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Phenylethylamine—Deamination by multiple types of monoamine oxidase

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Various types of monoamine oxidases (MAO; monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4) can be distinguished after differential inhibition with clorgyline [1]. Type A is defined as the most sensitive, while type B requires more clorgyline for full inhibition. Clorgyline-resistant activity has also been identified [2–4]. Conversely, deprenyl preferentially inhibits type B MAO [5].

2-Phenylethylamine (PEA) is regarded as a type B substrate [6–9] of high selectivity. For instance, in the rat brain [6], liver [10], lung [11, 12] and human brain [13, 14] PEA is selectively metabolized by MAO type B. The high preference of PEA for the B form of MAO has led several investigators to regard its deamination as a measure of type B activity in tissues. However, a statement made in a paper by Lyles and Callingham [2] indicates that PEA is deaminated by type A MAO in the rat heart. Since detectable type B activity in this organ is very limited [2, 15, 16], it might be assumed that an inherent low affinity of PEA for the type A enzyme had become exposed. Indeed, Ekstedt [10] has shown that, when type B MAO of rat liver is inhibited with deprenyl, PEA is still metabolized, but to a much smaller extent (4 per cent). Conversely, it is possible that MAO of the rat heart might differ from that in other organs.

Therefore, it was of interest to compare PEA deamination in the liver, ventricle, and vasa deferentia of rats. Unlike the heart, the liver [7, 9, 10] and vasa deferentia [4, 15, 17, 18] are reportedly well endowed with both type A and type B activity.

Experimental

Male Wistar rats (Hilltop Laboratory Animals, Inc., Scottsdale, PA) weighing between 200 and 400 g were used. The tissues were washed well in 0.9% (w/v) saline to remove blood, blotted, and homogenized in water. [^{14}C]PEA (51 mCi/m-mole) and kynuramine were used as substrates. The kynuramine assay was carried out as described previously [4]. For PEA, the assay mixture consisted of 0.1 ml homogenate (16.5 mg/ml for heart and vasa deferentia and 1.65 mg/ml for liver), 0.1 ml phosphate buffer (0.15 M, pH 7.4), 0.1 ml [^{14}C]PEA (0.5 mM) and 0.2 ml water. The vessels were preincubated with or without an inhibitor (clorgyline or deprenyl, made up in water) for 15 min at 37° prior to substrate addition. The reaction was allowed to proceed for 30 min and was stopped by plunging the reaction tubes into an ice–salt mixture (−5°). The entire contents of the tubes were passed over Bio-Rex 70 columns (0.75 × 1.5 cm, buffered to pH 6.0) and the effluent and water washes (3 × 2 ml) were combined. A 2-ml aliquot was counted by liquid scintillation spectrometry and total deaminated products were computed. In all experiments, [^{14}C]PEA was run both in the absence of tissue and in the presence of boiled homogenate. The [^{14}C]PEA was purified prior to use, and it was established that the columns retained in excess of 99.98 per cent of the purified amine. Both enzyme assays were shown to be linear with time and homogenate concentration. Substrate utilization over the 30-min incubation period did not exceed 5 per cent. All experiments were made twice, utilizing different tissue samples and duplicate determinations.

In the mixed substrate experiments, PEA (25, 50, 100 or 200 μM) was mixed with kynuramine (33 or 100 μM) in the presence of either 10^{-7} M clorgyline (to inhibit MAO type A) or 5×10^{-7} M deprenyl (to inhibit MAO type B). The lower kynuramine concentration approximates to the K_m of this substrate on both the A and B types of MAO in rat vasa deferentia.

Protein concentration was measured by the method of Lowry *et al.* [19] and all experiments were performed in duplicate.

Results and Discussion

Figure 1 shows that high concentrations of clorgyline were required to inhibit PEA deamination in the rat liver, even though considerable type A activity is known to exist in this organ [7, 9, 10]. In contrast, PEA deamination proceeded through both the A and B types of MAO in the vasa deferentia, as indicated by the biphasic curve. In the ventricles, PEA was deaminated by type A MAO, as shown by the high sensitivity to inhibition by clorgyline. Nearly 90 per cent inhibition occurred at 10^{-8} M clorgyline, whereas PEA metabolism in the liver was only very slightly affected (about 4 per cent inhibition) at the same inhibitor concentration. Mixed substrate experiments were made with PEA and the dual substrate kynuramine [4, 20] in the vasa deferentia (see Experimental). As would be expected from Fig. 1, PEA was found to be an effective inhibitor of 4-hydroxyquinoline formation on both the type A and type B activity.

The above results show that PEA can serve as a type A substrate, a dual substrate, or a type B substrate, depending upon the organ examined. Ekstedt [10] has shown that the K_m values for PEA on type A and type B MAO in rat

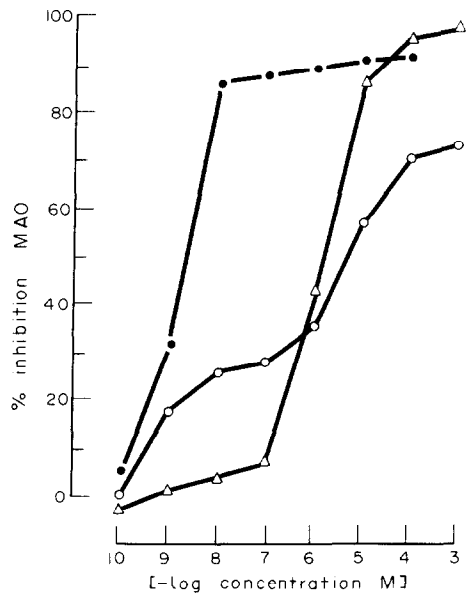

Fig. 1. Inhibition of 2-phenylethylamine deamination with clorgyline. Key: rat ventricles (●——●); vasa deferentia (○——○); and liver (△——△). The mean specific activities (nmoles deaminated/mg of protein/hr) were: 15.1, 16.4 and 95.2 respectively.

liver are 6.2×10^{-5} M and 4×10^{-6} M respectively. Thus, the concentration of PEA used in our experiments (1×10^{-4} M) would be expected to detect both types of MAO activity, providing sufficient amounts were present. Similar concentrations of PEA have been used by others for experiments in which PEA was determined to be a preferential type B substrate [6, 10, 21]. Thus, one explanation for the presently reported data is that differences exist in the kinetics of the type A and type B activity between the three organs examined. MAO has been postulated to exist in multiple forms [7, 9] and is believed to exhibit allosteric properties which might account for tissue selectivity [22, 23]. Additionally, since crude homogenates were used, the liberation of non-specific modulating factors and contamination with blood must be considered. However, in this latter regard, we have found that rat plasma is virtually devoid of deaminating activity toward PEA compared with the three organs tested.* Finally, the absolute amounts of type A and type B activity is another crucial factor. This in itself might account for PEA metabolism by type A in the ventricles but is less likely to be the sole explanation for the differences between the other two organs. Both the liver [7, 9, 10] and vasa deferentia [4, 15, 17, 18] exhibit considerable type A and type B activity.

PEA metabolism was not inhibited fully by clorgyline in the vasa deferentia. About 25 per cent of the measured activity remained uninhibited. This was not the case with kynuramine [4], or with PEA in the liver (Fig. 1). Thus, the vasa deferentia of rats contain a clorgyline-resistant species of MAO which can discriminate between PEA and kynuramine. This observation is intriguing since kynuramine will detect clorgyline resistance in the major blood vessels of the rat [4]. It seems, therefore, that this species of MAO may exist in more than one detectable form.

In another experiment, deprenyl (10^{-3} M) also failed to inhibit about 25 per cent of PEA deamination in the vasa deferentia. Previously, Yang and Neff [6] found clorgyline-resistant activity to PEA in the rat brain, but in their experiments deprenyl failed to reveal this same phenomenon. Roth and Gillis [11] have also shown that 30 per cent of PEA deamination is resistant to inhibition by pargyline in the perfused rabbit lung.

In summary, the present data show that the deamination of PEA can no longer be taken as a presumptive measure of type B activity in whole cell homogenates, unless additional proof is provided.

* Unpublished observations.

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