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MONOAMINE OXIDASE IN SINGLE NERVE CELL BODIES FROM LOCUS COERULEUS OF THE RAT

A MICROGASOMETRIC STUDY

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Abstract—The magnetic diver microgasometer was used for determination of MAO activity in single nerve cell bodies isolated from the locus coeruleus of the rat. Tyramine was used as a substrate. Both molecular forms of MAO, MAO A and MAO B, are present in single nerve cell as shown by clorgyline, a selective inhibitor of MAO A molecular form. The activity of MAO in nerve cell bodies from locus coeruleus was compared to the activities in seven other types of nerve cells.

Locus coeruleus, a noradrenergic nucleus of the pontine tegmentum [1], differs from other nuclei of the rat brain by showing the highest activity of monoamine oxidase (MAO; amine: oxygen oxidoreductase (deaminating) (flavin containing); EC 1.4.3.4.) [2]. This finding may indicate the importance of the enzyme in the nucleus. In nervous tissue, MAO occurs in multiple molecular forms which differ in their substrate affinities and can be selectively inhibited [3–5]. The forms A and B were found in the homogenates of the locus coeruleus region [6, 7]. However, in order to elucidate a possible functional role of MAO molecular forms in locus coeruleus it would be important to know which molecular forms are present in individual nerve cells. Therefore, the activity of MAO and of its molecular forms was determined in single nerve cell bodies isolated from locus coeruleus of the rat. The magnetic diver microgasometric method [8] was applied in order that the activity of MAO in such small samples could be measured.

MATERIALS AND METHODS

White female rats (Wistar, 160–200 g) were exsanguinated under ether anaesthesia between 10 and 11.30 a.m. Brains were isolated and the area in the brainstem containing locus coeruleus was exposed according to Zigmond *et al.* [9]. The cells were isolated as described by Hydén and Pigon [10]: a slice was cut from the brainstem and covered with 0.25 M sucrose solution containing methylene blue dye (0.5 mM). The slightly stained nerve cell bodies from the locus coeruleus were dissected free with a thin wire under the dissecting microscope and immersed in a drop of reaction medium containing: 1/15 M Na phosphate buffer pH 7.0, 10 mM tyramine and 10 mM semicarbazide. Measurements of MAO activity with lower concentrations of tyramine were not reliable. In a series of experiments, KCN (1 or 10 mM) was added to the incubation medium. The nerve cell body was then sucked into the ampulla, together with some medium and an air bubble, and the microgasometric measurement was performed (for method see refs. 8 and 11). Preincubation for 15 min at 30° was used in order to achieve equilibration of gases in the ampulla. Oxygen consumption was then measured every 10 min for an hour at the above mentioned temperature. Usually MAO activity in one isolated cell body was assayed but in experiments with higher concentration of inhibitor, two cells were pooled in order to increase the accuracy of the assay. The volume of oxygen consumed in the enzymic reaction was calculated and the activity of MAO expressed in pmols of oxygen/hr/cell. The activity of MAO was determined also in the presence of 0.1 nM–1 μM clorgyline, a selective inhibitor of the A form of the enzyme. In these experiments, a nerve cell body was first preincubated in a medium containing clorgyline but without tyramine for 20 min at room temperature. Further procedures were the same as described above except that the reaction medium contained clorgyline in the appropriate concentration. Iproniazide (1 μM), a nonselective inhibitor of MAO, was used in separate experiments in order to test the specificity of the assay.

Student's *t*-test was used for statistical evaluation of the results. The following substances were used in experiments: tyramine hydrochloride (Hoffman La Roche, Basel, Switzerland), semicarazide hydrochloride (Riedel-de-Haën, Hannover, F.R.G.), clorgyline hydrochloride (May and Baker, Dagenham, U.K.), iproniazide phosphate (Aldrich, Milwaukee, WI).

RESULTS

Oxygen consumption in samples assayed either with or without KCN (1 and 10 mM) did not differ significantly (Table 1). Therefore, we did not use KCN in assays of MAO activity in further experiments. The rate of oxygen consumption due to the MAO activity in isolated nerve cell body was con-

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Table 1. The activity of MAO (pmol O_2/hr/cell) and the influence of KCN in a single isolated nerve cell body from locus coeruleus of the rat (substrate: tyramine 10 mM)

KCN (mM)	Mean ± S.D.	N
10	10.3 ± 2.8[a]	23
1	12.1 ± 1.8[b]	8
0	11.3 ± 2.3[c]	38

a:b, b:c, a:c—$P > 0.05$.

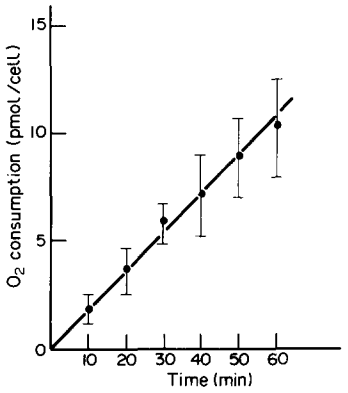

Fig. 1. The time course of oxygen consumption in a microgasometric MAO assay by a single nerve cell body isolated from locus coeruleus of the rat. Mean ± S.D., N = 10, substrate: tyramine 10 mM.

stant throughout the period of the assay (Fig. 1). The rate of the enzymic reaction was directly proportional to the number of cell bodies in the ampulla (Fig. 2). MAO activity in single nerve cell body from locus coeruleus was 11.3 ± 2.3 pmole O_2/hr/cell (N = 38). Population of nerve cells from this nucleus

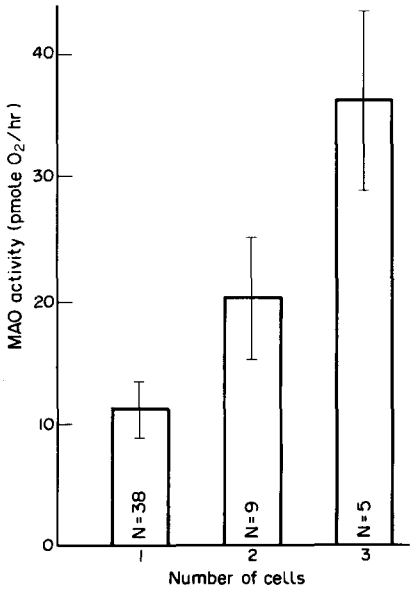

Fig. 2. The relationship between the number of isolated nerve cell bodies in the ampulla and the reaction rate. Mean ± S.D., substrate: tyramine 10 mM.

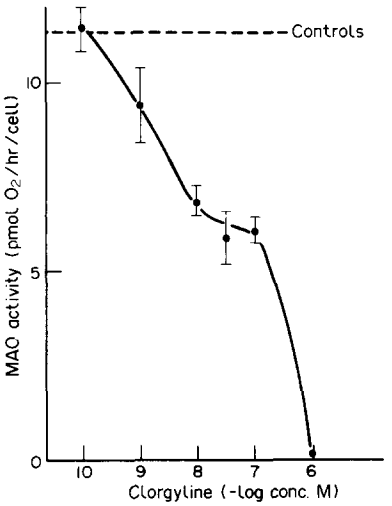

Fig. 3. Inhibition by clorgyline of MAO activity in a single nerve cell body isolated from locus coeruleus of the rat. Each point is the mean ± S.D. of 4–7 determinations. Substrate: tyramine 10 mM.

seems to be quite uniform as far as their MAO activities are concerned. Inhibition of MAO in presence of different concentrations of clorgyline is shown in Fig. 3. MAO activity left uninhibited at clorgyline concentration between 0.01 and 0.1 μmole/l (in the region of the plateau of the "log inhibitor concentration–% of inhibition" curve) represents the activity of the molecular form B. Using 10 mM tyramine as a substrate, the activity of MAO B represented about 60% of the total enzymic activity of MAO. No clorgyline resistant MAO could be detected in these cells. Iproniazide, a nonselective specific inhibitor of MAO, completely inhibited the enzyme at a μM concentration.

DISCUSSION

By using the microgasometric method it was possible to determine the total MAO activity and the activities of molecular forms A and B of the enzyme in a single nerve cell body isolated from the locus coeruleus of the rat. The sample containing only a nerve cell body was not contaminated with other structures containing MAO, e.g. capillaries [12, 13], neuropil and glia [14, 15]. This is not the case when MAO is assayed in tissue homogenates. The dendrites projecting in the neuropil and the axon are almost completely torn away during the isolation procedure and we are dealing mainly with the nerve cell soma. The axosomatic synapses are scarce in locus coeruleus [16]. Therefore, the number of the synaptic knobs attached to the isolated nerve cell bodies must be relatively small. The greater part of the plasmalemma is in contact with astrocytic processes [16] and some contamination of cell bodies with them was possible. However, the contamination of isolated cell bodies with glia was negligible as verified by phase contrast microscopy (unpublished observation). Small contamination with glial processes could not contribute considerably to the activity of the sample since the activity of glia was

Table 2. The activity of MAO (pmol O_2/hr/cell) in single isolated cell bodies from different neural tissues of the rat (substrate: tyramine 10 mM)

Cell (nucleus)	Mean activity of MAO (pmol O_2/hr/cell)	Reference
Locus coeruleus	11	this investigation
Nucl. caudatus	22	14
Globus pallidus	14	14
Substantia nigra	20	14
Glial clumps*	7	14
Purkinje cell	18	20
Nucl. giganto-cellularis pontis	17	20
Odontoblast	15	19

* Size of the nerve cell.

found to be lower than the activity of nerve cells ([14, 15]; Table 2). In "classical" gasometric assays of MAO activity semicarbazide (10 mM), an inhibitor of aldehyde dehydrogenase, and KCN (1 mM), an inhibitor of cytochrome oxidase and peroxidase, were added to the incubation medium; catalase remained uninhibited even at 10 mM concentration of cyanide [17]. Later Johnston [3] omitted KCN from the incubation medium in his gasometric assays of MAO activity because he found that cyanide did not influence the oxygen consumption during the reaction. We checked the influence of KCN also in our microgasometric assay. Only insignificant differences in oxygen consumption were found when KCN was omitted from the incubation medium. It seems that, under our experimental conditions, cytochrome oxidase and peroxidase, even in the absence of KCN, do not interfere significantly with the determination of MAO activity. The reaction rate was proportional to cell, i.e. enzyme concentration in the ampulla. The total inhibition of oxygen consumption by iproniazide and by higher concentration of clorgyline proves a high specificity of our experimental assay. Methylene blue which was used for the visualization of nerve cells during the isolation procedure is a reversible inhibitor of MAO [18]. However, after the isolated cell body was suspended in the incubation medium and sucked into the ampulla, the cell had been destained and the inhibitor diluted to an ineffective concentration.

Using this microgasometric assay, we found that both molecular forms of MAO were present in one and the same nerve cell. Both molecular forms have already been found in homogenates of locus coeruleus [6, 7]. The question remained, however, whether the B form belonged to glia or to noradrenergic nerve cells [7]. Our results clearly demonstrated the presence of B form in nerve cells of locus coeruleus.

MAO activity in nerve cell bodies of locus coeruleus is rather low when compared to activity in other cells of rat, isolated and assayed in the same manner [14, 19, 20] (Table 2). Therefore, the relatively high activity of MAO in locus coeruleus homogenates [2, 6, 7] could be ascribed to the dense packing of nerve cells in this nucleus [21–23] rather than to the high activity in individual cells.

The presence of MAO activity in the locus coeruleus nerve cells conforms well with the fact that this nucleus contains a high concentration of noradrenaline [21, 24, 25] which is a typical substrate for the A form of the enzyme. MAO B in this nucleus may be involved in degradation of dopamine [26] which is a precursor of noradrenaline but has no transmitter function in locus coeruleus neurons [24]. Other possible substrates for MAO B are phenylethylamine [17] and tele-methylhistamine [18] but there are no available data concerning the presence of these substrates in locus coeruleus.

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