given as early as 1950.[1] Later other authors also found that plasmin is involved in the release of kinin in plasma.[2–5] More recent experiments leave no doubt as to the kininogenase activity of plasmin.[6–9] Our previous work indicated that plasmin plays an important part in the release of kinin in plasma.[9] This observation led us to try and identify the kinins formed by this enzyme when incubated with a purified substrate. The results of these experiments are described in this paper.

*Material and methods.* Bradykinin acetate, synthetic, was obtained from Sigma Chemical Company, St. Louis, U.S.A.; Methionyl-lysyl-bradykinin was a gift from Dr. Schröder, A.G., Berlin; Kallidin was a gift from Sandoz, Ltd., Basel, Switzerland. Kininogen II[10] was prepared as previously described[11] from the supernatant of horse plasma precipitated at 6·7% (v/v) saturation with polyethyleneglycol, mol. wt. 6000.[12] Plasmin was prepared by the method of Robbins and Summaria[13] with minor modifications[9] from Fraction B (Cohn's II + III) from human plasma. Trypsin, crystalline, Spofa, Czechoslovakia, was used. The activity of the kinins released was assayed on the guinea pig ileum.[11] Incubation of kininogen with plasmin was made as follows: 20 ml solution of kininogen (15 mg protein) were incubated with 6 casein units[13] of plasmin for 150 min, as recommended by Budnitskaya *et al.*[9] The mixture was then immediately frozen and lyophilized. For the separation of