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Effects of brocresine (NSD-1055) and cycloheximide on amino acid decarboxylase activities in gastric mucosa of normal and vagally denervated rats

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Summary

1. Histidine decarboxylase activity of rat stomach fluctuates depending upon the functional state of the stomach. This varying enzyme activity poses special problems in assessing the effectiveness of enzyme inhibitors. After vagal denervation gastric histidine decarboxylase is markedly activated and remains at a high, stable level, which is unaffected by the functional state of the stomach. Thus it appears that vagally denervated rats are well suited for studies on histidine decarboxylase inhibitors.

2. *In vivo*, brocresine (NSD-1055) was found to be a more effective inhibitor of gastric DOPA decarboxylase than of gastric histidine decarboxylase. With the fairly high dose given (200 mg/kg) the inhibition of histidine decarboxylase was at most 75–85% and quite short-lasting. The DOPA decarboxylase activity, which was not affected by vagal denervation, was inhibited more than 95% by brocresine; this inhibition was longer-lasting.

3. Cycloheximide, which probably lowers gastric histidine decarboxylase activity by inhibiting enzyme synthesis, was maximally effective at a dose level as low as 1 mg/kg. Gastric DOPA decarboxylase was not inhibited by cycloheximide. Vagotomized rats and control rats responded similarly.

4. Combined treatment of vagally denervated rats with brocresine and cycloheximide resulted in a rapid and persistent reduction of the histidine decarboxylase activity. It is concluded that the failure of brocresine alone to induce a lasting inhibition of histidine decarboxylase is due to continuous, rapid synthesis of new enzyme.

5. The calculated half-life of gastric histidine decarboxylase was 75 min in vagally denervated rats and 45 min in normal fasted rats. The results suggest that the increased enzyme activity after vagal denervation is caused by an increased rate of enzyme synthesis.

Introduction

Histidine decarboxylase activity of rat stomach (EC 4.1.1.22) varies depending upon the functional state of the stomach (Kahlson, Rosengren, Svahn & Thunberg, 1964). In contrast, the activity of gastric DOPA decarboxylase (EC 4.1.1.26) does not seem to vary at all (Aures, Håkanson & Schauer, 1968; Aures, Davidson & Håkanson, 1969). Fasting reduces the gastric histidine decarboxylase activity while feeding or treatment with gastrin causes marked enzyme activation. The variation in the histidine decarboxylase activity poses special problems in assessing the effectiveness of inhibitors of this enzyme. Vagal denervation of the stomach results

in a powerful and persistent activation of the histidine decarboxylase. After vagotomy the enzyme activity is no longer affected by fasting, feeding or treatment with gastrin (Håkanson & Liedberg, 1970, 1971). Therefore, vagotomized rats appear particularly suitable for studies on histidine decarboxylase inhibitors.

Brocresine (NSD-1055) has been reported to inhibit histamine formation in the rat and to reduce the level of gastric histamine (Levine, Sato & Sjoerdsma, 1965). Its effectiveness as an inhibitor of histidine decarboxylase has been questioned (Johnston & Kahlson, 1967; Kobayashi, Kupelian & Maudsley, 1970; Mesch & Sewing, 1971). At best the brocresine-induced inhibition appears to be only partial and short-lasting (Kobayashi *et al.*, 1970). Brocresine is believed to interact with pyridoxal-5′-phosphate (Reid & Shepherd, 1963; Shepherd & Mackay, 1967; Leinweber, 1968; Ellenbogen, Markley & Taylor, 1969) which is the coenzyme of mammalian histidine decarboxylase (see Håkanson, 1963). Since pyridoxal-5′-phosphate is essential for most amino acid metabolism, it may be assumed that brocresine exerts a multitude of actions in the body (see e.g. Kobayashi *et al.*, 1970).

Cycloheximide, an inhibitor of protein synthesis, did not alter the fasting levels of histidine decarboxylase in rat stomach, but completely inhibited the enzyme activation that follows re-feeding or gastrin treatment (Kobayashi & Maudsley, 1968; Snyder & Epps, 1968). These results indicate that the increase in enzyme activity following stimulation is due to enhanced synthesis of new enzyme protein. From experiments with cycloheximide given to freely fed rats the half-life of gastric histidine decarboxylase was estimated to be 100–126 minutes. This value was identical with that calculated from the decline of the enzyme activity that follows its activation by feeding or by a single injection of gastrin (Kobayashi & Maudsley, 1968; Snyder & Epps, 1968).

In the present study the effects of brocresine and cycloheximide on the activities of gastric mucosal (oxyntic gland area) histidine decarboxylase and DOPA decarboxylase were compared in normal and vagally denervated rats.

Methods

Animals. A total of 317 male Wistar rats (weighing 150–250 g) were used. Vagal denervation was performed on 126 rats by cutting both vagal trunks immediately below the diaphragm. A pyloroplasty was always made at the same time to prevent gastric dilation. Operated rats were allowed to recover for at least 3 weeks before being used in the experiments.

Drugs. Brocresine (4-bromo-3-hydroxybenzyloxyamine dihydrogenphosphate; NSD-1055), a gift from Cyanamid Int., Wayne, New Jersey, was dissolved in 0·9% w/v NaCl (40 mg/ml) and injected intraperitoneally in a dose of 200 mg/kg body weight. Cycloheximide (actidione), purchased from Upjohn Co., Kalamazoo, Mich., was dissolved in 0·9% w/v NaCl (saline) and injected intraperitoneally in doses of 0·1–50 mg/kg body weight (in a volume of 0·5 ml/kg). In one experiment where both drugs were used in combination cycloheximide was given 5 min after brocresine.

Determination of histidine decarboxylase and DOPA decarboxylase activities

All rats were fasted for 48 h (with free access to water) before decapitation under light ether anaesthesia. The stomachs were immediately removed, cut open

along the greater curvature and washed with ice-cold saline. The mucosa of the oxyntic gland area was scraped off, weighed and homogenized in ice-cold 0·1 M phosphate buffer, pH 6·9, to a final concentration of 100 mg tissue (wet weight) per ml. The homogenate was centrifuged at 10,000 × *g* for 10 min at 0° C. The supernatant was used as the enzyme source. Histidine decarboxylase was assayed by incubating aliquots (0·4 ml) of the mucosal extract with 1-^{14}C-labelled L-histidine (4×10^{-4}M ; 1·3 mCi/mmol ; New England Nuclear) in the presence of pyridoxal-5′-phosphate (10^{-5}M) and glutathione (5×10^{-4}M) in a total volume of 0·5 ml at 37° C for one hour under nitrogen. DOPA decarboxylase was assayed by using 1-^{14}C-labelled DL-DOPA (8×10^{-4}M ; 0·2 mCi/mmol ; New England Nuclear). Incubation conditions were as above except that 0·2 ml aliquots of the tissue extracts were used and the incubation volume was made up to 0·5 ml with the phosphate buffer. The amount of $^{14}CO_2$ produced during the reactions was determined radiometrically (for details see Håkanson, 1970). The results were corrected for non-enzymatic decarboxylation determined by incubating boiled tissue extracts. Duplicate assays were run in all experiments. Enzyme activities were expressed as $^{14}CO_2$ produced in nmol per mg per hour.

Results

Effect of brocresine

With the dose given (200 mg/kg i.p.) brocresine caused inhibition of gastric histidine decarboxylase activity in both normal and vagally denervated rats. In both groups of animals the effect of brocresine was maximal 30–90 min after the injection (Fig. 1) at which time the enzyme activity was reduced by about 75–85%.

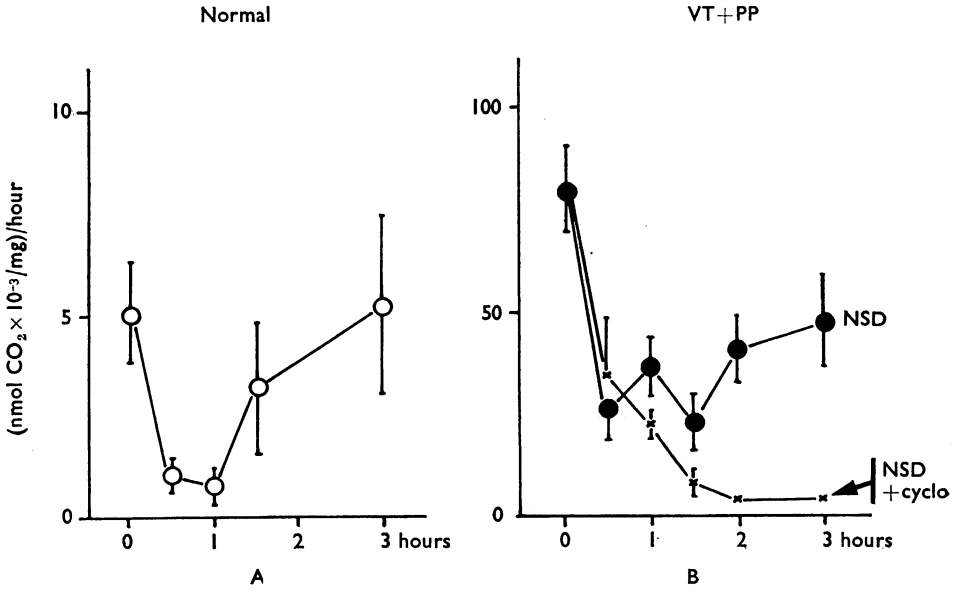

FIG. 1. Effect of brocresine (NSD, 200 mg/kg i.p.) on histidine decarboxylase activity in the oxyntic gland mucosa of normal (A:○) and vagally denervated (B:●) rats. VT, Truncal vagotomy ; PP, pyloroplasty. Effect of combined treatment with brocresine (200 mg/kg) and cycloheximide (cyclo, 10 mg/kg) in vagally denervated rats (B:+). Injections were made at zero time. Enzyme activities are expressed as (nmol $CO_2 \times 10^{-3}$ produced per mg mucosa)/hour. Means ± S.E.M. At least 5 animals in each group.

TABLE 1. *Effects of brocresine and cycloheximide on the activity of rat stomach DOPA decarboxylase**

Time after injection (min)	DOPA decarboxylase activity (nmol $CO_2 \times 10^{-3}$/mg)/h, mean±S.E.M. (*n*) Brocresine 200 mg/kg, i.p.	Cycloheximide 10 mg/kg, i.p.
0	153±36 (4)	153±21 (16)
20	—	185±35 (6)
30	0 (4)	—
40	—	229±40 (6)
60	3± 1 (4)	187±22 (8)
90	4± 1 (4)	228±30 (6)
120	—	204±43 (5)
180	5± 2 (4)	98±17 (7)
240	—	136 (2)
300	—	115 (2)

* Normal, fasted rats were used.

In normal rats the enzyme activity was back to normal after 3 h ; in the vagotomized rats the enzyme recovery was slower. The gastric DOPA decarboxylase activity of normal rats was very effectively inhibited by brocresine. The enzyme activity was reduced by more than 95% after 30 minutes. This inhibition lasted for the entire period of observation (Table 1).

Effect of cycloheximide

Vagally denervated rats were used for assessment of the histidine decarboxylase inhibition induced by cycloheximide in doses of 0·1–50 mg/kg given to the animals 3 h before killing. With the 50 mg/kg dose the rats were prostrate and had severe diarrhoea. With the lower doses, however, the rats exhibited no toxic symptoms during the 3 h of observation. Cycloheximide was found to cause a marked reduction of gastric histidine decarboxylase activity (almost 50% at a dose level of 0·1 mg/kg (Table 2)). Maximal enzyme inhibition was observed at 1 mg/kg. Contrary to previous reports (Snyder & Epps, 1968) cycloheximide lowered the histidine decarboxylase activity in normal, fasted rats (Table 2). The activity of gastric DOPA decarboxylase in normal and vagotomized rats was not significantly affected by all doses of cycloheximide (Tables 1 and 2). A dose of 10 mg/kg was used in the subsequent studies on the effect of cycloheximide (see below). After treatment with cycloheximide (10 mg/kg) the histidine decarboxylase activity decreased with time as shown in Figure 2.

TABLE 2. *Effect of various doses of cycloheximide on the activities of histidine decarboxylase and DOPA decarboxylase**

Cycloheximide mg/kg	Histidine decarboxylase (nmol $CO_2 \times 10^{-3}$/mg)/h, mean±S.E.M. (*n*) Control	Vagotomy	DOPA decarboxylase Control	Vagotomy
0	5·8±1·5 (12)	78·7±10·1 (13)	153±21 (16)	175±31 (4)
0·1	2·5±1·6 (3)	42·5±15·3 (7)	102±13 (6)	301±69 (2)
1·0	1·1±0·5 (3)	17·6± 6·8 (6)	127±27 (8)	304±118 (3)
10·0	0·4±0·2 (11)	18·1± 4·9 (5)	98±17 (7)	288±72 (2)
50·0	0 (3)	14·0± 6·9 (6)	—	—

* The rats (fasted) were killed 3 h after the injections.

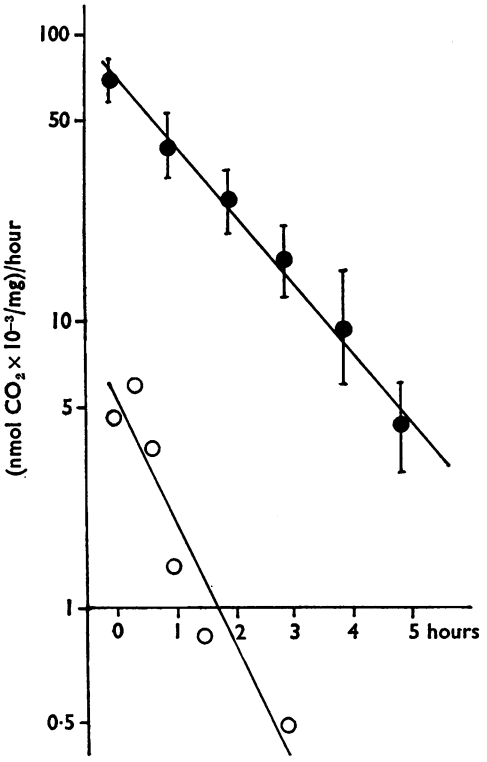

FIG. 2. Histidine decarboxylase activity in the oxyntic gland mucosa of normal (○) and vagally denervated (●) rats at various times after the injection of cycloheximide (10 mg/kg). Logarithmic scale on the ordinate. At least 5 animals in each group. The values for the denervated rats are the means of the logarithms ± S.E.M., the values for the normal rats are the logarithms of the means (because in some cases the enzyme activities recorded were 0).

Effect of brocresine+cycloheximide

Vagally denervated rats were injected with both brocresine (200 mg/kg) and cycloheximide (10 mg/kg). The activity of gastric histidine decarboxylase was rapidly reduced and the activity remained low for the 3 h of observation (Fig. 1).

Rate of histidine decarboxylase turnover in normal and vagally denervated rats

When the enzyme activities recorded after injection of cycloheximide were plotted on a logarithmic scale against time the curves appeared to be linear (Fig. 2) suggesting a first order reaction. The correlation and regression coefficients for the lines were calculated from the experimental data. The lines could be fitted to the equation $\log m = \log m_o - \frac{k}{2{\cdot}303} t$, where m = enzyme activity at given time t, m_o = enzyme activity at $t = 0$, and k = velocity constant (see e.g. Wallwork 1960). Rewriting the equation gives $k = \frac{2{\cdot}303}{t} \log \frac{m_o}{m}$; $m = \frac{m_o}{2}$ gives $t_{\frac{1}{2}} = \frac{2{\cdot}303}{k} \log 2$ where $t_{\frac{1}{2}}$ is the enzyme half-life. In the vagally denervated rats k was $0{\cdot}55 \times h^{-1}$, $t_{\frac{1}{2}}$ 1·26 h and the correlation coefficient 0·995. In the normal rats k was $0{\cdot}94 \times h^{-1}$, $t_{\frac{1}{2}}$ 0·73 h and correlation coefficient 0·939.

In the fasted rat (whether normal or denervated) the rate of enzyme synthesis must equal the rate of enzyme break-down to maintain a constant enzyme level. An estimate of the rate of enzyme synthesis can be made from the measured rates of inactivation if the following assumptions are made: the enzyme activity is proportional to the concentration of enzyme, and the rate of enzyme inactivation is the same in cycloheximide-treated as in untreated rats.

From experimental data the rate of enzyme synthesis (v) in normal and in vagally denervated rats was calculated from the formula $v=km_0$ and found to be 4·7 and 43 arbitrary enzyme units/h, respectively, i.e. the rate of synthesis appears to be 9 times higher in the denervated rats.

Discussion

Brocresine (NSD-1055) is a very effective inhibitor of mammalian histidine decarboxylase *in vitro* (Levine *et al.*, 1965 ; Shepherd & Mackay, 1967 ; Leinweber, 1968 ; Kobayashi *et al.*, 1970). The effectiveness of brocresine *in vivo*, however, has been questioned (Johnston & Kahlson, 1967 ; Mesch & Sewing, 1971). In the present study, brocresine (200 mg/kg i.p.) was found to lower the activity of gastric histidine decarboxylase by about 75–85% but only for a fairly short period of time. The concomitant inhibition of gastric DOPA decarboxylase was more pronounced (>95%) and much longer-lasting. It is suggested that *in vivo* brocresine is a more effective inhibitor of gastric DOPA decarboxylase than of gastric histidine decarboxylase. Levine & Watts (1966) first reported that brocresine is an irreversible inhibitor of mammalian histidine decarboxylase. Subsequently Leinweber (1968) showed that brocresine is competitive relative to both substrate and coenzyme, and that inhibition could be reversed by dialysis or gel filtration. Therefore it must be emphasized that the experimental conditions used do not simulate *in vivo* conditions. Pyridoxal-5′-phosphate is added in excess in the enzyme assay and the results do not give any information on the enzyme activity *in situ*. The transient nature of the reduction of gastric histidine decarboxylase activity seen with brocresine may be attributed to inactivation or elimination of the inhibitor. This might occur if the inhibitor is disengaged from the cofactor or the inhibitor-cofactor complex from the apoenzyme or if new enzyme protein is synthesized. The finding that the brocresine-induced inhibition of gastric DOPA decarboxylase, which presumably has a similar chemical basis, is long-lasting speaks against the first two mechanisms.

Cycloheximide, which is a potent protein synthesis inhibitor, reduced the gastric histidine decarboxylase activity of vagotomized rats by approximately 50% 3 h after injection of 0·1 mg/kg. The enzyme inhibition was maximal at a dose of 1 mg/kg. Even in normal fasted rats cycloheximide (10 mg/kg) caused a gradual decline in enzyme activity which is in contrast to observations of Snyder & Epps (1968). The activity of gastric DOPA decarboxylase, on the other hand, was not significantly lowered by cycloheximide. In contrast to treatment with brocresine alone, the combined treatment with brocresine and cycloheximide resulted in a more pronounced and persisting inhibition of the histidine decarboxylase activity. It is concluded that the incomplete inhibition and rapid return towards normal enzyme activities observed after treatment with brocresine alone is caused by continuous synthesis of new enzyme protein.

The activity of gastric histidine decarboxylase is markedly affected by a variety

of physiological and pharmacological stimuli (Kahlson *et al.*, 1964; Kahlson, Rosengren & Thunberg, 1967; Lilja & Svensson, 1967; Aures, *et al.*, 1968). Prolonged fasting causes a profound reduction in the enzyme activity. This reduction is reversed by feeding or by the injection of gastrin. The rapid changes in the enzyme activity make it difficult to obtain quantitative information on the effectiveness of histidine decarboxylase inhibitors. Vagal denervation which is without effect on gastric DOPA decarboxylase activity, causes a marked and lasting activation of gastric histidine decarboxylase (Håkanson & Liedberg, 1970, 1971). Moreover, after vagotomy, refeeding or pentagastrin treatment causes no further activation of the enzyme. Consequently, gastric histidine decarboxylase activity remains at a high, persistently stable level after vagotomy. There was no evidence that vagally denervated rats responded differently to treatment with enzyme inhibitors than normal fasted rats. These results support the view that vagally denervated rats are well suited for studies on the effectiveness of histidine decarboxylase inhibitors.

Cycloheximide has been used in experiments designed to give information on the half-life of gastric histidine decarboxylase (Kobayashi & Maudsley, 1968; Snyder & Epps, 1968). In those experiments freely fed rats were given cycloheximide (50 mg/kg) by intraperitoneal injection. The decline of the enzyme activity was plotted semi-logarithmically against the time elapsed after injection and from this curve the enzyme half-life was calculated to be between 100 (Kobayashi & Maudsley, 1968) and 108 min (Snyder & Epps, 1968). Inhibition of protein synthesis by cycloheximide might influence the degradation as well as the synthesis of gastric histidine decarboxylase. The enzyme half-life was similar to that calculated by following the decline in enzyme activity that follows after the activation induced by feeding or by injection of gastrin. This suggests that cycloheximide inhibits the synthesis much more than the degradation of the enzyme (see Snyder & Epps, 1968). In the present study, the half-life of gastric histidine decarboxylase was calculated in a similar manner, using cycloheximide (10 mg/kg) and comparing the values obtained with normal fasted rats with those from vagally denervated rats. The enzyme half-life in normal, fasted rats was 45 min; the corresponding value for vagally denervated rats was 75 minutes. Assuming a complete inhibition of enzyme synthesis following cycloheximide treatment, it can be estimated that the number of enzyme molecules produced per unit time in vagotomized rats is 9 times that produced in normal fasted rats.

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