THE DEVELOPMENT OF MONOAMINE OXIDASE IN RAT LIVER AND BRAIN

Timothy J. MANTLE, Nigel J. GARRETT and Keith F. TIPTON
Department of Biochemistry, Tennis Court Road, Cambridge CB2 1QW, England

Received 20 February 1976

1. Introduction

Two activities of membrane-bound monoamine oxidase (MAO) can be distinguished in mammalian tissues with the aid of substrate selective inhibitors. A serotonin oxidising activity that is sensitive to Clorgyline (*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxyl)propylamine)[1], Lilly 51641 (*N*-[2(*o*-chlorophenoxyl)-ethyl]-cyclopropylamine [2] and PCO (5-phenyl-3-(*N*-cyclopropyl)ethylamine 1,2,4-oxadiazole) [3] and a benzylamine oxidising activity that is sensitive to Deprenil (phenyisopropylmethylpropinylamine) [4]. Tyramine, tryptamine and dopamine are deaminated by both systems [5]. The nature of the two activities remains obscure. The concept of two distinct enzymes A and B, for the serotonin and benzylamine oxidising activities respectively, was suggested by Johnston [1] and has received support from several workers [6]. However no convincing separation of the two forms, a requisite for the Johnston model, has been demonstrated to date. Alternative models that have been suggested for the rat liver enzyme are a single enzyme in different membrane environments [7] and a two site enzyme [8]. Experiments to test these models are not straightforward. This is partly due to the fact that none of these models stipulates the nature of the hypothetical form(s) as isolated from the membrane environment, and is considerably complicated by the known allotopic properties of the enzyme [9]. Thus the isolation of a clorgyline sensitive enzyme that deaminates serotonin but not benzylamine, before being assigned as MAO-A [10] should be shown not to contain an inactive B protein (or subunit). The problem of selectively inactivating one of the two forms during purification is well known [11–13]. Therefore until schemes for purifying preparations of MAO that can account for both forms become available alternative approaches to delineate the nature of the two forms have to be considered.

While several developmental studies of MAO have been reported [14–17] these have generally been limited with respect to the substrates used and are consequently of little use in the context of multiple forms. An exception is the study by Callingham and Lyles [18] who have shown that the increase in rat heart MAO with age is due to an increase in the A-form (using the criterion of clorgyline sensitivity). It has recently been suggested [19] that in developing mouse brain the A and B forms follow different post-natal development patterns. This conclusion was based on the different rates of development of the specific activity of representative substrates of the two forms. We wish to report the results of a study on the development of MAO in rat liver and brain monitoring the A/B ratio with the selective inhibitors Clorgyline, Deprenil and PCO as well as the specific activity with appropriate substrates.

2. Materials and methods

[1-^{14}C]-labelled serotonin creatinine sulphate, tyramine hydrochloride, dopamine hydrochloride and [G-^{3}H] tryptamine hydrochloride were obtained from the Radiochemical Centre, Amersham, Bucks, U.K. and [1-^{14}C]-labelled benzylamine hydrochloride was obtained from ICN Pharmaceuticals, Irvine, Calif. USA. Clorgyline (M & B 9302) was a kind gift from May & Baker, Dagenham, Essex, U.K. and Deprenil and PCO were kind gifts from Roche Products Ltd., Welwyn Garden City, Herts, UK.

2.1. *Preparation of enzyme extracts*

Mitochondrial fractions from the pooled livers and brains of Wistar rats at various stages of development were stored at −20°C. Brain homogenates were used immediately or stored at −20°C.

2.2. *Monoamine oxidase activity*

MAO activity was assayed radioisotopically [3], extracting deaminated products into 0.6% PPO in toluene/ethyl acetate (1:1, v/v).

3. Results and discussion

The specific activity of foetal liver mitochondrial MAO (from 3 days prepartum) was similar to adult values (150 g and 350 g rats). Similar results were obtained over this developmental period when benzylamine, serotonin or dopamine were used as substrates. Vaccari et al. [14] have reported a similar result with kynuramine as substrate. The effect of PCO and Deprenil on the tyramine deaminating activity of foetal liver MAO (fig.1) shows the presence of both forms in similar proportions to those found in adult tissues [21]. These results demonstrate that the binary system in rat liver is fully developed at least 3 days prepartum. It is tempting to extrapolate these findings to rat liver parenchymal cells which have been shown to contain both forms of MAO in similar proportions to those found in whole liver [21]. In this connection the enzyme from these cells (a washed mitochondrial preparation) has been further characterised with regard to kinetic parameters, which for serotonin, tyramine, dopamine and phenylethylamine are similar to values obtained for whole liver (Mantle, Garrett and Tipton, unpublished observations).

In rat brain we observed a similar difference in the developmental patterns of the specific activity of A and B substrates as reported for mouse brain [19]. The most striking and quantitative demonstration of this delayed development of the B-form in rat brain came from experiments where the A/B ratio was determined with the aid of selective inhibitors (fig.2). By the criterion of Clorgyline sensitivity the foetal rat brain (6 days prepartum) has only 2.5–3% of the B-form. This proportion rises rapidly so that by day 25 it is approaching the adult value of 32%. Similar results were obtained using Deprenil or PCO with homogenates or washed mitochondrial fractions (fig.2). It is tempting to speculate that the predominance of the A-form in foetal rat brain may be related to reports from Murphy's group that neuroblastoma [22] and glioma [23] contain exclusively this form. If cultured neuroblastoma and glioma contain only a foetal form of the enzyme, differentiation of these cells may provide a useful model for MAO development in the brain. Preliminary studies in this laboratory (Mantle and Cantin, unpublished observations) have confirmed the results of Murphy's group but so far have been unsuccessful in inducing the B-form on differentiation. The attraction of such an inducible system for further studies on the nature and development of the B-form in brain is obvious.

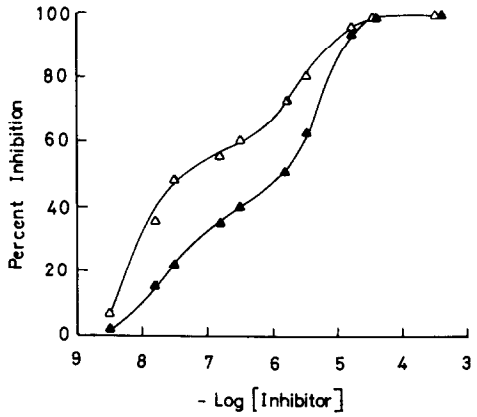

Fig.1. Effect of Deprenil and PCO on the MAO activity of two day prepartum rat liver mitochondria. Enzyme was preincubated in 0.1 M phosphate buffer pH 7.2 for 30 min at 30°C with the concentrations of Deprenil (△) or PCO (▲) indicated, prior to the addition of [^{14}C] tyramine (0.05 μCi= final concn 100 μM). After 20 min the reaction was terminated by the addition of 2 M-citric acid (0.5 ml) and the deaminated product extracted as described in the text. No inhibition occurred during preincubation in the absence of inhibitor. Results obtained with clorgyline on separate preparations were in agreement with those obtained with PCO and curves obtained with different preparations using Deprenil gave plateau values that did not differ by more than ± 5%.

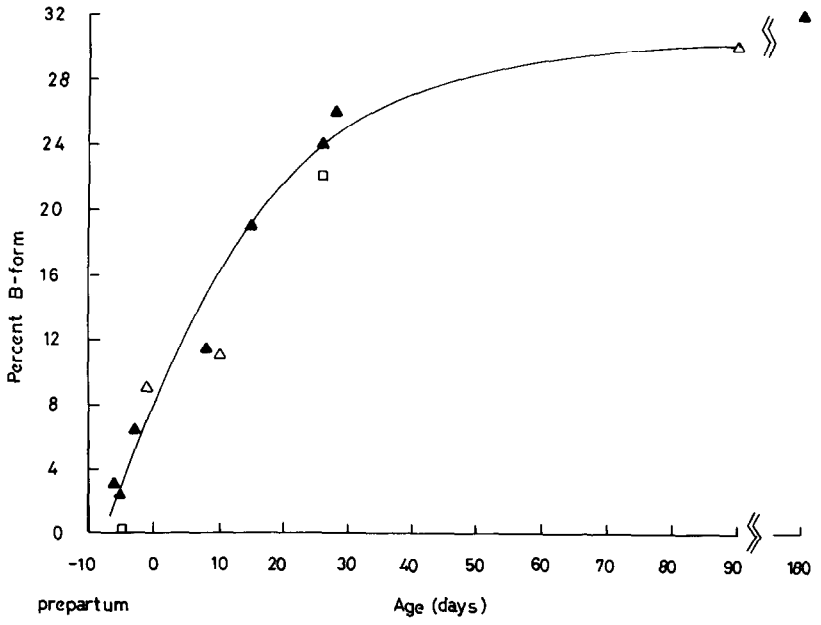

Fig.2. Age-dependence of the proportion of the B-form in rat brain. The proportion of the B-form was estimated from the clorgyline insensitive (▲) and Deprenil sensitive (□) fraction of brain homogenates and the PCO insensitive (△) fraction of brain mitochondrial fractions. MAO was assayed radioisotopically with tyramine as substrate in 0.1 M phosphate pH 7.2 The inhibitor concentration ranges were the same as in fig.1. Each preparation represented pooled foetal brains from 2–4 dams or 2–4 litters. The plateau values were each obtained from dose-response curves using not less than 10 inhibitor concentrations. The degree of error in estimating the plateau values was maximal at the highest proportion of the B form (± 5%) and decreased to < 1% at values below 10% of the B form.

Acknowledgement

We are grateful to the MRC for a grant in support of this work.

References

[1] Johnston, J. P. (1968) Biochem. Pharmacol. 17, 1285–1297.
[2] Fuller, R. W. (1968) Biochem. Pharmacol. 17, 2097–2106.
[3] Mantle, T. J., Wilson, K. and Long, R. F. (1975) Biochem. Pharmacol. 24, 2031–2038.
[4] Knoll, J. and Magyar, K. (1972) Adv. Biochem. Psychopharmac. 5, 393–408.
[5] Hall, D. W. R., Logan, B. W. and Parsons, G. H. (1969) Biochem. Pharmacol. 18, 1447–1454.
[6] Neff, N. H., Yang, H-Y.T. and Goridis, C. (1973) in: Frontiers in Catecholamine Research 133–137 (Snyder, S. H. and Usdin, E. eds.) pp. 133–137. Pergamon Press, London, New York.
[7] Houslay, M. D. and Tipton, K. F. (1973) Biochem. J. 135, 173–186.
[8] Mantle, T. J., Wilson, K. and Long, R. F. (1975) Biochem. Pharmacol. 24, 2039–2046.
[9] Tipton, K. F., Houslay, M. D. and Garrett, N. J. (1973) Nature 246, 213–214.
[10] McCauley, R. and Racker, E. (1973) Molec. Cell Biochem. 1, 73–81.
[11] Severina, I. S. and Zhivotova, N. I. (1973) Biokhimiya (Moscow) 38, 202–211.
[12] Shih, J. C. and Eiduson, S. (1973) J. Neurochem. 21, 41–49.
[13] Diaz Borges, J. M. and D'Iorio, L. (1972) Adv. Biochem. Psychopharmac. 5, 79–89.
[14] Vaccari, A., Maura, M., Marchi, M. and Cugurra, F. (1972) J. Neurochem. 19, 2453–2457.
[15] Bennett, D. S. and Giarman, N. J. (1965) J. Neurochem. 12, 911–918.
[16] Kuzuya, H. and Nagatsu, T. (1969) J. Neurochem. 16, 123–125.