The Effects of Amiflamine, a Reversible MAO-A Inhibitor, on the First Pass Metabolism of Tyramine in Dog Intestine

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Abstract—The effects of amiflamine on tyramine deamination were studied using isolated loops of intestine in anesthetized dogs. In the pretreatment experiment, dogs were dosed with amiflamine (3.5 mg/kg/day) once daily for 3 days, with the study being carried out 3 hr after the final dose. [14C] Tyramine (50 mg and 50 μCi) in 10 ml of normal saline was introduced into the isolated loops of gut, and tyramine and p-hydroxyphenylacetic acid in the venous blood were separated by HPLC and measured by scintillation spectrometry. In the untreated dogs, approximately 15% of the tyramine passed through the gut wall unchanged. When tyramine and amiflamine (0.06 to 3.5 mg/kg) were administered simultaneously to the gut loop, about 27 to 65% of the tyramine passed through the gut wall unchanged. On the contrary, after pretreatment with amiflamine for 3 days, percentage of tyramine passing through the gut wall was not increased in comparison with the control. These results suggest that pretreatment with amiflamine does not produce drug concentrations in the lining cells of the gut sufficient to effectively inhibit the deamination of oral tyramine, which is administered at least 3 hr after the final dose of amiflamine.

Monoamine oxidase (MAO) inhibitors are clinically used as antidepressants, but limitation of extensive use of these drugs is due to hypertensive crisis (cheese effect) occurring when the patient ingests certain foodstuff containing vasoactive amines such as tyramine. Circulating tyramine liberates noradrenaline from nerve endings and produces the pressor effect (1). It is generally assumed that such hypertensive crises occur as a result of inhibition of MAO in the intestine and to a lesser extent in the liver, allowing active tyramine to enter the systemic circulation, and of inhibition of intraneural

Fig. 1. Chemical structure of amiflamine.
MAO which normally degrades both tyramine and norepinephrine. It was previously demonstrated that in the dog, almost 80% of the oral dose of tyramine is deaminated during the first passage through the gut wall (2).

Amiflamine is a reversible inhibitor of MAO-A which is reported to exhibit additional selectivity by accumulating in the serotonergic neurons in the brain (3–6). In the present investigation, the effect of amiflamine on tyramine metabolism by the dog gut has been studied. The chemical structure of amiflamine is shown in Fig. 1.

### Materials and Methods

#### Treatment of animals and experimental procedure:
Greyhounds of either sex were used throughout the study. Jejunal loops isolated in situ were prepared in anesthetized dogs as previously described (2). A scheme of this procedure is shown in Fig. 2. In pretreatment experiments, the dogs were dosed with amiflamine (3.5 mg/kg/day) once daily for 3 days with the study commencing 3 hr after the final dose.

$[^{14}C]$ Tyramine (50 mg and 50 μCi) in 10 ml of normal saline was introduced into the isolated loop, and venous blood from the loop was collected over three 10 min intervals. In simultaneous experiments, $[^{14}C]$ tyramine and amiflamine (0.06 to 3.5 mg/kg) were introduced together into the isolated loop and venous blood was collected over six 5 min intervals. Arterial blood samples (10 ml) were collected from the abdominal aorta over three 10 min intervals so that the effectiveness of the isolation procedure could be assessed by comparing the amount of radioactivity in the arterial and venous plasma. Total radioactivity in plasma was measured in a liquid scintillation counter using Instagel scintillant. Tyramine and p-hydroxyphenylacetic acid (p-OHPA) were separated from venous plasma samples by high pressure liquid chromatography on a C$_{18}$-Bondapak column as previously described (2).

#### Preparation of tissue homogenates and mitochondria:
For tissue homogenates, 1–2 g liver or brain was homogenized with 9 vol. of 0.05 M phosphate buffer (pH 7.4) using a Polytron Model PT 10–35 homogenizer. The homogenates were centrifuged at 600 g for 10 min, and the supernatant fraction was retained. For mitochondria, 3–5 g of intestine was homogenized as above in 4 vol. of 0.25 M Tris-EDTA buffer.
M sucrose. Following sedimentation of nuclei and cell debris by centrifugation at 600 g for 10 min, the mitochondrial pellet was obtained by further centrifugation at 10,000 g for 20 min. All procedures were carried out at 4°C. Mitochondria were resuspended in the phosphate buffer.

Assay of MAO activity: MAO activity was determined at 37°C using the radiometric assay of Wurtman and Axelrod (7). The incubation mixture contained 20 μl of the MAO preparation and 20 μl of substrate in a total volume of 100 μl of 0.1 M phosphate buffer (pH 7.4). The reaction was started by adding 20 μl of [14C] tyramine or [14C] 5-HT (1 μCi/ml). Incubations were carried out for 20 min at 37°C. The reactions were stopped by adding 3 N HCl (20 μl). The mixtures were extracted by shaking with 1 ml of ethylacetate : toluene (1:1) for 15 sec. After centrifugation for 3,000 rpm, 0.5 ml of the organic layer was mixed with 10 ml of Instagel scintillation liquid. The radioactivity was expressed as disintegrations per minute (DPM). MAO activity was determined under conditions which were linear with respect to time and tissue concentration.

Drug: Amiflamine was obtained from Astra Lakemedel AB Sweden.

Results

Effects of amiflamine on MAO activity in vitro: The effects of amiflamine on MAO activities in dog intestine, brain and liver were investigated with 5-HT and tyramine as substrates (Fig. 3). Amiflamine inhibited 5-HT oxidation to a greater extent than it did tyramine oxidation in all tissues examined. The I₅₀ values for 5-HT oxidation were approximately 3×10⁻⁶ M for all three different tissues. On the other hand, the degree of inhibition of tyramine oxidation was quite different for the three tissues. Amiflamine at a concentration of 10⁻³ M inhibited MAO activity in the liver, brain and intestine by 27%, 66% and 89%, respectively.

Effects of amiflamine on the deamination of tyramine in isolated loops of dog gut: In untreated dogs, almost 80% of the radioactivity appearing in the venous effluent was present as p-OHPA, indicating that extensive deamination of tyramine as it passed through the gut wall had occurred (Table 1). Pretreatment of dogs with amiflamine (3.5 mg/kg) for 3 days did not have a dramatic effect on the deamination of tyramine in the gut wall (Table 1).

The direct interaction between amiflamine and tyramine was also examined. The ratio of p-OHPA/tyramine in venous plasma from isolated intestinal loops is shown in Fig. 4. In control dogs, there was a tendency for the ratio of p-OHPA/tyramine in venous plasma to increase with time and then attain a steady maximum of approximately 6 after about 10 min. When amiflamine was administered together with tyramine into the intestinal loop, the ratio was decreased with an increase.

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**Fig. 3.** Effects of amiflamine on MAO activity in dog intestine, brain and liver in vitro. Substrates: 100 μM tyramine (●—●). 100 μM 5-HT (○—○). Data represent the mean±S.E. in percent of control MAO activity for 3 dogs.
Table 1. Deamination of tyramine in isolated loops of gut in dogs treated with amiflamine

| Treatment                      | Tyramine  | p-Hydroxyphenylacetic acid |
|-------------------------------|-----------|---------------------------|
| None (n=4)                    | 15.6±3.6  | 78.0±3.8                  |
| Amiflamine (n=3) 3.5 mg/kg    | 21.5±4.1  | 70.1±5.8                  |

Dogs were treated with amiflamine once daily for 3 days, and the study was carried out 3 hr after the final dose. The results are the mean±S.E.

Fig. 4. Ratio of p-hydroxyphenylacetic acid/tyramine in venous plasma from isolated dog intestinal loops.

in the dose of amiflamine. The lowest dose of amiflamine (0.06 mg/kg) had a moderate inhibitory effect on the gut wall deamination of tyramine and the ratio was about 2.5. At 0.19 mg/kg, 35% of the tyramine passed through the gut wall without deamination. This was increased to more than 50% for doses of 1.2 and 3.5 mg/kg. It was observed that there was a corresponding fall in the concentration of p-OHPA.

Discussion

Amiflamine has been shown to be a specific MAO-A inhibitor exhibiting both competitive and reversible properties in rats (3, 4) and in humans (6, 8).

Tyramine is a substrate for both MAO-A and MAO-B in most tissues (9); therefore, it would be expected that both A and B selective MAO inhibitors would potentiate the effect of intravenously administered tyramine. The potentiation of tyramine's effects has been demonstrated for the MAO-A selective inhibitor clorgyline (10, 11), but not for the MAO-B selective inhibitor l-deprenyl (12). It is reported that MAO-A is responsible for the bulk of the tyramine deamination in both dog and rat intestine (2, 13). In this inhibition study with amiflamine in vitro, the I_{50} value for tyramine oxidation...
in the intestine was similar to that for oxidation of 5-HT, which is a specific substrate of MAO-A (14). On the contrary, tyramine oxidation was inhibited only 27% with $10^{-3}$ M amiflamine, while 5-HT oxidation was almost completely inhibited with the same concentration of amiflamine in the liver. Previous reports (15, 16) indicate that MAO-A is predominant in dog intestine, while MAO-B is predominant in dog liver. These facts indicate that tyramine is deaminated in the intestine mainly by MAO-A and by MAO-B in the liver. Thus, since tyramine deamination is carried out mainly by MAO-A in rat and dog intestine, it would be expected that an MAO-A selective inhibitor would potentiate the effects of oral tyramine on blood pressure. However, amiflamine only produces tyramine potentiation at doses much greater than those required for MAO-A inhibition (17, 18).

In in vitro and the simultaneous experiments of this study, amiflamine in a sufficiently high concentration inhibited the gut wall deamination of tyramine. Thus, amiflamine appears to be an inhibitor of tyramine deamination by the dog gut wall. The pharmacokinetics of amiflamine is important when determining tissue concentration (19). Peak blood concentrations of amiflamine are attained 1–1.5 hr after oral administration in the dog and rat. Blood elimination half-life in the dog is approximately 2 and 1.3 hr in the dog and rat, respectively. This indicates a relatively rapid elimination of the drug from the tissue. However, a maximal increase in rat brain 5-HT is produced by amiflamine (3.8 mg/kg, p.o.) at 4–8 hr after administration (20). So, experiments were started at 3 hr after the final administration of amiflamine in this study. However, no inhibition of tyramine deamination was observed 3 hr after the last dose in the 3 day pretreatment experiments. These results indicate that high local concentrations of the drug are needed to produce this inhibition. The amiflamine doses producing 50% inhibition of brain MAO-A activity are 1.3 mg/kg, p.o., in rat (3) and 1.9 mg/kg, p.o., in dogs (H. Yasuhara et al., unpublished data). The results of our study suggest that pretreatment of dogs with doses of amiflamine sufficient to inhibit brain MAO-A (3.5 mg/kg) does not produce concentrations of the drug in the lining cells of the gut which are sufficient to effectively inhibit the deamination of oral tyramine. Furthermore, rapid elimination of amiflamine from the tissue may account for the low interaction between amiflamine and oral tyramine.

The tyramine dose producing a pressure reaction is considerably higher than that reported for irreversible inhibitors such as tranylcypromine and phenelzine (21–23). Since amiflamine is a competitive inhibitor of MAO, high concentrations of tyramine are liable to displace the inhibitor (12). According to this hypothesis, there is a large increase in tyramine in the intestine after oral ingestion of the amine which causes the displacement of amiflamine from the intestinal MAO-A active center. The result of this is that some tyramine will be metabolized, therefore, the concentration of unmetabolized tyramine reaching the circulation will be lower than if an irreversible or a non-competitive reversible inhibitor had been used. Namely, inhibition of intestinal MAO with a competitive and reversible MAO inhibitor such as amiflamine can be overcome by the oral tyramine itself. It would thus seem that this hypothesis provides an explanation for the low degree of interaction between tyramine and amiflamine.

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References
1 Marley, E.: Monoamine oxidase inhibitors and drug interactions. In Drug Interactions, Edited by Grahame-Smith, D.G., p. 171–194, Biological Council, London (1977)
2 Ilett, K.F., George, C.F. and Davies, D.S.: The effect of monoamine oxidase inhibitors on 'First pass' metabolism of tyramine in dog intestine. Biochem. Pharmacol. 29, 2551–2556 (1980)
3 Ask, A.-L., Hogberg, K., Schmidt, L., Kiessling, H. and Ross, S.B.: (+)-4-Dimethylamino-2, a-dimethylphenethylamine (FLA 336(+)), a selective inhibitor of the A form of monoamine oxidase in the rat brain. Biochem. Pharmacol. 31, 1401–1406 (1982)
4 Ask, A.-L., Fagervall, I. and Ross, S.B.: Selective inhibition of monoamine oxidase in monoamin-
ergic neurons in the rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 324, 79-87 (1983)

5 Ask, A.-L., Fagervall, I., Jonze, M., Kelder, D., Nygren, R. and Ross, S.B.: Effect of acute and repeated administration of amiflamine on monoamine oxidase inhibition in the rat. Biochem. Pharmacol. 33, 2839-2847 (1984)

6 Tipton, K.T., O’Carroll, A.-M., McCrodden, J.M. and Fowler, C.J.: Monoamine oxidase-A selective inhibition in human hypothalamus and liver in vitro by amiflamine and its metabolites. J. Pharm. Pharmacol. 37, 352-354 (1985)

7 Wurtman, R.J. and Axelrod, J.: A sensitive and specific assay for the estimation of monoamine oxidase. Biochem. Pharmacol. 12, 1439-1441 (1963)

8 Fowler, C.J. and Oreland, L.: Substrate and stereoselective inhibition of human brain monoamine oxidase by 4-dimethylamino-α, 2-dimethylphenethylamine (FLA 336). J. Pharm. Pharmacol. 33, 403-408 (1981)

9 Hall, D.W.R., Logan, B.W. and Parsons, G.H.: Further studies on the inhibition of monoamine oxidase by M&B 9302 (clorgyline). 1. Substrate specificity in various mammalian species. Biochem. Pharmacol. 18, 1447-1454 (1969)

10 Lader, M.H., Sakalis, G. and Tansella, M.: Interactions between sympathomimetic amines and a new monoamine oxidase inhibitor. Psychopharmacologia (Berlin) 18, 118-123 (1970)

11 Pickar, D., Cohen, R.M., Jimerson, D.C. and Murphy, D.L.: Tyramine infusions and selective monoamine oxidase inhibitor treatment. 1. Changes in pressor sensitivity. Psychopharmacology (Berlin) 74, 4-7 (1981)

12 Elsworth, J.D., Glover, V., Reynolds, G.P., Sandler, M., Lees, A.J., Phuapradit, P., Shaw, K.M., Stern, G.M. and Kumar, P.: Deprenyl administration in man: a selective monoamine oxidase B inhibitor without cheese effect. Psychopharmacologia (Berlin) 57, 33-38 (1978)

13 Strolin Benedetti, M., Boucher, Th., Carlson, A. and Fowler, C.J.: Intestinal metabolism of tyramine by both forms of monoamine oxidase in the rat. Biochem. Pharmacol. 32, 47-52 (1983)

14 Neff, N.H. and Yang, T.-Y.: Another look at the monoamine oxidase and the monoamine oxidase inhibitor drugs. Life Sci. 14, 2061-2074 (1974)

15 Sato, H.: Studies on monoamine oxidase (report 75): monoamine oxidase in dog intestine. J. Showa Med. Assoc. 40, 416-422 (1980)

16 Kimura, K.: Effects of oxygen and some drugs on monoamine oxidase in dog liver, Folia Pharmacol. Japon. 81, 305-313 (1983) (Abs. in English)

17 Ögren, S.-O., Ask, A.-L., Holm, A.-C., Florvall, L., Lindom, L.-O., Lundström, J. and Ross, S.B.: Biochemical and pharmacological properties of a new selective and reversible monoamine oxidase inhibitor, FLA 336. In Monoamine Oxidase Inhibitors—The State of the Art, Edited by Youdim, M.B.H. and Paykel, E.S., p. 103-112. John Wiley and Sons Ltd., Chichester (1981)

18 Lindom, L.-O., Norrman, S. and Hellström, W.: Low interaction between amiflamine and oral tyramine. Animal studies. In Monoamine Oxidase and Disease, Edited by Tipton, K.F., Dostert, P. and Strolin Benedetti, M., p. 602-603. Academic Press, London (1984)

19 Graffner, C., Alvan, G., Grind, M., Lake-Bakaar, D., Lindgren, J.-E., Lindström, J. and Sander, H.: Pharmacokinetics and metabolism of amiflamine in rat, dog and man. In Monoamine Oxidase and Disease, Edited by Tipton, K.F., Dostert, P. and Strolin Benedetti, M., p. 193-201. Academic Press, London (1984)

20 Ask, A.-L., Hellström, W., Norrman, S., Ogren, S.-O. and Ross, S.B.: Selective inhibition of the A form of monoamine oxidase by 4-dimethylamino-α-methylphenylalkylamine derivatives in the rat. Neuropharmacology 21, 299-308 (1982)

21 Siwers, B., Alvan, G., Graffner, C., Grind, M., Gustafsson, L., Helleday, J., Lindgren, J.-E. and Sander, H.: Investigation of the possible interaction between amiflamine and oral tyramine in man. In II. World Conference on Clinical Pharmacology and Therapeutics. Abstract, p. 125 (1983)

22 Dollery, C.T., Brown, M.J., Davies, D.S. and Strolin Benedetti, M.: Pressor amines and monoamine oxidase inhibitors. In Monoamine Oxidase and Disease, Edited by Tipton, K.F., Dostert, P. and Strolin Benedetti, M., p. 429-441. Academic Press, London (1984)

23 Finberg, J.P.M. and Youdim, M.B.H.: Reversible monoamine oxidase inhibitors and the cheese effect. In Monoamine Oxidase and Disease, Edited by Tipton, K.F., Dostert, P. and Strolin Benedetti, M., p. 479-485. Academic Press, London (1984)