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The effect of brocresine (NSD-1055) on the histidine decarboxylase activity in the rat gastric mucosa*

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The effect of the histidine decarboxylase inhibitor brocresine (NSD-1055) on the specific histidine decarboxylase in the gastric mucosa was investigated in rats. The inhibiting potencies of brocresine were compared after oral and intraperitoneal administration with and without 2-deoxy-D-glucose. Furthermore, different doses of brocresine were added directly to an incubation medium containing an homogenate of the gastric mucosa of untreated animals. The gastric histidine decarboxylase of the brocresine-pretreated animals was not inhibited. Addition of brocresine to the incubation medium produced a dose dependent blockade of the enzyme. 50% inhibition was accomplished by a concentration of $1{\cdot}4 \times 10^{-6}$M. The results demonstrated an inhibition of the rat stomach histidine decarboxylase *in vitro*, but not *in vivo*, indicating the inability of brocresine to interfere with the biosynthesis of histamine in the rat stomach.

4-Bromo-3-hydroxybenzyloxyamine dihydrogenphosphate (brocresine, NSD-1055) has been reported to be a potent histidine decarboxylase inhibitor. Its effectiveness *in vitro* is well established (e.g. Levine & Watts, 1966; Johnston & Kahlson, 1967; Thayer & Martin, 1967). *In vivo*, however, the effect is not unequivocal (Levine, Sato & Sjoerdsma, 1965; Levine, 1966; Johnston & Kahlson, 1967; Johnson & Burfine, 1968; Johnson, 1969).

We have investigated the effect of different routes of administration of brocresine on the histidine decarboxylase of the rat stomach and the effect of different concentrations of brocresine on the histidine decarboxylase of the rat stomach was studied *in vitro*.

METHODS

The experiments were performed in a randomized order on female rats (FW 49, 180–340 g). In the first group, 10 animals were injected with brocresine (100 mg/kg, i.p.) 4 h before the experiments, 10 control animals received 10 ml/kg phosphate buffer in the same way. In the second group, 10 animals were given an oral dose of brocresine (100 mg/kg) by gastric tube 4 h before the experiments. The control animals received 10 ml/kg phosphate buffer in the same way. Animals in the third group were treated similarly to those in group 1 with the addition of an injection of 2-deoxy-D-glucose (100 mg/kg, i.p.). Animals in group 1–3 were re-fed after pretreatment. In separate experiments, brocresine, to give final concentrations of $1{\cdot}3 \times 10^{-8}$, 4×10^{-7} and 4×10^{-6}M was added to an incubation medium (N = 10 for each concentration).

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Preparation of mucosa homogenates and the incubation medium. The abdominal cavity was opened under ether anaesthesia and the stomach dissected. The glandular stomach was separated from the rumen and opened along the lesser curvature. After rinsing with demineralized water the gastric mucosa (about 0·63 g) was scraped off, suspended in 20 ml phosphate buffer and homogenized in a glass homogenizer.

The incubation medium [(M) phosphate buffer (pH 7·0) 1×10^{-1}, L-histidine 5×10^{-4}, aminoguanidine sulphate $1{\cdot}3 \times 10^{-4}$, nicotinamide 1×10^{-2}; pyridoxal-5-phosphate 10 μg/ml, glucose 10 mg/ml] was preincubated for 30 min and after addition of the substrate incubated in a Warburg apparatus for 60 min at 37° under nitrogen (the main vessel contained homogenate 1·0, pyridoxal-5-phosphate 0·1, aminoguanidine sulphate 0·5, nicotinamide 0·5, glucose 0·4 ml. The side-arm contained either L-histidine or phosphate buffer. The final volume was 3 ml).

Histamine extraction and estimation. At the end of the incubation period the reaction was stopped by addition of 9 volumes 0·4N perchloric acid. Histamine was estimated fluorometrically (Shore, Burkhalter & Cohn, 1959).

Compounds. 4-Bromo-3-hydroxybenzyloxyamine dihydrogenphosphate (brocresine, NSD-1055), American Cyanamid Company, Pearl River, N.Y. and Smith & Nephew Research Ltd., Gilston; pyridoxal-5-phosphate and 2-deoxy-D-glucose, EGA-Chemie Steinheim; aminoguanidine sulphate, nicotinamide and *o*-phthaldialdehyde, Fluka, Buchs; L-histidine, Schuchardt, Munich.

The histidine decarboxylase is expressed in nmol of histamine formed per g mucosa per hour. For statistical analysis all results were treated with the *t*-test for pairs.

RESULTS

The histidine decarboxylase activity of the rat gastric mucosa of treated and untreated animals is summarized in Table 1. It is evident that brocresine has no effect on the histidine decarboxylase activity of the rat gastric mucosa when it was given by different routes of administration. There was also no brocresine effect when 2-deoxy-D-glucose was given.

Table 1. *The effect of brocresine on the rat gastric mucosal histidine decarboxylase activity.* A = amount of histamine present in the tissue or formed by decarboxylation of endogenous histidine (or both) expressed in nmol histamine per g mucosa. B = the amount of histamine present in the tissue or formed by decarboxylation of endogenous and exogenous histidine (or both) expressed in nmol histamine per g mucosa. B–A = histidine decarboxylase activity expressed in nmol histamine formed per 1 h by 1g tissue. Values mean ± s.e.

Pretreatment	n	A	B	B–A	*P*
Control	10	922·4 ± 89·1	1149·1 ± 77·0	226·5 ± 29·4	>0·5
Brocresine i.p.	10	939·0 ± 89·2	1161·0 ± 93·2	221·8 ± 31·8	
Control	10	824·9 ± 71·8	1011·8 ± 79·7	186·9 ± 52·8	>0·5
Brocresine oral	10	747·3 ± 79·2	963·5 ± 103·3	216·3 ± 40·7	
Control + 2-Deoxy-D-glucose	10	938·3 ± 82·0	1075·7 ± 91·3	157·5 ± 33·6	>0·5
Brocresine i.p. + 2-Deoxy-D-glucose ..	10	793·1 ± 60·9	928·4 ± 74·4	135·6 ± 27·7	

When brocresine was added to the incubation medium with mucosa homogenate it exhibited a dose-dependent inhibition of the histidine decarboxylase activity in the rat stomach—at brocresine concentrations of $1{\cdot}3 \times 10^{-8}$, 4×10^{-7}, 4×10^{-6}M the % enzyme activity was 90, 62 and 38% respectively.

DISCUSSION

The results demonstrate that brocresine was unable to interfere with the biosynthesis in the rat stomach *in vivo* and that it was a potent inhibitor of the gastric histidine decarboxylase when added directly to the incubation medium.

Although there is general agreement that the rat stomach histidine decarboxylase can be inhibited by brocresine *in vitro* (Levine & Watts, 1966; Johnston & Kahlson, 1967; Thayer & Martin, 1967), the concentration producing a 50% inhibition differed considerably. The one we reported ($1{\cdot}4 \times 10^{-6}$M) is the highest. This could be due to a large excess of pyridoxal-5-phosphate, since brocresine is known to compete with pyridoxal-5-phosphate as well as with histidine (Ellenbogen, Markley & Taylor, 1969).

The lack of inhibition *in vivo* is supported by Johnson & Burfine (1968) who demonstrated in female rats that brocresine had neither an effect on the histamine level of the gastric mucosa nor on the disappearance rate of exogenous [^{3}H]labelled histamine. These results were confirmed by Johnson (1969).

Even after a long-term treatment with brocresine (100–200 mg/kg, i.p.) for several days the excretion of free histamine in the urine remained constant (Johnston & Kahlson, 1967).

In contrast, Levine & others (1965) described a strong inhibition accompanied by a decline of the histamine contents in heart and stomach and the excretion of histamine in the urine with an inhibition maximum between 3 and 6 h. One year later no such an inhibition could be observed by the same author in another laboratory (Levine, 1966) suggesting a different susceptibility of nearly related strains of rats.

After oral administration of brocresine the histidine decarboxylase-inhibiting activity of the human plasma is maximal 30–45 min after the intake of the drug and declines rapidly afterwards (Wustrack & Levine, 1969). Obviously not all pharmacological activity disappears so quickly since we could demonstrate an increased histamine-stimulated gastric acid response in rats 4 h after intraperitoneal brocresine administration (Becker & Sewing, 1971). Whether these different activities can be attributed to the same compound remains to be elucidated.

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