Biochemical Pharmacology, Vol. 20, pp. 1501–1506. Pergamon Press, 1971. Printed in Great Britain

ASSAY AND RAPID DISAPPEARANCE OF BROCRESINE ACTIVITY IN BLOOD

LEON ELLENBOGEN, C. S. STUBBS, JR., E. MARKLEY and R. J. TAYLOR, JR.

Department of Cardiovascular-Renal Pharmacology, Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y., U.S.A.

(*Received* 4 *July* 1970; *accepted* 21 *August* 1970)

Abstract—Upon administration of brocresine to man, rat or dog, the blood plasma becomes inhibitory to a preparation of fetal histidine decarboxylase *in vitro*. The inhibitory activity of the plasma quickly disappears 2 hr after treatment. Brocresine when added to normal plasma and assayed immediately inhibits histidine decarboxylase to the same extent as brocresine assayed in the absence of plasma. This inhibitory activity quickly disappears when the brocresine is incubated in plasma prior to assay. It is concluded that brocresine is rapidly cleared from the plasma and/or metabolized.

THE RECENT development of sensitive techniques for measuring histidine decarboxylase (L-histidine-carboxy-lyase, EC 4.1.1.22), the enzyme responsible for the formation of histamine, has facilitated the studies on the physiological importance of this amine.[1–5] Agents which inhibit histidine decarboxylase would also contribute to the studies of histamine physiology and might prove to be useful in clinical conditions associated with elevated histamine levels.

Brocresine (NSD 1055: 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate) inhibits histidine decarboxylase *in vitro* and histamine synthesis in man.[6, 7] Various experimental procedures have been used to measure histidine decarboxylase inhibition *in vivo*.[8–12] In the present report it is shown that after administration of brocresine the plasma from man, rat and dog inhibit the activity of a preparation of rat fetal histidine decarboxylase *in vitro*. This method for measuring the inhibitory activity of plasma from treated animals were also used to show that the inhibitory activity of brocresine rapidly disappears indicating a rapid clearance probably because of metabolism of this compound. This technique for measuring plasma levels of histidine decarboxylase inhibitory activity has been used independently in man by Wustrack and Levine.[13]

MATERIAL AND METHODS

Histidine decarboxylase was prepared from whole fetal rats (20–21 days of gestation) by the Levine and Watts modification[14] of the method of Hakanson,[15] using acetate buffer at pH 5·5 instead of pH 4·5. The supernatant II fraction was used. The reaction vessel and procedure for the assay of histidine decarboxylase was previously described.[5] All assays were performed in duplicate. The L-histidine carboxyl-^{14}C was obtained from Calbiochem. Instead of Bray's solution, a mixture consisting of 4 g PPO, 200 mg dimethyl POPOP, 800 ml toluene and 200 ml methanol was found to be more suitable and resulted in considerably more reproducibility on repeated counting.

The heparanized plasma (usually 0·1–0·2 ml) was always added to the incubation mixture after a 10-min preincubation of enzyme and pyridoxal-5-phosphate. The plasma of untreated animals or human subjects was usually found to inhibit fetal histidine decarboxylase slightly (less than 10–15 per cent). This inhibition was always corrected by the use of pretreatment plasma samples. The per cent inhibition was calculated as:

$$100\left[1-\frac{\text{(activity of post-treatment plasma)}}{\text{(activity of pretreatment plasma)}}\right].$$

The molar I_{50} values were obtained from a plot of the points obtained from the mean of duplicate analyses of at least three inhibitor concentrations as previously described.[5]

The eight human subjects used in these studies were normal male volunteers, ages 21–45.

Male Sprague–Dawley rats (150–200 g) were obtained from Carworth Farms, New City, N.Y.

RESULTS

In preliminary experiments it was found that when brocresine was added to rat plasma and assayed immediately for histidine decarboxylase inhibitory activity, the inhibition observed was identical to that observed in the absence of plasma (Table 1). Similarly, in another experiment, the concentration of brocresine required to give 50 per cent inhibition of the fetal enzyme was 10^{-8} M, approximately the same molar I_{50} found with no plasma added. If, however, the mixture of plasma and brocresine was incubated for 3 hr and then assayed, the inhibitory activity decreased from 84 to 15 per cent (Table 1). Preincubation of brocresine alone at 37° prior to addition to plasma had no effect on the inhibitory activity of the drug. Conversely, preincubation of plasma prior to the addition of brocresine had no effect on the inhibitory activity. Similar results were obtained with whole blood and serum of rats and with whole blood and plasma of dogs. Inhibitory activity completely disappeared when 0·2 ml of whole blood was preincubated at 37° with brocresine prior to assay (Table 2).

The instability of brocresine in plasma suggested that plasma samples from brocresine-treated animals would have inhibitory activity for only a short time after dosing. To investigate this hypothesis, rats were orally dosed with brocresine (60 mg/kg). Groups of six rats were exsanguinated at 15, 30 and 60 min and the plasma assayed for histidine decarboxylase inhibitory activity. The plasma of rats orally intubated

TABLE 1. EFFECT OF PREINCUBATION OF RAT PLASMA WITH BROCRESINE ON INHIBITION OF RAT FETAL HISTIDINE DECARBOXYLASE ACTIVITY

Incubation mixture	Per cent inhibition
Brocresine*	82
Brocresine + 0·1 ml plasma assayed immediately	84
Brocresine + 0·1 ml plasma preincubated for 3 hr at 37°	15
Brocresine preincubated 2 hr at 37° and then mixed with 0·1 ml plasma and assayed immediately	72
Brocresine added to 0·1 ml plasma preincubated for 3 hr at 37°	84

* The concentration of brocresine in each incubation was 5×10^{-8} M.

TABLE 2. EFFECT OF PREINCUBATION OF DOG BLOOD WITH BROCRESINE ON INHIBITION OF FETAL HISTIDINE DECARBOXYLASE ACTIVITY

Incubation mixture	Per cent inhibition
Brocresine*	74
Brocresine + 0·2 ml whole blood assayed immediately	94
Brocresine + 0·2 ml whole blood preincubated 1 hr at 37° and then assayed immediately	0
Brocresine preincubated 2 hr at 37°, then mixed with 0·2 ml whole blood and assayed immediately	72

* The amount of brocresine used in each incubation was 5×10^{-8} M.

with water (the vehicle for brocresine) served as a control for each of the time periods. As can be seen in Fig. 1, the inhibitory activity at 60 min is less than half of that observed 15 min after oral dosing.

The fact that considerable inhibitory activity is observed in 15 min indicates that the brocresine or some active metabolite is rapidly absorbed. Similar results have been obtained after the intraperitoneal injection of the drug.

In Table 3 it can be seen that the inhibitory effect observed in rats appears to be dose-dependent. The plasma of rats gavaged with 20 mg/kg of brocresine inhibited histidine decarboxylase significantly less than animals treated with 400 mg/kg.

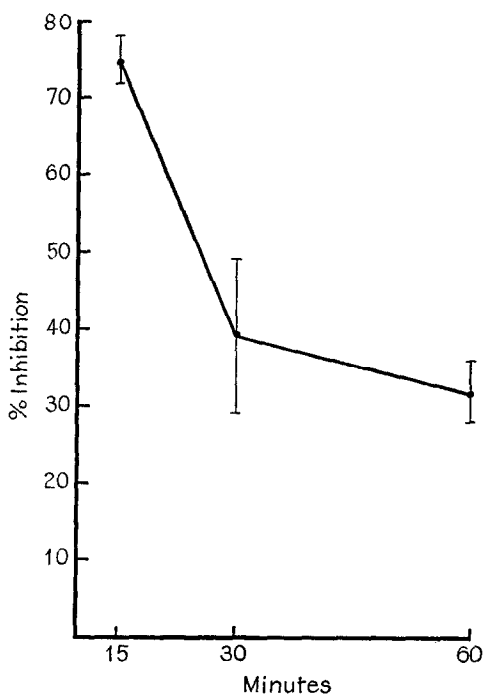

FIG. 1. Inhibition of histidine decarboxylase activity by rat plasma at various times after oral administration of a single dose of 60 mg/kg of brocresine.

TABLE 3. HISTIDINE DECARBOXYLASE INHIBITORY ACTIVITY OF PLASMA FROM RATS DOSED ORALLY WITH BROCRESINE—(DOSE DEPENDENCY)

Dose (mg/kg)	Per cent inhibition
20	30 ± 7
400	66 ± 12

* The results are expressed as per cent inhibition ± standard error (five animals/group). Rats were bled 30 min after administration of dose.

Similar results were observed in another experiment in which three dogs were dosed orally (10 mg/kg) with brocresine and three were dosed with the free base of brocresine, equivalent to 10 mg/kg. The plasma of the dogs was assayed for inhibitory activity at 30 min, 1 and 2 hr after dosing. The pretreatment plasma sample of each dog served as the control. As can be seen from Table 4, the inhibitory activity rapidly disappears. Only about 20 per cent of the inhibitory activity observed at 30 min was present 2 hr after dosing. No differences were observed between the dogs treated with brocresine and those treated with the brocresine free base.

TABLE 4. HISTIDINE DECARBOXYLASE INHIBITORY ACTIVITY OF PLASMA FROM DOGS TREATED WITH BROCRESINE AND BROCRESINE-FREE BASE*

Drug	Dog no.	Per cent inhibition		
		0·5 hr	1 hr	2 hr
Brocresine	794	87	66	9
	873	78	82	23
	994	71	55	16
Brocresine-free base	823	78	70	5
	875	100	77	17
	898	99	67	22

* The assays were performed with 0·1 ml of plasma. Pretreatment plasma samples of each dog served as the controls. Animals were dosed with 10 mg/kg of brocresine and 6·8 mg/kg of brocresine-free base (10 mg/kg equivalent).

A similar study was conducted in normal male volunteers 21–45 years old given a single oral dose of 400 mg of brocresine. Again, the pretreatment sample of each man served as control. The data are shown in Table 5. As was observed with the dogs 2 hr after dosing, 0·1 ml of plasma had about 20 per cent of the inhibitory activity observed at ½ hr. The numbers in parentheses at 30 min and 1 hr are the values observed when the plasma was frozen and reassayed 2 days later. The sharp drop in inhibitory activity observed with stored plasma lends further support to the fact that all assays on plasma after treatment with brocresine must be performed immediately after withdrawal of the blood.

Preliminary studies of the correlation between inhibitory activity and the concentration of brocresine in blood indicate that the inhibition is because of brocresine *per*

TABLE 5. HISTIDINE DECARBOXYLASE INHIBITORY ACTIVITY OF PLASMA FROM HUMANS TREATED WITH BROCRESINE

Subject	Per cent inhibition*		
	0·5 hr	1 hr	2 hr
1	79 (37)	40 (37)	38
2	92 (47)	70 (10)	15
3	100 (50)	82 (14)	11
4	98 (80)	80 (24)	15
5	100 (78)	95 (38)	35
6	100 (42)	74 (12)	24
7	100 (72)	88 (24)	28
8	100 (69)	77 (19)	8

The values in parentheses at 30 min and 1 hr are the values observed when the plasma was frozen and reassayed 2 days later. All assays were performed with 0·1 ml of plasma. Pretreatment plasma samples of each human served as control. Each subject was given an oral dose of 400 mg of brocresine.

TABLE 6. EFFECT OF BROCRESINE-ACETONE OXIME ON HISTIDINE DECARBOXYLASE INHIBITORY ACTIVITY

Incubation mixture	Per cent inhibition
Brocresine (10^{-8} M) + 0·1 ml normal rat plasma assay immediately	67
Rat plasma (0·1 ml) obtained 15 min after i.v. administration (40 mg/kg) and assayed immediately	100
Rat plasma (0·1 ml) + 0·001 ml acetone obtained 15 min after i.v. administration (40 mg/kg) and assayed immediately	16
Brocresine–acetone oxime (10^{-5} M)	50

se. Acetone reacts rapidly with brocresine to form a brocresine–acetone oxime which is 0·1 per cent as active as brocresine in inhibiting fetal histidine decarboxylase activity. When 0·1 ml of plasma from rats given brocresine i.v. (40 mg/kg) was mixed with 0·001 ml of acetone, the inhibitory activity dropped sharply (Table 6).

DISCUSSION

These studies suggest an additional method for assay of histidine decarboxylase inhibitory activity of a drug and may prove to be an adjunct to the method of Johnson[12] in which the disappearance of ^{3}H histamine is measured. However, in Johnson's study and those of others,[16] the effect of the drug was not measured shortly after its administration.

The method of measuring histidine decarboxylase inhibitory activity in plasma is of value in estimating the dosing schedule necessary to obtain therapeutic efficacy. With dogs, rats and humans, it would be necessary to give brocresine frequently to maintain a high plasma concentration of inhibitory activity. The rate of disappearance of inhibitory activity was shown independently in humans by Wustrack and Levine.[13]

Equally important is the information that is obtained with regard to the rate of metabolism and/or disappearance of the drug from the plasma.

It has been established that pyridoxal or pyridoxal phosphate forms an oxime complex with brocresine.[17] Brocresine could exert its inhibitory effect by formation of an oxime with pyridoxal or pyridoxal phosphate. However, it has been shown that these oximes are considerably less inhibitory than brocresine *in vitro*.[17] It is more likely that the rapid disappearance of histidine decarboxylase is because of the reaction of blood with brocresine or to the metabolism of brocresine *in vivo*. A rapid disappearance and/or metabolism of brocresine is also suggested by the report of Kobayashi *et al.*[18]

REFERENCES

1. Y. KOBAYASHI, *Analyt. Biochem.* **5**, 284 (1963).
2. D. AURES and W. G. CLARK, *Analyt. Biochem.* **9**, 35 (1964).
3. R. J. LEVINE and D. E. WATTS, *Biochem. Pharmac.* **15**, 841 (1966).
4. F. J. LEINWEBER and L. A. WALKER, *Analyt. Biochem.* **21**, 131 (1967).
5. L. ELLENBOGEN, E. MARKLEY and R. J. TAYLOR, *Biochem. Pharmac.* **18**, 683 (1969).
6. J. D. REID and D. M. SHEPHERD, *Life Sci.* **1**, 5 (1963).
7. R. J. LEVINE, *Science, N.Y.* **154**, 1017 (1966).
8. G. KAHLSON, E. ROSENGREN and R. THUNBERG, *J. Physiol., Lond.* **169**, 467 (1963).
9. R. J. LEVINE, T. L. SATO and A. SJOERDSMA, *Biochem. Pharmac.* **14**, 139 (1965).
10. D. P. HIGH, D. M. SHEPHERD and B. G. WORDCOCK, *Life Sci.* **4**, 787 (1965).
11. M. A. REILLY and R. W. SCHAYER, *Br. J. Pharmac.* **32**, 567 (1968).
12. H. L. JOHNSON, *Biochem. Pharmac.* **18**, 651 (1969).
13. K. O. WUSTRACK and R. J. LEVINE, *Biochem. Pharmac.* **18**, 2465 (1969).
14. R. J. LEVINE and D. E. WATTS, *Biochem. Pharmac.* **15**, 841 (1966).
15. R. HAKANSON, *Biochem. Pharmac.* **12**, 1289 (1963).
16. G. KAHLSON and E. ROSENGREN, *Physiol. Rev.* **48**, 155 (1968).
17. F. J. LEINWEBER, *Mol. Pharmac.* **4**, 337 (1968).
18. Y. KOBAYASHI, J. KUPELIAN and D. W. MAUDSLEY, *Biochem. Pharmac.* **19**, 1761 (1970).