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DISTRIBUTION OF TYPE A AND TYPE B MONOAMINE OXIDASE ACTIVITIES IN RAT AND CHICKEN SKELETAL MUSCLE AND NERVES

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Abstract—The monoamine oxidase (MAO) activities and proportions of type A and type B MAO in rat and chicken skeletal muscles, sciatic nerves, phrenic nerves, brachial plexi, spinal cords and motor cortices were studied. Skeletal muscle contained mainly or exclusively type A MAO. The MAO in neural tissue of both species was 30–95 per cent type A, with chicken neural tissue tending to have more type B MAO than the rat. There was no evidence of a correlation between the total MAO activity of particular nerves and the MAO activity of the skeletal muscles they innervate. The MAO activity of the skeletal muscles did not correlate with their frequencies of contraction.

Monoamine oxidase (MAO, EC 1.4.3.4) is a mitochondrial enzyme which has been known to exist in multiple forms which differ in substrate specificity [1,2], inhibitor sensitivity [3–5] and electrophoretic mobility [6,7]. The molecular basis for the two forms is not known for certain. The different forms have been reported to contain proteins of the same molecular weights with varying lipid moieties attached [8]. Different regional distributions of brain biogenic amines [9,10], together with the relatively selective substrate specificity and regional distribution of the two forms of MAO [1,2] indicate that there may be physiological significance associated with this differentiation of enzymatic activity [11].

In a previous paper, we reported that rat vastus lateralis muscle contained only type A MAO [12]. Suzuki and Yagi [13] reported that four rat brain areas (cerebral hemispheres, cerebellum, medulla plus pons, and brain stem) and the spinal cord had greater activity toward serotonin (5-HT), a preferred substrate of type A MAO, than toward phenethylamine (PEA), a preferred substrate of type B MAO. Chick spinal cord also deaminated 5-HT more actively than PEA, but the same four areas of chick brain showed greater activity toward PEA than 5-HT. We were interested in determining if a variety of rat muscles and chick muscles also had only type A MAO and, if not, in comparing the proportion of type A to type B MAO in skeletal muscles, as well as total MAO activity to the relative amount and activity of these forms in the motor nerves which innervate the muscles.

METHODS

Two male Sprague–Dawley rats (Sprague–Dawley, Inc., Madison, WI), weighing 150–200 g, were housed in a temperature controlled (26°) colony where lights were on from 5:00 a.m. to 7:00 p.m. and Purina rat chow and water were available *ad lib*. Following decapitation, aliquots of the following muscles or neural tissues were taken: vastus, soleus, gastrocnemius, extensor digitorum longus (EDL), diaphragm, intercostal, pectoralis and biceps muscles; the sciatic and phrenic nerves; and the brachial plexus, spinal cord and cerebral hemispheres. Following excision, muscle tissue was immersed in buffer for 5 min [14]. The buffer contained 0.1 M KCl, 2mM EGTA,* 10 mM Tris and 1 mM Na-pyrophosphate, pH 7.4. The muscle, nerve or cerebral hemisphere was homogenized in the same medium (5%,w/v) with a glass homogenizer. Two adult chickens of the white leghorn strain, obtained from a local slaughterhouse, were also killed, and the same muscles and nerves described above, as well as the cerebral hemispheres, were removed and homogenized in the same medium. All samples were analyzed in duplicate and the averages from the two animals were determined.

Monoamine oxidase activity was determined by the modified radioisotopic method of McCaman *et al.* [15], as reported earlier [12], using [^{14}C]-5-hydroxytryptamine as substrate for MAO A, [^{14}C]benzylamine for MAO B, and [^{14}C]tyramine, a mixed substrate, for both type A and type B. The ratio of type A to B was determined by evaluating the effect of increasing concentrations, of clorgyline, which is a specific inhibitor of type A MAO [2], on tyramine metabolism, a mixed substrate, and then plotting the log of inhibitor concentration vs percentage inhibition, as described by Johnston [2].

Tyramine HCl, benzylamine HCl and serotonin creatine sulphate were purchased from the Sigma Chemical Co. (St. Louis, MO). The radioactive substrates [2-^{14}C]-5-HT binoxalate (sp. act. 27 mCi/mmole) and [1-^{14}C]tyramine HCl (sp. act. 55 mCi/mmole) were purchased from the Amersham/Searle Corp. (Arlington Heights, IL). [1-^{14}C]Benzylamine HCl (sp. act. 4.0 mCi/mmole) was purchased from New England Nuclear (Boston MA). All the other chemicals used were of analytical grade quality.

Protein concentration in muscle homogenate was determined by the method of Lowry *et al.* [16] using bovine serum albumin as the standard.

* EGTA = ethyleneglycolbis (aminoethylether) tetraacetate.

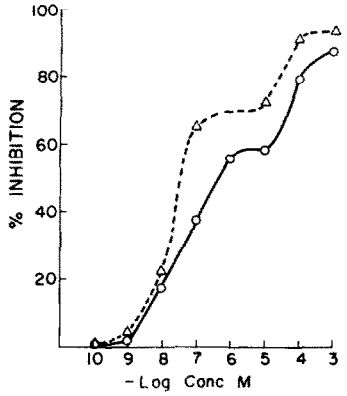

Fig. 1. Inhibition of the deamination of tyramine by increasing concentrations of clorgyline. Sciatic nerve homogenates were preincubated with clorgyline at 37° for 15 min and then tyramine (0.65 mM) was added. The incubation was continued for an additional 30 min. The degree of inhibition was calculated from the difference in the number of counts/min in the sample with and without the clorgyline. Data are presented as the mean (duplicate) of percentage inhibition of MAO activity. Key: (○) rat sciatic nerve, and (△) chicken sciatic nerve.

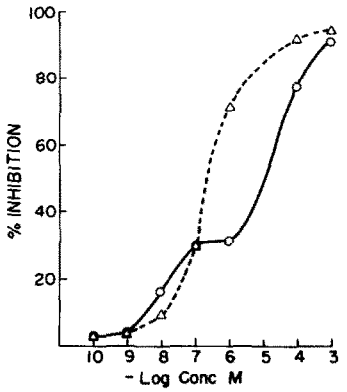

Fig. 2. Inhibition of the deamination of tyramine by increasing concentrations of clorgyline. Brachial plexus homogenates were preincubated with clorgyline for 15 min at 37° and then tyramine (0.65 mM) was added. The incubation was continued for an additional 30 min. Data are presented as the mean (duplicate) of percentage inhibition of MAO activity. Key: (○) rat brachial plexus, and (△) chicken brachial plexus.

RESULTS

The MAO activities of rat and chicken muscles, sciatic nerves, phrenic nerves, brachial plexi, spinal cords and motor cortices are given in Table 1. To avoid confusion, we wish to indicate again that the percentage of each type of MAO was not calculated from the MAO activities with serotonin and benzylamine as substrates but by the method of Johnston [2], in which the extent of inhibition of tyramine oxidation by various concentrations of clorgyline, a specific type A MAO inhibitor, is determined. This is illustrated in Fig. 1 where the effects of increasing concentrations of clorgyline on tyramine deamination by rat and chicken sciatic nerves are shown. As evident from Fig. 1, the percent inhibition increases as the concentration of clorgyline increases until a plateau is reached at 60 per cent (rat) and 70 per cent (chicken) inhibition and then increases again as the concentration of clorgyline is raised. This indicates the presence of two forms of MAO in rat sciatic nerve in the ratio of 60:40 and in chicken sciatic nerve in the ratio of 70:30. A simple sigmoid curve would indicate the presence of only one type of MAO. The correlation between %A calculated from the clorgyline plateau and from the serotonin/serotonin + benzylamine ratio was $r = 0.941$ (N = 13) for rat tissues ($P < 0.01$) and $r = 0.0384$ (N = 13) for chicken tissues (P = not significant). The correlation in the rat was solely due to the muscle ($r = 1.0$, N = 8, $P < 0.01$). For the rat neural tissue, the correlation was 0.0717, N = 5, P = not significant.

Rat sciatic nerve, phrenic nerve and brachial plexus had 60, 70 and 30 per cent type A MAO activity,

Table 1. Type A and Type B MAO activities in rat and chicken nerves, muscles and cerebral cortex

Tissue	Rat (nmoles/mg/hr) Serotonin deamination	Rat (nmoles/mg/hr) Benzylamine deamination	Rat %A*	Chicken (nmoles/mg/hr) Serotonin deamination	Chicken (nmoles/mg/hr) Benzylamine deamination	Chicken %A*
Sciatic nerve	68.3 ± 1.5†	33.4 ± 1.2	60 ± 5	31.9 ± 0.8	2.2 ± 0.04	70 ± 5
Vastus M.	4.2 ± 0.2	0	100	0.79 ± 0.01	0	100
Soleus M.	3.4 ± 0.13	0	100	2.94 ± 0.14	0.24 ± 0.02	100
Gastrocnemius M.	2.8 ± 0.08	0	100	1.1 ± 0.03	0.11 ± 0.02	90 ± 2
Extensor digitorium longus M.	3.8 ± 0.08	0	100	1.5 ± 0.01	0	90 ± 3
Phrenic nerve	15.2 ± 0.72	12.6 ± 0.84	70 ± 5	8.6 ± 1.1	5.2 ± 1.2	80 ± 5
Diaphragm M.	16.5 ± 1.2	0	100	24.9 ± 1.6	2.4 ± 1.4	50 ± 5
Brachial plexus	9.3 ± 0.18	9.1 ± 0.26	30 ± 5	21.3 ± 1.4	3.4 ± 0.5	95 ± 2
Intercostal M.	1.8 ± 0.2	0.4 ± 0.07	90 ± 4	5.2 ± 0.8	1.6 ± 0.6	90 ± 3
Pectoralis M.	2.2 ± 0.06	0	100	0.41 ± 0.01	0.15 ± 0.02	100
Biceps M.	1.6 ± 0.07	0	100	0.19 ± 0.04	0	100
Cerebral hemispheres	24.3 ± 1.2	14.8 ± 1.2	55 ± 5	13.0 ± 1.2	2.2 ± 0.5	95 ± 2
Spinal cord	17.6 ± 0.87	7.0 ± 0.7	65 ± 8	36.1 ± 1.4	2.8 ± 0.7	87 ± 2

* By the method of Johnston [2].
† S.D.

respectively, indicating significant variability among these three neural tissues. All the muscles innervated by these nerves had 100 per cent type A MAO activity with the possible exception of the intercostal muscle which had 90 ± 4 per cent (S.D.) type A MAO activity. The sciatic nerve had much greater MAO activity than the phrenic nerve or the brachial plexus, but the highest MAO activity of any of the muscles in the rat was in the diaphragm. Thus, the correlation between muscle and nerve MAO activity was relatively poor. The rat spinal cord and cerebral hemispheres were 65 and 55 per cent type A MAO, respectively—close to the proportion found in the sciatic and phrenic nerves but greater than that of the brachial plexus.

Chicken sciatic nerve, phrenic nerve and brachial plexus were 70, 80 and 95 per cent type A MAO. Thus, these chicken tissues had a relatively higher proportion of MAO A activity than their rat counterparts. This was particularly striking for the brachial plexus which for the chick was 95 per cent type A, and for the rat, 30 per cent type A (Fig. 2). Four chick muscles had some type B MAO: the gastrocnemius (10 per cent), extensor digitorum longus (10 per cent), diaphragm (50 per cent) and intercostal muscle (10 per cent), compared to only one rat muscle, the intercostal, which had approximately 10 per cent type B MAO activity.

The chick sciatic nerve, like the rat sciatic nerve, had the highest MAO activity of the three nerves studied. The chicken phrenic nerve had the lowest MAO specific activity of any neural tissue, but the chick diaphragm had the highest MAO activity of any of the muscles studied. The diaphragm also had the highest MAO activity of any rat muscle. The intercostal muscle in the chick also had relatively high MAO activity. With the exception of the diaphragm and intercostal muscles, the chick muscles had lower MAO activity than the corresponding rat muscle. The chick spinal cord and cerebral hemispheres were 87 and 95 per cent type A MAO, respectively—slightly higher percentages than found in the rat. Chick spinal cord MAO activity was much greater than chick cerebral hemisphere MAO activity, whereas the opposite was found in the rat.

Rat sciatic nerve, phrenic nerve and cerebral hemisphere MAO activity was greater than that of the chick, but chick brachial plexus and spinal cord MAO activities were greater than those of the rat.

DISCUSSION

We confirmed the previous finding of Suzuki and Yagi [13] that rat and chick spinal cord and rat cerebral hemisphere have greater type A MAO activity than type B MAO activity. However, in the chick cerebral hemisphere, we also found higher type A MAO activity, whereas Suzuki and Yagi found type B MAO activity to be greater than type A. We also found a much smaller proportion of type B MAO in chick spinal cord and relatively more type B MAO in rat cerebral hemispheres and spinal cord than did Suzuki and Yagi [13]. Factors that could contribute to the difference in these results are substrate difference (benzylamine vs phenethylamine), difference in assay conditions, and differences in strains of rats and chickens. Furthermore, phenethylamine may not be as useful a substrate to distinguish between type A and type B MAO as benzylamine since recent studies have found phenethylamine may be a preferred substrate for some variants of type A MAO [17]. Suzuki and Yagi [13] have reported the amount of each form of MAO by determining the specific activity for serotonin and phenethylamine, whereas we have used the method of Johnston [2] which may be a more reliable way to compare the proportion of MAO A and B than the activity toward relatively selective substrates [11].

The marked differences between the percentages of MAO B in rat sciatic and phrenic nerves (40 and 30 per cent) and brachial plexus (70 per cent) are of interest. It is not yet known if these differences have any physiological significance. If one role of MAO is to inactivate biogenic amines, these results may indicate that PEA, the major endogenous substrate of type B MAO, or substrates of both MAO A and B such as dopamine, may have a significant physiological function in the brachial plexus which is absent in the other two nerves. Alternatively, the higher MAO B activity in the rat brachial plexus may be needed to prevent toxicity from PEA. In contrast to the rat, the brachial plexus of the chick had less type B MAO (5 per cent) than the sciatic nerve (30 per cent) or the phrenic nerve (20 per cent). The significance of the striking difference of the proportion of type B MAO in rat and chick brachial plexi will require further study.

Almost all rat muscle MAO activity was the type A form. Thus, skeletal muscle is the first tissue which has MAO A alone. Previously, mouse neuroblastoma and some other cell lines grown in tissue culture have been reported to contain only type A MAO [18–20]. This is additional evidence that type A and type B MAO are genuinely independent forms of MAO. This contrasts with the corresponding motor nerves which had 30–70 per cent type B MAO. Since the MAO of sympathetic nerves which are mingled with the motor nerves is type A MAO [21], the type B MAO of motor nerves apparently has little, if any, significance for the type of MAO in the skeletal muscles they innervate. One can conclude from this that the monoamine oxidases of muscle and nerve have independent functions.

The rat spinal cord has 65 per cent type A MAO, which agrees fairly closely with the proportion of type A MAO in sciatic nerve and phrenic nerve. The proportion of type A MAO in chick spinal cord was also comparable to that in sciatic nerve, phrenic nerve and brachial plexus.

The high activity of MAO in the diaphragms of both the chick and the rat is noteworthy. These muscles are phasically active and are rich in mitochondria. However, the intercostal muscles, which contract with each breath, had much lower MAO activity, suggesting that no simple relationship with activity is likely to explain the variations in MAO activity which have occurred during evolution.

There have been various suggestions that biogenic amines, such as dopamine or epinephrine, have a role in neuromuscular transmission [22,23] or a trophic effect on skeletal muscle [24]. On the basis of inhibitor studies, it has been suggested that MAO is involved in the regulation of axoplasmic transport [25,26]. This was not confirmed in a recent study [27]. A role for MAO, presumably type A MAO, in the degradation of dopamine and norepinephrine, which escapes from ves-

icles being actively transported in motor nerves, has been postulated [28]. On the basis of the large amounts of MAO A in skeletal muscle of rats and chickens and the rich activity and varying proportions of MAO A and B in motor nerves, further studies of the role of MAO in skeletal muscle and motor nerves are clearly indicated.

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