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# Deamination of hordenine by monoamine oxidase and its action on vasa deferentia of the rat

C. J. BARWELL, A. N. BASMA, M. A. K. LAFI*, L. D. LEAKE*, *School of Pharmacy and Biomedical Sciences and *School of Biological Sciences, Portsmouth Polytechnic, Portsmouth, Hampshire, UK*

**Abstract**—The selectivity of the naturally occurring amine, *N,N*-dimethyltyramine (hordenine) for monoamine oxidase (MAO) and its action upon isolated vasa deferentia of the rat was investigated. Hordenine was deaminated by rat liver MAO with a Michaelis constant of 479 μM and maximum velocity of 128 nmol (mg protein)$^{-1}$ h$^{-1}$ compared with 144 μM and 482 nmol (mg protein)$^{-1}$ h$^{-1}$ for tyramine. Studies, with selective irreversible inhibitors of MAO, showed that hordenine was a highly selective substrate for MAO-B of liver and that it was not deaminated by the MAO-A of intestinal epithelium. In contrast to tyramine, hordenine did not produce contractions of isolated vasa deferentia. However, 25 μM hordenine potentiated contractile responses of vasa, from control animals, to submaximal doses of noradrenaline and inhibited responses to tyramine. It did not alter responses, to noradrenaline, of vasa denervated by chronic pretreatment of rats with guanethidine. Therefore, it appears that hordenine acted as an inhibitor of noradrenaline uptake, in isolated vasa deferentia. These results indicate that dietary-hordenine is unlikely to be deaminated by intestinal MAO as this is predominantly MAO-A. Consequently, it is likely to be absorbed and could affect the sympathetic nervous system, by virtue of its action as an inhibitor of noradrenaline uptake.

Tyramine and *N,N*-dimethyltyramine (hordenine) occur naturally. Tyramine is commonly present in cheeses, fish products and wines (Youdim 1977) and plants (Wheaton & Stewart 1970). Hordenine occurs in barley, some cacti and citrus fruit (Wheaton & Stewart 1970) and at relatively high levels, of 1-5 mg g$^{-1}$, in the marine alga *Mastocarpus stellatus*, which is incorporated into the food product carrageen (Barwell & Blunden 1981). Tyramine is a substrate for both the A and B forms of monoamine oxidase (MAO) (amine:oxygen oxidoreductase (deaminating) (flavin-containing) EC 1·4. 3·4) (Kinemuchi et al 1984) and normally, dietary-tyramine is deaminated in the intestinal epithelium by MAO, which is predominantly MAO-A (Barwell & Canham 1988). Irreversible inhibitors of MAO-A eliminate this physiological barrier so that dietary-tyramine is absorbed and may induce hypertension, by virtue of its pharmacological action as an indirectly acting sympathomimetic amine (Patil et al 1967). Hordenine has been shown to be deaminated by hepatic MAO (Alles & Heegaard 1943) but it is not clear whether it is a substrate for both the A and B or only one form of the enzyme. There is apparently no reported study of its pharmacological activity. We have investigated the selectivity of hordenine, as a substrate, for MAO-A and MAO-B and its action upon the isolated vas deferens of the rat.

## Materials and methods

*Isolation of liver mitochondria and intestinal epithelium.* Livers from four male Sprague-Dawley rats (200–300 g) were homogenized in 0·3 M sucrose and mitochondria isolated by differential centrifugation, then resuspended in 0·1 M sodium phosphate pH 7·4 and stored at minus 20°C. The pyloric to caecal end of the intestine, was used to isolate epithelium which was homogenised and stored as described by Barwell & Canham (1988).

*Measurement of amine oxidase activity.* Activity was measured at pH 7·4 and 37°C with a Clark-type oxygen electrode in 0·05 M sodium phosphate pH 7·4 saturated with oxygen from air. The proportion of tyramine- and hordenine-deaminating activity, in liver mitochondria, due to MAO-A and MAO-B was determined by titration with the selective irreversible inhibitor of MAO-A, Lilly 51641 (N-[2-(*O*-chlorophenoxy)ethyl]cyclopropylamine) (Fowler & Ross 1984). The selective irreversible inhibitor of MAO-B, selegiline ((−)-deprenyl) (Fowler & Ross 1984) was used to selectively inhibit the MAO-B activity of liver mitochondria and intestinal epithelium homogenate. Tissue preparations were preincubated with inhibitors at 37°C for 30 min. Preliminary time courses of the inhibition showed that it was complete within 25 min. There was no loss of enzyme activity for samples preincubated with buffer.

*Vas deferens preparation.* Vasa deferentia were isolated from male Sprague-Dawley rats (200–300 g) and cumulative dose-response curves of isometric contractions, induced by (−)-noradrenaline and tyramine, obtained as described previously (Lafi & Leake 1988).

*Amine depletion and denervation.* This was carried out by chronic guanethidine treatment of animals as described by Lafi & Leake (1988). Vasa from guanethidine-treated animals were checked for the presence of amines by HPLC and UV-fluorescence, both before and after subsequent exposure to α-methylnoradrenaline (Lafi & Leake 1988), thus testing for a neuronal uptake mechanism.

*Analysis of data.* Michaelis constants and maximum velocities were obtained from Hanes plots (s/v against s) constructed with five substrate concentrations ranging from approximately one third to four times the Michaelis constant. Lines of best fit were obtained by unweighted linear regression. Results were expressed as mean ± s.e., calculated from values obtained with different preparations. Statistical comparisons were made using Student's *t*-test with $P < 0.05$ being taken as significant.

## Results

*Deamination by liver and intestine amine oxidases.* Isolated rat liver mitochondria deaminated tyramine and hordenine. The Michaelis constant for hordenine (479 ± 39 μM) was about three times higher than for tyramine (144 ± 10 μM) and the maximum velocity (128 ± 11 nmol (mg protein)$^{-1}$ h$^{-1}$) was about four times lower than for tyramine (482 ± 15 nmol (mg protein)$^{-1}$ h$^{-1}$). Fig. 1 shows the effect of Lilly 51641, at concentrations from $10^{-8}$M to $10^{-3}$M, upon tyramine and hordenine deamination by rat liver mitochondria. With tyramine as substrate, there was a biphasic inhibition curve with a distinct plateau between $10^{-6}$M and $10^{-5}$M. With hordenine as substrate a monophasic inhibition curve was obtained with inhibition occurring at concentrations of Lilly 51641 above $10^{-5}$M. Liver mitochondria and homogenate of intestine epithelium, preincubated with $10^{-6}$M selegiline did not deaminate either 0·1 mM benzylamine or 5 mM hordenine, under conditions where the rate of deamination of 1 mM 5-hydroxytryptamine was 970 ± 18 μM h$^{-1}$ and 376 ± 10 μM h$^{-1}$, respectively.

Correspondence to: C. J. Barwell, Pharmacology Division, St. Michaels Building, White Swan Road, Portsmouth PO1 2DZ, UK.

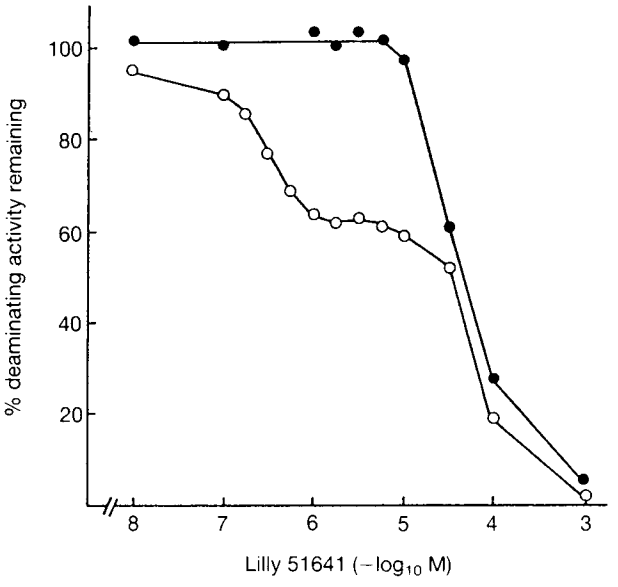


FIG. 1. In-vitro inhibition by Lilly 51641 of tyramine and hordenine deamination by rat liver mitochondria. Each point represents the mean of duplicate determinations made with a mitochondria preparation isolated from livers of four male rats. Enzyme preparation was preincubated with Lilly 51641 at 37°C for 30 min after which deaminating activity was measured with 5 mM tyramine (○) and 5 mM hordenine (●) at 37°C and pH 7·4 as described in Materials and methods.

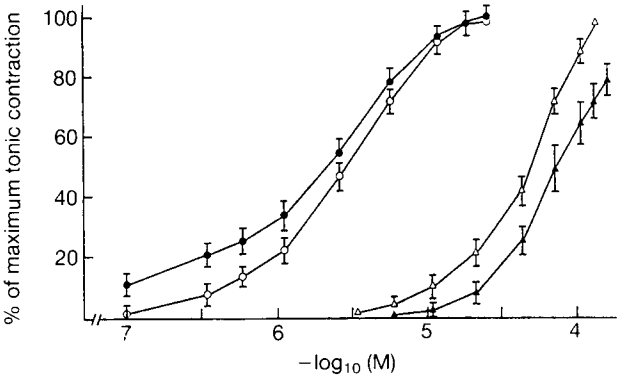


FIG. 2. Affect of hordenine (25 μM) upon dose-response curves of tonic contractions to noradrenaline and tyramine of vas deferentia from control rats. Each point represents the mean ± s.e., n = 4. The dose-response curves were obtained as indicated in Materials and methods. Noradrenaline (○), noradrenaline plus hordenine (●), tyramine (△), tyramine plus hordenine (▲).

*Action on the isolated vas deferens.* Chronic guanethidine pretreatment depleted the noradrenaline content of the tissue. The level in vasa from treated rats was 0·11 ± 0·01 μg (g wet weight)$^{-1}$, compared with 3·67 ± 0·01 μg (g wet weight)$^{-1}$ in controls. This treatment also destroyed the neuronal uptake system for α-methylnoradrenaline.

Exogenous doses of noradrenaline (0·1–25 μM) and tyramine (1–160 μM) produced concentration-dependent tonic contractions of vasa deferentia from control rats (Fig. 2). Half-maximal contractions were produced by 3 μM noradrenaline and 50 μM tyramine. In contrast, hordenine at concentrations up to 150 μM produced no measurable contraction but it did affect responses to both noradrenaline and tyramine. Fig. 2 shows that 25 μM hordenine potentiated significantly, the tonic responses to submaximal doses of noradrenaline and inhibited those to tyramine. For example, responses to 1 μM noradrenaline were increased by 65% and responses to 20 μM tyramine reduced by 70%. Chronic guanethidine pretreatment increased the sensitivity of vasa to noradrenaline by about thirty fold and completely abolished responses to tyramine. Hordenine did not produce any contraction but, also, did not now alter the responses of denervated vasa to noradrenaline.

## Discussion

Selective irreversible inhibitors of MAO-A and MAO-B and the substrate tyramine, may be used to determine the activity of MAO-A and MAO-B in a preparation. Samples are preincubated with various concentrations of inhibitor and the MAO activity remaining measured with a saturating concentration of tyramine, which is deaminated at a similar rate by both forms. If a preparation contains both the A and B forms, a biphasic inhibition curve is obtained with a distinct plateau, which indicates their relative activities (Kinemuchi et al 1984). With a selective inhibitor of MAO-A, such as Lilly 51641 (Fowler & Ross 1984), the first phase of the inhibition is due to inhibition of MAO-A and the second to inhibition of MAO-B. A preparation demonstrated to contain both forms of MAO may be used to determine the contribution of each to the deamination of other amines. In addition, titration with an inhibitor establishes a concentration which may be used to selectively inhibit one form of MAO. In this investigation, titration of rat liver mitochondria with Lilly 51641 and assay of remaining activity with tyramine, yielded a biphasic inhibition curve and the plateau indicated that the preparation contained approximately equal activities of MAO-A and MAO-B. MAO-A was selectively inhibited at concentrations of Lilly 51641 below $10^{-6}$M and MAO-B was inhibited at concentrations above $10^{-5}$M. With hordenine as substrate, a monophasic inhibition curve was obtained at concentrations of inhibitor above $10^{-5}$M indicating that it was deaminated by only one form of MAO and that this was MAO-B. Mitochondria and homogenate of intestinal epithelium, pretreated with selegiline so as to selectively inhibit MAO-B but which contained MAO-A activity, as indicated by deamination of 5-hydroxytryptamine, did not deaminate hordenine. Therefore, it appears that hordenine is a highly selective substrate for the B form of rat liver MAO and that it is not deaminated by MAO-A of intestinal epithelium.

Sympathomimetic amines produce contractions of the isolated vas deferens either directly or indirectly. Directly-acting sympathomimetic amines affect postjunctional receptors, whereas indirectly-acting sympathomimetic amines produce contractions by being taken up into prejunctional neurons and displacing endogenous neurotransmitter (Trendelenburg 1972). Thus, an amine with a direct action should produce contractions in both control vasa and those depleted of noradrenaline by pretreatment with guanethidine, whereas an amine with only an indirect action should produce contractions only in control vasa. In this investigation, tyramine produced contractions only in control vasa. Hordenine did not produce contractions of vasa from either control or guanethidine-pretreated animals indicating that it had neither direct nor indirect sympathomimetic activity, in contrast to tyramine and *N*-methyltyramine, which are indirectly acting sympathomimetic amines (Patil et al 1967; Lafi & Leake 1988). However, it reduced responses of control vasa to doses of tyramine and increased their responses to submaximal doses of noradrenaline. Since tyramine must be taken up into neurons to produce a contraction and responses to submaximal doses of exogenous noradrenaline are normally modulated by neuronal uptake (Trendelenburg 1972) these results indicated that hordenine acted as an inhibitor to neuronal amine-uptake. This interpretation is supported by the fact that it

did not alter responses, to noradrenaline, of vasa from guanethidine-pretreated animals.

During passage along the gut it is possible that a compound such as hordenine could be metabolized by microsomal enzymes which catalyse reactions such as *N*-demethylation (Lindeke & Cho 1982) and glucuronide formation (Mulder 1982). The activity of these enzymes is generally low in the intestine (Hanninen et al 1987) and in the rat, where hepatic-microsomal enzyme activity is higher than in intestine, hepatic-microsomal metabolism of hordenine was not detectable (Barwell et al 1984). Dietary-hordenine might be deaminated by MAO, which is present in the epithelium in high calalytic activity. However, more than 90% of this activity is due to MAO-A (Barwell & Canham 1988) which this study has shown does not deaminate hordenin. Therefore, intestinal deamination of hordenine would be dependent upon MAO-B. Studies with selective irreversible inhibitors of MAO-A have shown that intestinal MAO-B activity is apparently insufficient to prevent absorption of oral-tyramine (Youdim 1977). Therefore, it is unlikely that intestinal MAO-B activity is sufficient to deaminate dietary-hordenine, which is likely to be absorbed. Under the in-vitro conditions of this investigation hordenine produced a significant effect, upon responses to noradrenaline, at 25 $\mu$M. This was similar to the concentration of tyramine (50 $\mu$M) which produced half maximum contractions of the vasa. Therefore, like tyramine, dietary hordenine is likely to produce adverse pharmacological effects, upon the sympathetic nervous system.

Lilly 51641 was a gift from Lilly Research Laboratories, Indianapolis, Indiana 46285, USA.

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# Rapid production of ulcerative disease of the colon in newly-weaned guinea-pigs by degraded carrageenan

A. J. MARCUS, S. N. MARCUS, R. MARCUS, J. WATT, *Department of Pathology, University of Liverpool Duncan Building, P.O. Box 147, Liverpool L69 3BX, UK*

**Abstract**—In a dose-response study, degraded carrageenan (*Eucheuma spinosum*) was supplied in the drinking fluid at 1,2 and 3% concentrations over two weeks to young adult guinea-pigs. Ulceration of the large bowel was produced in 100% of animals, the severity and extent of damage probably being dose-related. In a time-course study, 3% degraded carrageenan solution supplied to newly-weaned guinea-pigs produced in 100% of animals ulceration in the caecum by four days and in the ascending colon by seven days. The onset of ulceration occurred as early as the second day. This model is convenient and economic for the screening of drugs of potential therapeutic value in human ulcerative colitis.

An experimental model for the investigation of ulcerative disease of the colon has become available in recent years following the recognition that certain sulphated polysaccharides fed to a variety of animal species will produce ulceration of the large bowel in a high proportion of animals (Marcus & Watt 1969; Mottet 1972; Watt & Marcus 1973). One of the most active ulcerogenic agents is carrageenan, derived from the red seaweed *Eucheuma spinosum*. When carrageenan, either in its native form or as a degraded product, is supplied in the drinking fluid, ulceration of the colonic mucosa occurs after a period which may range from about two weeks to as long as three months or more, depending on the nature and concentration of carrageenan supplied and the particular animal species used (Watt & Marcus 1973). In studying the effects of pharmacological and therapeutic agents in relation to experimental ulcerative disease of the colon, there are distinct advantages in having an animal model in which ulceration begins within a few days rather than weeks.

In this paper we describe a dose response in young guinea-pigs and a time-course study in newly-weaned animals, the results of which provide a convenient experimental model for the rapid production of carrageenan-induced ulcerative disease of the colon in 100% of animals by four days.

Correspondence to: R. Marcus, 193 Menlove Avenue, Liverpool L18 3EF, UK.