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THE INFLUENCE OF AGE ON THE RECOVERY OF CARDIAC MONOAMINE OXIDASE AFTER IRREVERSIBLE INHIBITION*

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Abstract—The level of monoamine oxidase (MAO) activity of rat heart is dependent on the age of the animal. Activity appears at 1–2 weeks after birth and increases progressively for the following 16–18 weeks. Thereafter the enzyme level remains fairly constant. Irreversible inhibition of cardiac MAO by administration of pargyline is followed by complete recovery of the enzyme, but the rate and duration of recovery are dependent on the age at which inhibition is produced. Thus, pargyline administration to 1-week-old rats resulted in a recovery curve which did not differ from that of controls. Total recovery of cardiac MAO, which was inhibited in rats 8 and 16 weeks old, required 6–8 weeks, with the initial rates of recovery being greater for the older animals.

THE ENZYME, monoamine oxidase (MAO), has been the subject of much research since its original discovery in 1928.[1] As a result, many of its properties and possible functions are now understood. A major breakthrough in MAO biochemistry and pharmacology in recent years has been the discovery of a number of irreversible inhibitors, some of which have found use as therapeutic agents. These compounds also have been employed extensively as tools to study various aspects of the biogenic amines, and this in turn has shed further light on the functions of MAO.

However, relatively little work has appeared on the formation and regulation of MAO in which the irreversible inhibitors have been employed. It would appear that advantage could be taken of this irreversible property to study the regeneration of the enzyme under various conditions. Previous studies from various laboratories have shown that after irreversible inhibition of MAO complete recovery to normal levels requires days to weeks, depending upon the tissue and species of animals.[2–5] Wiseman-Distler and Sourkes[6] have investigated in rats the rate of MAO regeneration during riboflavin deficiency after inhibition with iproniazid. Their results indicate that riboflavin deficiency does not alter the rate of recovery of MAO, but the sensitivity of MAO to inhibition is increased. Other than the preceding reference, little has been done in the way of determining the various factors that modify or control the rate of MAO regeneration.

The present study has employed an irreversible inhibitor of MAO to determine the influence of age on the recovery of MAO in the rat heart and other organs. The heart

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is of special interest in that its MAO increases with age,[7, 8] suggesting that different rates of enzyme synthesis might be involved during different ages of the rat's life. Recovery of MAO activity after inhibition then, would possibly be influenced by the age of the animal.

METHODS

Male Sprague–Dawley rats of ages ranging from 1 to 28 weeks were employed in this study. The animals were divided into two groups, one serving as the control while the other received a single injection of 25 mg/kg of pargyline (expressed as the base) at 1, 8 or 16 weeks of age. Animals were sacrificed at various intervals during the 28-week period and the livers, brains and hearts were assayed for MAO activity. In the 1-week-old rats, the hearts of several animals were pooled in order to obtain sufficient tissue for the enzyme assay. Beyond the first week, each heart was determined individually.

MAO activity was determined by employing tissue homogenates with 5-hydroxytryptamine (5HT) creatinine sulfate as the substrate. One ml of tissue homogenate (liver, heart 5 per cent; brain 10 per cent) was incubated with 3 μmole 5HT and pH 7·4 buffer in a final volume of 3·0 ml. After various intervals of incubation, the reaction mixture was inactivated with trichloroacetic acid and aliquots were assayed for residual serotonin content according to the method of Udenfriend *et al.*[9] MAO activity is expressed as μmoles 5HT metabolized/g of tissue in 1 hr. Growth rates of all rats were recorded by following weight gain over the designated periods.

RESULTS

Heart MAO. The heart of the male rat shows an age-dependent increase in MAO activity during at least the first 16–20 weeks, after which there is some degree of stabilization. While little or no MAO activity was exhibited in hearts from 1-week-old rats, by 8 weeks the activity had increased to values exceeding that in the brain (10 μmole/g/hr) and in 18 weeks it was almost equal to that of liver (28–35 μmole/g/hr). Thereafter it stabilized at activities of approximately 30 μmole 5HT metabolized/g in 1 hr (Fig. 1).

When pargyline was administered to 1-week-old animals, the recovery of cardiac MAO was complete within a week. Actually there was no significant difference in enzyme activity between the control and inhibited groups throughout the entire period of 8 weeks. Recovery of enzyme activity to control levels after induction of irreversible inhibition at 8 weeks of age required approximately 4–6 further weeks. Recovery proceeded at an essentially linear rate with time, and while at 16 weeks there appears to be an overshoot above normal, this was not a consistent or statistically significant observation. Pargyline again produced complete inhibition of cardiac MAO in 16-week-old animals, but the rate of recovery was markedly different from those of the 1- and 8-week-old rats. During the first week after pargyline administration, heart MAO had returned to activities of approximately 12–13 μmole/g tissue per hr, which represent about twice the value in 8-week-old animals one week after pargyline administration. Thereafter, the recovery progressed at a less rapid rate, and total recovery to control levels required some 6–8 weeks after MAO inhibition (see Fig. 1 and Table 2). The resulting recovery curve for animals given pargyline at 16

weeks is hyperbolic as compared to the linear relationship seen with the 8-week-old rats.

Brain and liver MAO. The activities of brain and liver in 1-week-old male rats are developed to about 75 per cent of the adult animal. By the fourth week of life, the

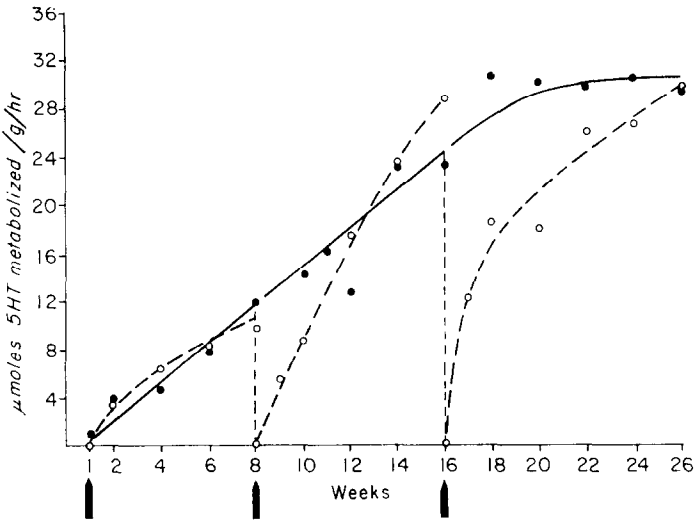

FIG. 1. Graph representing the progressive increase in heart MAO activity with increasing age of the rat (solid line). At 1, 8 and 16 weeks, pargyline sulfate (25 mg/kg) was administered i.p., and the rate of MAO recovery was followed until recovery to control levels (dotted lines). Each point represents the mean MAO activities derived from hearts from 3–10 animals.

enzyme levels have reached their maximum and are maintained for at least the following 20–30 weeks. Brain MAO levels are remarkably stable and consistent, as seen in Table 1, their activities stabilizing at approximately 10 µmole/g/hr. The liver enzyme

TABLE 1. INFLUENCE OF AGE ON THE DEVELOPMENT OF MONOAMINE OXIDASE IN THE RAT HEART, LIVER AND BRAIN

Age (weeks)	No.	Mean MAO activity (µmoles 5HT metabolized/g in 1 hr ± S.D.)		
		Heart	Liver	Brain
1	5	1·0	23·0 ± 0·7	5·8 ± 0·2
2	3	3·9 ± 1·5	25·4 ± 4·5	8·8 ± 0·2
4	5	4·7 ± 1·0	30·2 ± 2·4	9·8 ± 1·3
6	5	8·0 ± 1·4	28·6 ± 1·8	9·3 ± 0·3
8	10	12·0 ± 3·6	30·2 ± 2·3	9·3 ± 0·5
10	5	14·3 ± 1·9	31·0 ± 2·1	9·2 ± 0·4
11	5	16·2 ± 2·3	33·0 ± 1·2	9·5 ± 0·2
12	4	12·8 ± 0·7	34·5 ± 0·8	9·4 ± 0·1
14	5	23·3 ± 3·1	32·3 ± 4·8	9·6 ± 0·3
16	10	23·4 ± 3·7	32·5 ± 1·5	9·6 ± 0·7
17	5	21·2 ± 2·3	31·0 ± 1·5	9·2 ± 0·7
18	5	30·8 ± 4·5	29·9 ± 1·6	9·4 ± 1·1
20	10	30·3 ± 4·3	33·7 ± 1·6	9·2 ± 0·3
22	10	29·9 ± 3·6	33·8 ± 2·8	9·1 ± 0·5
24	5	30·6 ± 4·9	31·1 ± 2·0	9·4 ± 0·3
26	5	29·5 ± 1·6	28·0 ± 1·5	9·2 ± 0·2
28	5	27·4 ± 4·6	28·9 ± 2·6	9·1 ± 0·5

exhibits a greater degree of variation from week to week and from animal to animal. As indicated in Table 1, normal levels of MAO activity in this organ range from 28 to 35 μmole/g tissue/hr.

The administration of pargyline (25 mg/kg, i.p.) results in total inhibition of MAO in all tissues studied. Recovery of MAO in the liver is quite rapid, showing almost complete recovery within 1 week irrespective of age. Brain MAO, however, requires at least 4 weeks to return to normal levels. In both organs there appeared to be no

TABLE 2. EFFECT OF PARGYLINE SULFATE (25 mg/kg, i.p.) AT AGES 1, 8 AND 16 WEEKS ON THE REGENERATION OF MONOAMINE OXIDASE IN THE RAT HEART, LIVER AND BRAIN

Age (weeks)	No.	Mean MAO activity (μmoles 5HT metabolized/g in 1 hr ± S.D.) Heart	Liver	Brain
1 (pargyline)	5	0	0	0
2	5	3·5 ± 0·7	23·1 ± 1·9	7·0 ± 0·3
4	5	6·4 ± 1·7	31·8 ± 5·8	9·9 ± 1·0
6	5	8·2 ± 0·7	29·8 ± 2·3	9·1 ± 0·4
8	5	9·8 ± 1·5	32·9 ± 1·6	9·5 ± 0·7
8 (pargyline)	5	0	0	0
9	5	5·5 ± 1·8	26·0 ± 2·1	4·8 ± 0·6
10	5	8·8 ± 2·5	28·8 ± 5·6	6·0 ± 1·6
12	5	17·5 ± 3·8	25·7 ± 2·0	6·5 ± 0·2
14	4	23·6 ± 8·1	30·8 ± 1·8	8·0 ± 0
16	5	29·0 ± 5·3	35·4 ± 1·2	9·2 ± 0·7
16 (pargyline)	5	0·1 ± 0·3	1·7 ± 1·4	0·4 ± 0·1
17	5	12·4 ± 1·5	25·2 ± 2·5	4·0 ± 0·6
18	5	18·7 ± 1·5	34·1 ± 1·3	6·6 ± 0·3
20	5	18·2 ± 6·5	32·2 ± 1·2	7·1 ± 0·5
22	5	26·2 ± 4·1	32·9 ± 1·8	7·9 ± 0·5
24	5	26·8 ± 2·2	31·0 ± 1·2	8·3 ± 0·5
26	5	29·9 ± 5·3	28·0 ± 1·8	8·5 ± 0·3
28	5	32·5 ± 4·9	33·8 ± 2·3	9·2 ± 0·4

difference in the rate of recovery if the pargyline had been given at the 8- or 16-week ages (Table 2). The only exception was seen in the recovery of brain MAO in rats given pargyline at the age of 1 week. These brains exhibited a much more rapid return of MAO activity, showing some 80 per cent recovery within a week after pargyline administration.

DISCUSSION

Most tissues of the rat which contain MAO reach their maximum level of enzyme activity within a few weeks after birth and presumably maintain this level during their lifetime. The heart represents an exception in that its MAO continues to increase with age and weight, suggesting that the rate of synthesis of the enzyme exceeds its rate of catabolism. Because of this fact, it was of interest to investigate the rate of recovery of cardiac MAO at different age levels after irreversible inhibition. This study was intended to determine: 1) whether recovery of cardiac MAO after irreversible inhibition would follow from the point of total inhibition a course parallel to that of the newborn

rat, but never reaching MAO levels of control animals of corresponding age; or 2) recovery would occur by an increase in the rate of MAO synthesis and result in an eventually complete recovery to the same level as that of control animals of corresponding age. The present investigations demonstrate that the latter speculation is true. Whether the heart MAO was inhibited at 1, 8 or 16 weeks of age, it eventually meets the progressively increasing control curve some time later. When pargyline was given at the 8-week or 16-week period, it required about 5 and 8 further weeks, respectively, for the enzyme to reach the levels of control animals of the same age. Thus, the older rats exhibited a much greater initial recovery of MAO than the younger animals, suggesting that the former had greater MAO synthesis taking place.

The very rapid recovery of MAO activity in the 1-week-old rats may be attributed to the fact that there was very little if any enzyme present to inhibit. Consequently, enzyme development proceeded as if no inhibition were present.

The recovery of brain and liver MAO requires little discussion. Previous investigations have amply demonstrated the return to normal of MAO after irreversible inhibition. The time required for total recovery of MAO in liver is between 1 and 2 weeks, while for brain it requires 4 to 6 weeks. Two differences exist between the recovery of MAO in heart and that in liver or brain. First, the heart is progressively increasing in MAO activity with increasing age, and consequently, when irreversible inhibition is exerted in such a system, recovery to normal means recovery to a point beyond its pre-inhibition level. Such is not the case with the liver or brain. Their enzymes are at a constant level, and recovery is merely returning to that point. Age does not influence this recovery rate as in the heart, except in the very young animal (1-week-old). Second, recovery from irreversible inhibition of cardiac MAO is affected by age. Younger rats have less to "catch-up" than older ones, since the latter animals have a much higher MAO content, which must be restored.

These studies are based on the findings of other workers that pargyline is an irreversible inhibitor of MAO.[10, 11] It is conceivable that the results described in this paper could be explained by a progressive decrease with age in the rate of pargyline metabolism by the rat heart. However, this is unlikely, since the use of other irreversible MAO inhibitors, unrelated in chemical structure to pargyline, resulted in durations of MAO inhibition similar to that described above (unpublished results). From all of these observations it appears that the recovery of cardiac MAO after irreversible inhibition is a highly age-dependent phenomenon, and consideration must be given to this fact in any investigations of cardiac MAO in the rat.

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