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NEURONAL AND HORMONAL INFLUENCES ON THE TURNOVER OF MONOAMINE OXIDASE IN SALIVARY GLAND*

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Abstract—The rate of formation of monoamine oxidase (MAO) in rat submaxillary gland was evaluated from the return of MAO activity after treatment with the irreversible inhibitor drug pargyline. We found that parasympathetic decentralization or sympathetic denervation of the gland had no significant effect on the half-life or the specific activity of the enzyme. The weight of the gland, however, was reduced after both surgical procedures; consequently, the rate of formation of MAO per gland was diminished in direct proportion to the loss of weight. Reserpine treatment, in contrast, had no effect on the weight of the gland, but it increased the $T_{1/2}$ of the enzyme from 3·4 to 5·2 days and decreased its specific activity resulting in a diminuation of the rate of formation of the enzyme. Thyroidectomy had no effect on the $T_{1/2}$ or the specific activity of the enzyme. Treatment with L-3,3′,5-triiodothyronine increased the activity of MAO in the salivary gland of normal and thyroidectomized rats, and it accelerated the return of enzyme activity after pargyline treatment in thyroidectomized animals.

WE HAVE studied some of the factors that influence the rate of formation of monoamine oxidase [MAO; monoamine:O_2 oxidoreductase (deaminating); EC 1.4.3.4] in the rat submaxillary gland. The return of enzyme activity after administering the irreversible inhibitor pargyline was used as a measure of MAO formation.[1–4] We chose the submaxillary gland for our studies because most of the MAO activity of the gland is associated with the mitochondria of the parencyhmal cells[5] and because the influence of adrenergic and cholinergic nerves on the formation of the enzyme can be studied easily, as either innervation can be surgically interrupted. We also studied the effect of reserpine treatment and thyroidectomy on MAO formation. Reserpine disrupts mitochondrical membranes[6] and induces the formation of abnormal protein in the salivary gland of the rat.[7] Thyroidectomy and treatment with L-3,3′,5-triiodothyronine (T3) change the activity of MAO and other mitochondrial enzymes, most likely by altering the synthesis of mitochondrial protein.[8–13]

METHODS

Estimation of the half-life of monoamine oxidase. The $T_{1/2}$ of MAO in the rat submaxillary gland was estimated from the return of enzyme activity after an intravenous injection of pargyline hydrochloride (10 mg/kg) as described previously.[1] Rats were killed by cervical dislocation at the time intervals shown in Results. The submaxillary

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glands were removed, frozen and then stored at −20° until assayed for MAO activity. Preliminary experiments had shown that salivary glands could be stored up to 3 weeks at −20° without loss of activity. Glands were homogenized for 15 sec with a Polytron high frequency homogenizer (Brinkman Instruments, Westbury, N.J.) in 1:25 (w/v) phosphate buffer 67 mM (pH 7·2). The crude homogenate was assayed for MAO activity using tyramine (2·1 mM) as substrate as described previously.[1] Protein was determined by the method of Lowry *et al.*[14]

We calculated the $T_{1/2}$ of MAO from the equation:

$$\log [(MAO)_0 - (MAO)] = -\frac{kt}{2{\cdot}303} + c \qquad (1)$$

where $(MAO)_0$ represents the steady-state activity of the enzyme, (MAO) the activity of MAO at time t after injecting pargyline, k the fractional rate constant for the normal loss of enzyme and c the constant of integration. The derivation and application of this equation have been presented in detail previously.[1]

A plot of log $[(MAO)_0 - (MAO)]$ vs t gives a linear relationship with the slope $-k/2{\cdot}303$. For convenience we plotted percentage inhibition of MAO activity vs time on semi-logarithmic graph paper. The method of least squares was used to evaluate the best fit curves shown in the figures and to calculate k. The $T_{1/2}$ of MAO was calculated from the relationship $T_{1/2} = 0{\cdot}693/k$.

Surgical procedures. Normal male Sprague–Dawley rats (180–200 g), rats with unilateral superior cervical ganglionectomy and rats with unilaterally severed chorda tympani, were purchased from Zivic–Miller Labs, Allison Park, Pa. Surgery was performed on the left side, and the right salivary gland served as a control tissue. All animals were maintained in our laboratory for 14–17 days before experimentation. Thyroidectomy was performed on 3-week-old rats by Zivic–Miller Labs. The animals were used for experimentation 4 weeks after surgery. At 7 weeks the thyroidectomized rats weighed 100–120 g. Normal rats of the same age, which served for control tissues, weighed 280–300 g.

Treatment with reserpine or 3,3′,5-triiodothyronine. Reserpine (Aldrich Chemical Company, Milwaukee, Wis.) was dissolved in 10% acetic acid and injected intraperitoneally. We administered 2 mg/kg initially and 1 mg/kg every second day. Pargyline was administered 24 hr after the first dose of reserpine. Saline was administered to control animals.

T3 (3,3′,5-triiodothyronine, monosodium salt, CalBiochem., La Jolla, Calif.) was suspended in 0·9% sodium chloride solution and the solution was adjusted to pH 8·5 with NaOH. Four weeks after surgery, thyroidectomized rats or normal rats of the same age were injected subcutaneously with T3. The initial dose was 0·2 mg/kg, on the second day the dose was 0·15 mg/kg, and on subsequent days the dose was 0·1 mg/kg. Control animals received saline. Pargyline was administered 48 hr after the first dose of T3.

RESULTS

Effect of chorda tympani section or superior cervical ganglionectomy. Severing the chorda tympani or superior cervical ganglionectomy did not change the slope of the graphs in Fig. 1 where percentage inhibition of MAO activity was plotted vs days after pargyline treatment. Thus, the calculated fractional rate constants and half-lives

for the enzyme were unchanged (Table 1). In contrast, the weights of the glands were reduced by both surgical procedures, most drastically after severing the chorda tympani. The deamination of tyramine per gland was also significantly reduced by the surgical procedure. There was a slight decrease of the specific activity of MAO after sympathetic denervation. Therefore, both procedures changed the total MAO activity in the gland, but not the $T_{1/2}$ of the enzyme that remained after surgery.

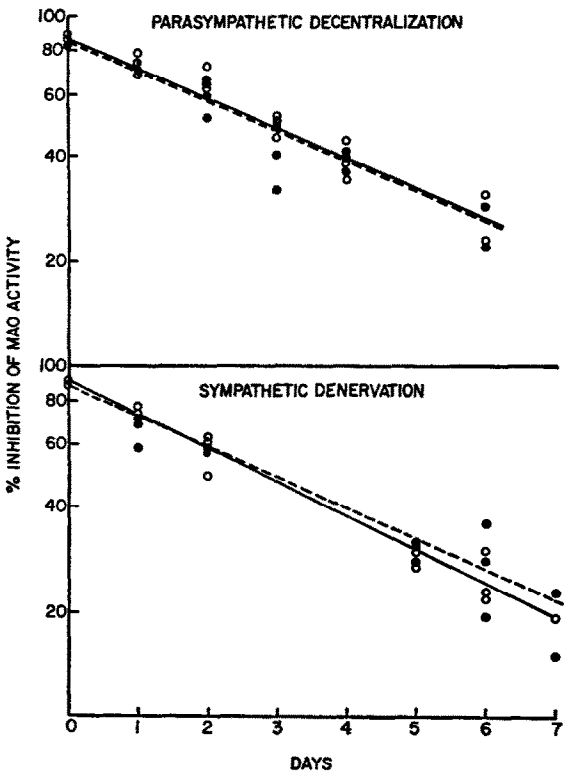

FIG. 1. Return of MAO activity after pargyline treatment (10 mg/kg, i.v.) in the submaxillary gland of rats with: unilaterally severed chorda tympani (parasympathetic decentralization) and unilateral superior cervical ganglionectomy (sympathetic denervation). The closed circles show the values for the surgically altered gland, the open circles the contralateral gland. The curves shown were obtained by the method of least squares analysis. There was no significant difference between the slopes for the control gland and the surgically altered gland.

Effect of treatment with reserpine. After treatment with reserpine, MAO activity of the gland was reduced, and the activity could be maintained at a lower than normal value if reserpine was administered every 48 hr (Fig. 2). There was no apparent change of gland weight, but the specific activity of MAO was reduced by about 30 per cent (Table 2). When the administration of reserpine was terminated, enzyme activity approached normal values in about 3 days (Fig. 2). The return of MAO activity in reserpine-treated animals, after pargyline treatment, is shown in Figs. 3 and 4. Reserpine treatment delayed the return of MAO to normal values (Fig. 3). When the data were plotted according to equation (1) (Fig. 4), it was apparent that

TABLE 1. EFFECT OF SYMPATHETIC DENERVATION AND PARASYMPATHETIC DECENTRALIZATION ON SUBMAXILLARY GLAND MONOAMINE OXIDASE*

	Sympathetic denervation			Parasympathetic decentralization		
Surgical procedure	Ganglionectomy	Control gland	Change (%)	Severed chorda tympani	Control gland	Change (%)
Gland wt (mg)	222 ± 11† (14)	261 ± 8 (15)	−15	163 ± 22† (13)	237 ± 7 (12)	−31
Tyramine metabolized (nmoles/gland/hr)	1240 ± 58† (14)	1736 ± 42 (15)	−29	979 ± 30† (13)	1571 ± 54 (12)	−38
Tyramine metabolized (nmoles/mg protein/hr)	41 ± 2† (14)	48 ± 1 (15)	−16	48 ± 2 (13)	52 ± 2 (12)	− 9
Rate constant, k ($days^{-1}$)	0·18 ± 0·03	0·23 ± 0·01		0·21 ± 0·02	0·21 ± 0·01	
Half-life (days)	3·8	3·1		3·3	3·3	

* All values are expressed as mean ± S.E. with the exception of the half-life which was calculated from the relationship $T_{1/2} = 0{\cdot}693/k$. The number of animals used is given in parentheses. The rats were injected with pargyline (10 mg/kg) intravenously 2 weeks after surgery. The animals were killed at the times shown in Fig. 1. MAO activity was assayed as described in Methods. Rate constants were calculated by the method of least squares. The difference between the control gland and the surgically altered gland was compared using a paired t-test.

† $P < 0{\cdot}01$.

the rate constant and consequently the $T_{1/2}$ (Table 2) were significantly reduced by reserpine treatment. Reserpine treatment, therefore, reduced the specific activity of the enzyme and increased the $T_{1/2}$ of the enzyme. It had no apparent effect on the weight of the salivary gland.

Effect of thyroidectomy and 3,3',5-triidothyronine administration. Thyroidectomized rats weighed between 55 and 67 per cent less than control animals and, therefore, their salivary glands weighed less (Table 3). The metabolism of tyramine was about the same when compared per mg of protein but was significantly less in the thyroidectomized rats when compared per mg of tissue (Table 3). The rate constants and half-lives were similar in thyroidectomized and control animals (Fig. 5 and Table 3).

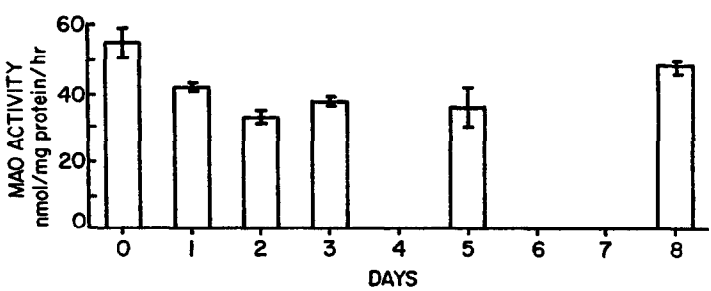

FIG. 2. Effect of reserpine on the activity of rat submaxillary gland MAO. Data are presented as the mean together with the range for three animals. Reserpine was administered i.p. as follows: day 0, 2 mg/kg; day 2, 1 mg/kg; day 4, 1 mg/kg.

TABLE 2. EFFECT OF RESERPINE ON SUBMAXILLARY GLAND MONOAMINE OXIDASE*

Treatment	Reserpine treated	Control rats
Gland wt (mg)	254 ± 12 (5)	261 ± 22 (5)
Tyramine metabolized (nmoles/mg protein/hr)	38 ± 2 (5)†	55 ± 2 (5)
Rate constant, k ($days^{-1}$)	0·13 ± 0·02† (13)	0·21 ± 0·02 (13)
Half-life	5·2	3·4

* Data are expressed as mean ± S. E. The number of animals used is given in parentheses. Rate constants were calculated by the method of least squares analysis. Half-life was calculated from the relationship $k = 0{\cdot}693/k$. Reserpine was injected intraperitoneally starting on day 0 with 2 mg/kg and continued on days 2 and 4 with 1 mg/kg. Solvent was administered to control animals. Pargyline (10 mg/kg, i.v.) was injected on day 2. The animals were killed at the times shown in Fig. 3. MAO activity was assayed as described in Methods.

† $P < 0{\cdot}01$ when compared with control glands.

Katyare *et al.*[12] reported that administration of T3 (see Methods for dosage schedule) to thyroidectomized rats had no obvious toxic effect. We made similar observations; however, there may have been biochemical changes that we were not aware of. In contrast, normal rats given the same doses of T3 lost weight, developed diarrhea and showed alopecia. MAO activity of the salivary glands from thyroidectomized animals was unchanged during the first 4 days of treatment with T3 and increased when measured on day 6 (Fig. 6). In contrast, MAO activity was elevated in normal animals when measured 3 days after initiating treatment with T3.

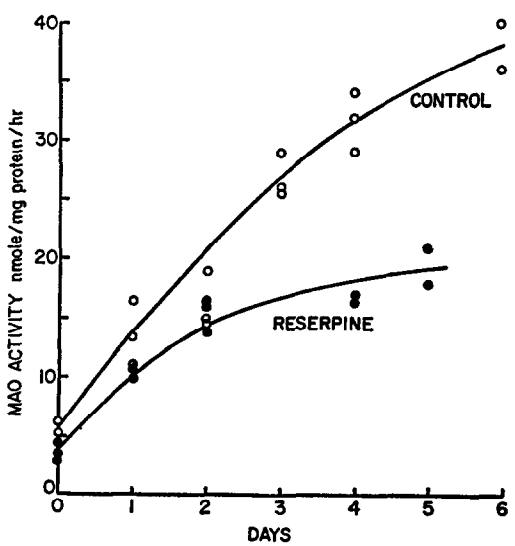

FIG. 3. Return of MAO activity in rat submaxillary gland after pargyline treatment alone or in combination with reserpine. Reserpine was injected intraperitoneally as described in Methods. Pargyline was injected (10 mg/kg, i.v.) 24 hr after the first reserpine injection. The days refer to the time after pargyline injection.

TABLE 3. EFFECT OF THYROIDECTOMY ON RAT SUBMAXILLARY GLAND MONOAMINE OXIDASE*

Treatment	Thyroidectomized rats	Control rats
Gland wt (mg)	124 ± 16 (8)†	235 ± 7 (8)
Tyramine metabolized (nmoles/mg tissue/hr)	5·1 ± 0·2 (8)†	6·3 ± 0·1 (8)
Tyramine metabolized (nmoles/mg protein/hr)	40 ± 2 (8)	43 ± 1 (8)
Rate constant, k ($days^{-1}$)	0·20 ± 0·02 (16)	0·20 ± 0·02 (8)
Half-life (days)	3·5	3·5

* Data are expressed as mean ± S. E. The number of animals used is given in parentheses. Rate constants were calculated by the method of least squares analysis, and half-lives were calculated from the relationship $T_{1/2} = 0{\cdot}693/k$. Thyroidectomy was performed on 3-week-old rats and the experiment was initiated 4 weeks later. Normal rats of the same age were used for control tissue. Pargyline (10 mg/kg, i.v.) or saline was injected and the animals were killed at the time intervals shown in Fig. 5. The submaxillary glands were removed and assayed for MAO activity as described in Methods.

† $P < 0{\cdot}01$ when compared with control gland.

Pargyline was administered to thyroidectomized rats 48 hr after initiating T3 treatment, a time when synthesis of mitochondrial protein is maximally stimulated by T3 treatment.[12] The $T_{1/2}$ of the enzyme could not be estimated by following the return of activity after pargyline as described in Methods because during this period a steady-state enzyme activity was never attained (Fig. 6). The net rate of return of MAO activity in the salivary glands of thyroidectomized rats and T3-treated thyroid-

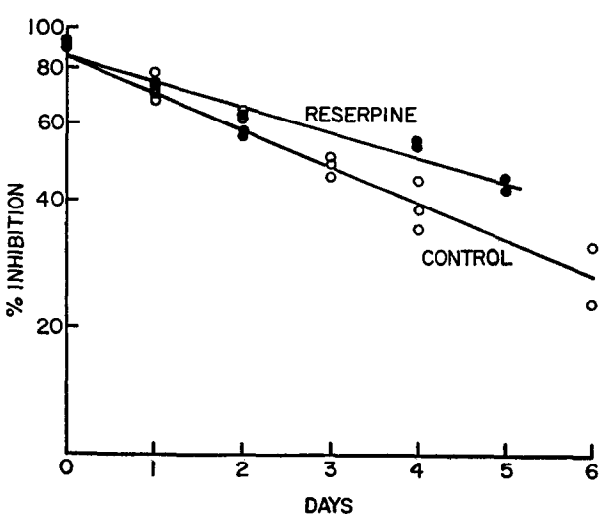

FIG. 4. Return of monoamine oxidase activity in rat submaxillary gland after pargyline treatment alone or in combination with reserpine. The experimental procedures are the same as described in Fig. 3. The curves were calculated by the method of least squares analysis.

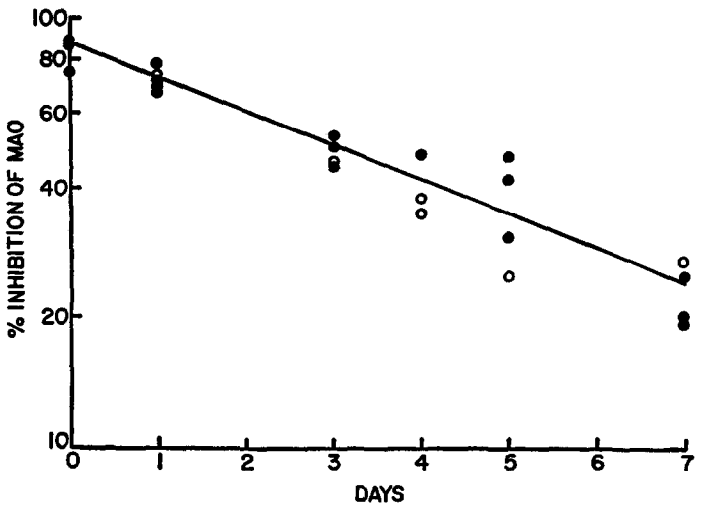

FIG. 5. Return of MAO activity after pargyline treatment in submaxillary gland of normal and thyroidectomized rats. Thyroidectomy was performed on 3-week-old rats and the experiment started 4 weeks later. Control rats were of the same age. The closed circles show the values for thyroidectomized rats, the open circles the control values. The curves coincided when analyzed by the method of least squares.

ectomized rats after pargyline treatment is shown in Fig. 7. Data for normal control animals, from the same study, followed a curve that could be superimposed on the curve for untreated thyroidectomized rats. T3 treatment accelerated the return of MAO activity after administering pargyline when compared to thyroidectomized rats (Fig. 7). For example, without T3 replacement therapy, activity returned to about 50 per cent of the steady-state activity in 3 days (Fig. 7 and Table 3). After treatment with T3, activity returned to about 75 per cent of the steady-state activity in the same time period.

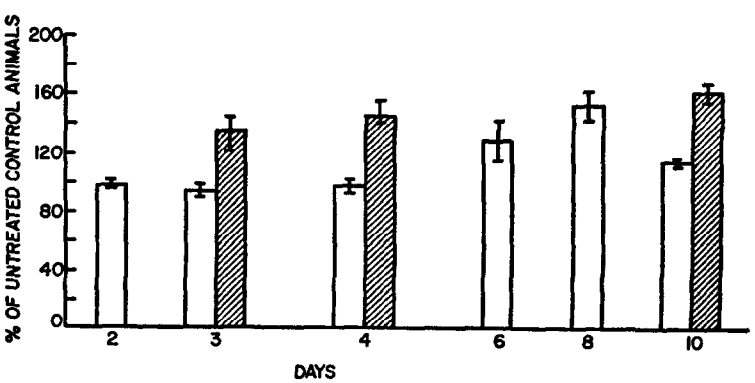

FIG. 6. Effect of T3 on the MAO activity of rat submaxillary gland of normal and thyroidectomized animals. See Methods for experimental details. Data are presented as mean per cent of the activity of thyroidectomized and normal animals. Bars show the range for three or four animals.

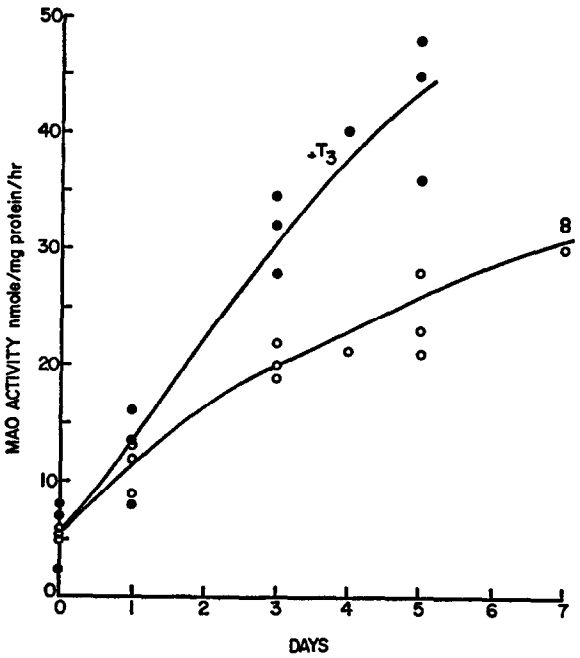

FIG. 7. Return of MAO activity after pargyline treatment in submaxillary glands of thyroidectomized rats treated with T3 or solvent. The closed circles represent T3-treated thyroidectomized animals and the open circles represent solvent-treated thyroidectomized animals. The days refer to the time after the pargyline administration.

DISCUSSION

The submaxillary gland of the rat provides a suitable model for studying the influence of neuronal activity on MAO. Most MAO in rat submaxillary gland occurs in parenchymal cells.[5] The preganglionic parasympathetic nerves to the gland are found in the chorda tympani while the sympathetic afferent nerves originate in the superior cervical ganglion. Sectioning these nerves leads, therefore, to either parasympathetic decentralization or sympathetic denervation of the gland. Following the return of MAO activity after inhibition by pargyline is a reliable method for estimating the $T_{1/2}$ of the enzyme.[1-4] The $T_{1/2}$ of MAO in salivary gland appears to be independent of afferent nerve activity. No significant changes followed ganglionectomy or sectioning of the chorda tympani despite the atrophy resulting from the surgical procedures. MAO activity was only reduced in correspondence to the reduction in gland weight (Table 1). A small but significant decrease of the specific activity occurred after superior cervical ganglionectomy (Table 1). The decrease of activity may have been caused by the lack of adrenergic nerve impulses or the degeneration of the adrenergic nerve endings which might contain MAO of a higher specific activity than in the whole gland. Our results suggest, therefore, that the MAO that remains after both surgical procedures is formed and destroyed by enzyme systems that are essentially the same as the enzyme systems of a normal gland. The consequence of the surgical procedures was atrophy of the gland and, therefore, the rate of formation of MAO per gland was diminished in direct proportion to the loss of weight.

Reserpine treatment is often regarded as a procedure to alter the effects of adrenergic neuron activity. We found that during repeated reserpine administration that the specific activity, the $T_{1/2}$ and the synthesis rate of salivary gland MAO were considerably reduced. The reduction of the synthesis rate is most obvious when the net rates of return of MAO activity are compared after pargyline inhibition (Fig. 3). Our studies imply that reserpine treatment disrupts the synthesis as well as the degradation of MAO in the gland. A reduction of MAO activity in the mesenteric artery after reserpine treatment has been reported by Tarver *et al.*[15] Apparently, the reduction of MAO synthesis and degradation in the gland are caused primarily by a direct toxic effect of reserpine and is not a consequence of reduced sympathetic output. This conclusion is consistent with the observation that reserpine has a deleterious effect on mitochondrial membranes[6] and the observation that abnormal protein accumulates in the gland after reserpine treatment.[7]

Most of MAO of the salivary gland is associated with mitochondria;[5] however, there may be some activity associated with the microsomes.[3] The synthesis of salivary gland MAO can, therefore, be regarded as an example of the turnover of a specific mitochondrial protein. The thyroid hormones, thyroxine and T3, have a pronounced effect on the metabolism of mitochondria, the activities of mitochondrial enzymes and the synthesis of mitochondrial protein.[11,16] For example, the incorporation of ^{14}C-leucine into mitochondrial fractions of rat liver is increased after T3 treatment and lowered in thyroidectomized rats.[12] Increased activities of several mitochondrial enzyme systems during the administration of thyroid hormones have been reported.[9,16] With the dosage schedule used in our studies, Katyare *et al.*[12] reported that T3 was not toxic to the animals and that protein synthesis was stimulated during the experimental period.

Thyroidectomy had no significant effect on the specific activity or $T_{1/2}$ of salivary gland MAO. There was, however, a fall in the weight of the gland and a decrease of enzyme activity per milligram of tissue, an observation implying that the synthesis of total protein was abnormal after thyroidectomy. Administering T3 to thyroidectomized rats resulted in an increase of MAO activity (Fig. 6) and the rate of recovery of salivary gland MAO after pargyline was accelerated (Fig. 7). T3 administration also increased enzyme activity in normal animals. Our results suggest that the thyroid plays an important role in regulating the rate of formation of salivary gland MAO but not in regulating its degradation.

Reports have appeared that are compatible with the existence of multiple forms of MAO.[17] We made no attempt to determine whether the synthesis rate of a specific form(s) of enzyme was induced by T3 treatment.

In conclusion, the steady-state activity of MAO in the rat submaxillary gland is apparently regulated by three major factors: (1) the viability of the parenchymal cells; (2) the rate of formation of the enzyme; and (3) the rate of destruction of the enzyme. Afferent nerves influence the viability of the parenchymal cells, but the enzymatic mechanisms that remain after nerve sectioning appear normal. Reserpine, in contrast, apparently disrupts the synthetic and degradative pathways for MAO. Thyroid hormones have little influence on the degradation of MAO, but they apparently have a profound influence on the synthesis of mitochondrial protein, including MAO.

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