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MONOAMINE OXIDASE ACTIVITY OF MACROPHAGES AT REST AND DURING PHAGOCYTOSIS

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Abstract—Mouse macrophages contain monoamine oxidase (MAO) A activity and traces of MAO B, as judged by a strong deamination of 5-hydroxytryptamine and tyramine and a marginal one of benzylamine. Significant inhibition of MAO activity occurred in the presence of the specific inhibitors clorgyline and deprenyl. MAO A activity was considerably depressed in phagocytizing cells.

Monoamine oxidase (MAO) (EC 1.4.3.4) has been shown to occur in many mammalian organs and tissues. This enzyme exists in multiple forms differing in substrate specificity, sensitivity to inhibitors and electrophoretic mobility. The substrate range of MAO is wide and includes "biogenic amines" such as 5-hydroxytryptamine (5-HT) and other primary and secondary amines [1].

5-HT occurs in the platelets, plasma and whole blood of various vertebrates [2, 3]. Organs particularly rich in MAO are the liver, kidneys, intestines, stomach and aorta [4]. Relatively high MAO activity is present in blood platelets [5] but only low activity in blood plasma and erythrocytes [4].

As known, degranulation of mast cells is followed by phagocytosis of the granules by macrophages and other cells [6, 7]. In previous studies [8, 9] we have demonstrated that leucocytes deactivate two mast cell components, namely, histamine and heparin. In the present study, MAO activity was investigated in mouse macrophages at rest and during phagocytosis. The isoenzyme specificity was determined by using the substrates 5-HT, tyramine and benzylamine, and the inhibitors deprenyl and clorgyline.

MATERIALS AND METHODS

Reagents. The radioactive substrate [^{3}H]5-HT (sp. act. 14 Ci/mM) was obtained from Radiochemical Centre, Amersham, U.K., [1-^{14}C]tyramine hydrochloride (sp. act. 56.2 mCi/mM) was purchased from New England Nuclear, Boston MA, and [methylene-^{14}C]benzylamine hydrochloride (sp. act. 12.5 mCi/mM) was purchased from ICN Chemical and Radioisotope Division, Irvine, CA. 5-HT creatinine sulphate, tyramine HCl and benzylamine-HCl were purchased from Sigma Chemical Co., St. Louis, MO, M & B 9302 (clorgyline) was a gift of May & Baker Ltd., Dagenham, U.K., and deprenyl was kindly donated by Professor Sandler of Queen Charlotte's Maternity Hospital, London.

Macrophage preparations from mice. Enriched macrophage suspensions were obtained by harvesting the peritoneal exudate of mice previously injected with 1 ml of 2.5% thioglycollate (Difco). The cells (1.6×10^7 cells/mouse) were maintained in 10 cm Petri dishes in M 199 medium containing 15% calf serum. 2.5×10^7 cells were cultured in 10 ml medium for 72 hr, in a (5%) CO_2 incubator before start of the experiment. The medium was replaced 24 hr after seeding of the cells.

Enzyme assay. Enzyme activity was estimated radiochemically using [^{3}H]5-HT, [^{14}C]tyramine or [^{14}C]benzylamine as substrates. Since serum was found to deaminate the various amines the following steps were carried out after the initial (3-day) incubation of the macrophages *in vitro*: the cells were washed several times with Tyrode's solution containing 1/6 M Na–lactate 5:1 v/v and then 10 ml of the lactate–Tyrode solution containing 50 μl of labelled substrate were added (approximately 1×10^6 cpm 5-HT; 50,000 cpm [^{14}C]tyramine and 50,000 cpm [^{14}C]benzylamine). In some experiments freshly prepared solutions of the unlabelled amines were employed in order to obtain higher substrate concentrations. The Petri dishes were incubated at 37° for 2 hr in a (5%) CO_2 incubator following which aliquots (0.6 ml) of the supernatant were acidified with 0.6 ml of 2 M citric acid. The [^{3}H] and [^{14}C] deaminated products were extracted by shaking with 12 ml of extraction fluid containing PPO (Benzene–ethylacetate as the solvent for the 5-HT and tyramine products or toluene for the benzylamine [10]). The organic layer was separated from the aqueous layer, poured into a scintillation vial and counted. The blank runs were done with reaction mixtures from which the cells were omitted.

Inhibition studies. The cells were first incubated with the appropriate inhibitors at 37° for 30 min, then substrates were added and the mixture incubated for another 2 hr.

Incubation with latex particles. Latex particles (Difco) were added to macrophage cultures in maintenance medium in the ratio of 4.5×10^8 particles–Petri dish containing 1.5×10^7 cells and the plates were incubated at 37° for 1 hr. The cells were then washed several times with Tyrode's solution as previously described and 5-HT added immediately. In other experiments the cells were incubated in M 199 medium for 24 hr and then MAO activity was assayed.

Table 1. Comparison of macrophage MAO activity with three different substrates*

Substrate	MAO activity (μM/1.5 × 10^7 cells)
Tyramine	6.7 ± 0.06
5-HT	0.5 ± 0.05
Benzylamine	Traces

* Macrophages maintained in culture (1.5 × 10^7 cells) were incubated with the following labelled amines: [^{14}C]tyramine (sp. act. 56.2 mCi/mM). [^{3}H]5-HT (sp. act. 14 Ci/mM) and [^{14}C]benzylamine (sp. act. 12.5 mCi/mM). These were dissolved and used at a final concentration of 4 × 10^{-5} M. The metabolites were extracted and counted as described in the text. The mean values (of two different runs done in triplicates) are expressed as μmoles of product formed/1.5 × 10^7 cells/2 hr.

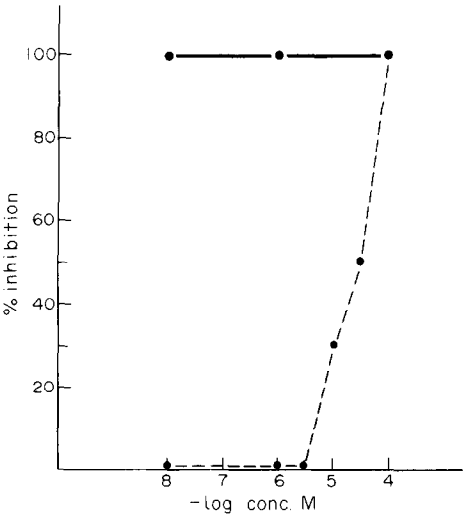

Fig. 1. Inhibition of 5-HT deamination at increasing concentrations of clorgyline and deprenyl. Macrophages in culture (1.5 × 10^7 cells) were preincubated with clorgyline (●—●) and deprenyl (·----·) at 37° for 30 min and then [^{3}H]5-HT (sp. act. 14 Ci/mM) was added. The incubation was continued for another 2 hr. The presented data are means of the percentage inhibition of MAO activity, as derived from two separate runs done in triplicate.

RESULTS

MAO A and B activity in mouse macrophages. Incubation of the macrophages with the various radioactive amines for 2 hr (Table 1) resulted in deamination products whose quantity differed considerably in each case. Thus tyramine was much more strongly deaminated by mouse macrophages than was 5-HT, while only traces of benzylamine were deaminated by the enzyme.

Increasing the substrate concentration from 4 × 10^{-9} M to 4 × 10^{-6} M did not affect the enzyme activity. Further increase of the concentration to 4 × 10^{-4} M did not increase the deaminative activity proportionately to the 5-HT concentration; however, the cells were not affected.

Inhibition studies. To confirm that macrophages contain essentially only MAO A, specific MAO inhibitors (i.e. clorgyline for MAO A and deprenyl for MAO B) were employed. With 5-HT as substrate, the addition of clorgyline resulted in complete inhibition of the deamination already at the low concentration of 10^{-8} M (Fig. 1). In contrast, deprenyl at low concentration did not inhibit the oxidation of 5-HT but did so at high concentrations. Thus there was no inactivation of the enzyme at a deprenyl concentration of 10^{-6} M but at 10^{-4} M, complete inactivation occurred as expected [11].

Similar results were obtained with tyramine as substrate: Clorgyline at a concentration of 10^{-8} M completely inhibited tyramine deamination but deprenyl at this concentration produced no inactivation. Again, a 100 per cent inactivation occurred at 10^{-4} M (data not shown).

Deamination of 5-HT in the presence of latex particles. Deamination of 5-HT by macrophages maintained in culture in the presence of latex particles was also determined. After 1 hr incubation the number of ingested latex particles ranged between 5–20 per cell. A 70 per cent decrement in 5-HT deamination occurred upon the addition of 5-HT.

In other experiments the free latex particles were first removed by rinsing and then the cells were incubated in medium for 24 hr. When exposed to 5-HT (after the replacement of the medium by Tyrode's solution) a partial recovery of the deaminating activity was noted; however the extent of this recovery fluctuated between 0–60 per cent.

DISCUSSION

Our investigation has been concerned with the determination of MAO activity in mouse macrophages. The findings seem to indicate the presence in mouse macrophages of MAO A which is active toward 5-HT as substrate and is inhibited by clorgyline. As expected the enzyme was found to act on tyramine but not on benzylamine. Such disparity in substrate specificity is not surprising as it is well known that the proportions of the two forms of MAO isozymes, namely A and B, may differ widely in various organs and that some organs may contain only a single form of MAO. The A enzyme abounds in the human placenta [12] as well as in rat spleen [13], whereas platelet MAO is predominantly of the B type.

Our findings are in agreement with those of Glenn [14] who very recently showed that guinea pig macrophages contain a MAO type A enzyme. In our study the activity of MAO B, using benzylamine as substrate, was very low (the radioactivity in the product was only twice that in the blank) so for all practical purposes mouse macrophages may be considered to contain only the MAO A isozyme.

The decline in 5-HT deaminative activity by phagocytizing macrophages suggests the possibility that macrophages contain surface receptors which become internalized during the process of phagocytosis. Internalization of receptors was shown to occur in the case of lectin binding sites on rabbit polymorphonuclear leucocytes [15] but the reappearance of receptor sites was not investigated. In our experiments however, a partial reappearance of receptors occurred within 24 hr.

Another possibility is that the substances become sequestered within the latex-containing lysophagosomes and are not available to the mitochondrially located MAO enzymes.

The present study is part of a wider investigation of the association between macrophages and mast cells. It is generally accepted that during mast cell degranulation, the granules are phagocytized by various leucocytes including macrophages [6]. Macrophages were shown by Zeiger [16] to transform histamine into various methylated imidazole derivatives. We have previously shown [9] that macrophages desulphate heparin and in the present work we report the deamination of 5-HT. Taken together, these findings suggest that the macrophages not only ingest the released mast cell granules but also deactivate their biological active amines.

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