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MODULATION *IN VITRO* OF MONOAMINE OXIDASE ACTIVITY BY THYROID HORMONES

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Abstract—Experiments *in vitro* using whole rat brain homogenate revealed that L-thyroxine (T_4) and L-triiodothyronine (T_3) can increase indoleacetic acid (IAA) formation from the oxidative deamination of tryptamine. This effect was dependent upon (a) the homogenate concentration, (b) the hormonal concentration, (c) temperature and (d) pre-incubation of the hormones with the homogenate prior to the addition of tryptamine. For T_4, the extent of the increase in IAA production was correlated with the specific activity of the particular homogenate under test but no such correlation existed with T_3. The influence of the thyroid hormones was shown to occur at the monoamine oxidase (MAO) step and appears to be mediated indirectly so as to increase MAO activity. The nature of the interaction is reversible but the precise mechanism(s) involved have not been elucidated. It is postulated that the thyroid hormones and/or their metabolites either suppress an endogenous inhibitor or function to dissociate aggregated MAO. A similar augmentation of IAA production was found in the liver, less in the kidney, and only slight increases were obtained in the heart. Thus, a degree of organ selectivity exists. The present finding describe a new effect of the thyroid hormones which may be of importance in the modulation of monoamine metabolism.

Thyroid hormones are known to modify monoamine oxidase [MAO; monoamine: oxygen oxidoreductase (deaminating); EC 1.4.3.4] activity *in vivo* [1–14] but the mechanism or mechanisms by which changes are mediated have not been resolved fully. An increased synthesis of FAD [15], the co-factor for mitochondrial MAO [16], has been reported, as well as possible modulating influences on the biogenesis of mitochondria [14]. Additionally, thyroid hormones can increase the synthesis of MAO itself, in the heart [12] and salivary glands [11] of rats. Conversely, rat liver MAO activity is decreased after thyroid administration [10, 13], even in low, non-toxic doses [13]. Thus, the mechanism of thyroid-induced influences can vary. Evidence suggesting additional mechanisms to interference with enzyme or co-factor synthesis has been presented previously. After L-thyroxine (T_4) administration, rat kidney MAO activity increased rapidly, an effect which appeared to be related to an activation of the enzyme [13].

In the present study we have investigated the effect of the thyroid hormones on the activity *in vitro* of MAO from rat brain, liver, kidney and heart. Although it is generally accepted that T_4 and L-triiodothyronine (T_3) fail to alter MAO activity *in vitro* [3, 10, 12], the present findings show that tryptamine deamination is increased markedly.

EXPERIMENTAL

Male albino rats (Sprague–Dawley descendants; Texas Inbred, Houston, TX 77047) were used. The rats weighed 200–300 g and were housed at 22–24° in pairs and were given food and water *ad lib.*

All experiments were made on whole tissue homogenates utilizing 0.25 M sucrose and a Tissuemizer homogenizer (Tekmar Co., Cincinnati, OH 45222). Thus, an attempt was made to preserve all cellular components since the activity and properties of MAO can vary markedly according to the local environment [17]. The homogenization procedure was conducted over ice and standardized at a fixed speed for a total time of 3 min (5 sec on and 5 sec off). Homogenization in water or in a 0.2% aqueous solution of Triton X-100 failed to increase product formation in either control or thyroid-treated homogenates, suggesting that mitochondrial disruption was adequately achieved in sucrose and that maximal enzymatic activity was being measured under the prevailing environmental conditions.

MAO activity was assayed by determining the indoleacetic acid (IAA) formed from tryptamine in the presence of excess aldehyde dehydrogenase, as first described by Lovenberg *et al.* [18]. The IAA was measured spectrofluorometrically (ex. 280 nm; em. 364 nm; uncorrected) and the values were corrected for extraction losses by running a set of standards through the entire procedure. The recovery of IAA from tissue homogenates was increased over that reported by Klingman and Klingman [19] by adding 4.5 g NaCl to aliquots of the acidifed incubating mixture and ranged between 60 and 75 per cent. Product identification was made by spectral analysis versus authentic IAA and by descending paper chromatography. The solvent was the toluene layer of a two-phase system containing toluene, acetic acid and water (4:1:5), the aqueous layer serving as the stationary phase. Complete inhibition of IAA production was obtained using 60 μg/ml of pargyline hydrochloride.

Unless stated othersiwe, the incubation mixture contained 20 mg/ml of tissue homogenate (1 ml),

14 μmoles NAD (0.7 ml), 60 μmoles nicotinamide (0.5 ml) and aldehyde dehydrogenase preparation (0.4 ml). T_3, T_4 or vehicle was added to ice-cold incubation mixtures and the reaction was initiated after a 15-min pre-incubation at 37° by adding 14 μmoles tryptamine hydrochloride (0.4 ml). The final volume of the incubating mixture was made equal to 5.0 ml. The reaction was run for 20 min, under air, in a Dubnoff metabolic shaker. T_3, T_4 or the appropriate vehicle did not interfere with the extraction or fluorescence of IAA.

The source of aldehyde dehydrogenase was the 78,000 *g* supernatant of 1:4 sucrose (0.25 M) homogenate of guinea pig kidney obtained from animals killed 18–24 hr after receiving tranylcypromine sulfate, 20 mg/kg, i.p., a long-lasting MAO inhibitor. The aldehyde dehydrogenase preparation exhibited no inherent MAO activity or residual MAO inhibitor activity.

The drugs used were: sodium L-thyroxine (Synthroid, Flint Laboratories) made up in NaCl injection, U.S.P.; sodium L-triiodothyronine (Cytomel, Smith Kline & French Laboratories) dissolved in 0.01 N NaOH and made up to volume with NaCl injection U.S.P.; indoleacetaldehyde sodium bisulfite (Regis Chemical Co.) made up in water.

RESULTS

Figure 1 shows the relationship between tissue concentration (brain) and IAA formation both in the absence and presence of the thyroid hormones. It can be deduced from the figure that the lack of linearity in the control curve was not due to substrate insufficiency since the thyroid hormones enhanced product formation. This point was further proven in a separate experiment by decreasing the substrate concentration by 10-fold to 1.4 μmoles tryptamine. The same

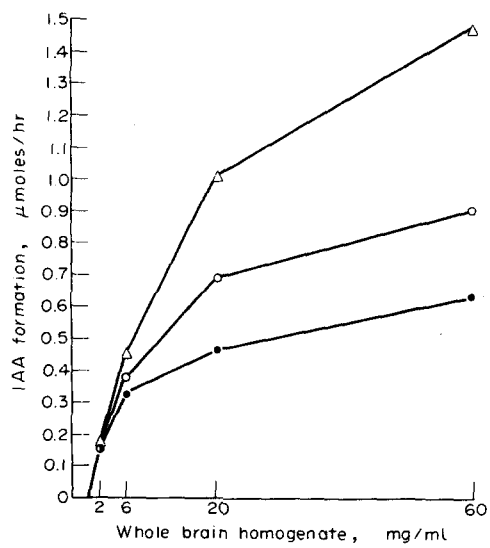

Fig. 1. Effect of L-triiodothyronine (O——O) or L-thyroxine (Δ——Δ) on indoleacetic acid (IAA) formation from tryptamine using increasing concentrations of whole rat brain homogenate. Control, no hormone = ●——●. Each value is the mean of at least two experiments. A hormone (10 μg/ml) or vehicle was added 15 min prior to the addition of tryptamine. Reaction time = 20 min.

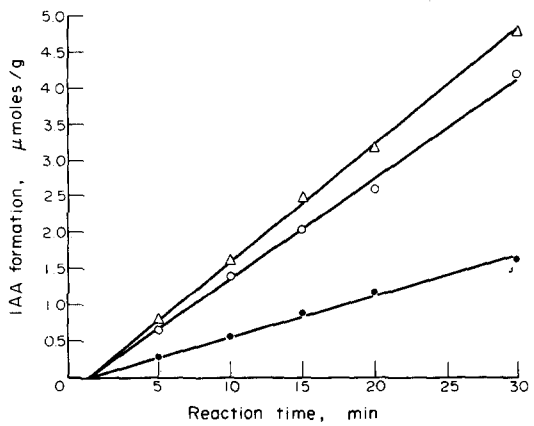

Fig. 2. Effect of L-triiodothyronine (O——O) or L-thyroxine (Δ——Δ) on indoleacetic acid (IAA) formation from tryptamine using increasing reaction times. Control, no hormone = ●——●. Each value is the mean of at least two experiments. A hormone (10 μg/ml) or vehicle was added 15 min prior to the addition of tryptamine. Whole rat brain homogenate, 20 mg/ml.

specific activity of MAO was obtained with 20 mg/ml of brain homogenate as with 14 μmoles tryptamine. Furthermore, the increase in IAA production with T_3 (10 μg/ml) was identical (100 per cent) at the two different substrate concentrations. Thus, at least a 10-fold substrate excess, rather than insufficiency, was present in the assay mixture.

Figure 2 shows no evidence for the production *in vitro* of a rate-limiting substance(s) with incubation time, since linearity was obtained for the three conditions tested. In a similar but separate experiment, the tryptamine concentration was decreased to 0.7 μmole. Evidence of substrate insufficiency occurred between 20 and 30 min of incubation (20 mg/ml of brain homogenate). However, T_3 (10 μg/ml) still enhanced IAA formation compared with the entire control curve, indicating the effect of T_3 is not dependent upon the presence of excess substrate.

The assay system requires that secondary oxidation via aldehyde dehydrogenase is not rate-limiting. Table 1 shows this to be the case and demonstrates that the action of T_3 and T_4 upon IAA formation cannot be ascribed to an activation of aldehyde dehydrogenase.

Table 1. Effect of altered aldehyde dehydrogenase (AD) concentrations upon thyroid-stimulated production of indoleacetic acid (IAA) from tryptamine in whole rat brain homogenate (20 mg/ml)

	IAA† (μmoles/g/hr)		
Condition*	Control (no hormone)	T_3 (10 μg/ml)	T_4 (10 μg/ml)
1 × AD	5.6	10.2	10.6
No AD	0.9	1.8	2.0
2 × AD	5.8	10.5	11.0

* The notation 1 × AD = 0.4 ml, aldehyde dehydrogenase preparation (see Experimental).

† Mean values for at least two experiments.

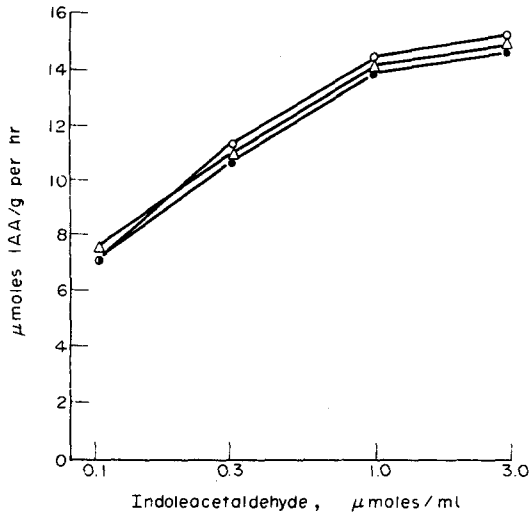

Fig. 3. Effect of L-triiodothyronine (O——O) or L-thyroxine (Δ——Δ) on the formation of indoleacetic acid (IAA) from indoleacetaldehyde. Control, no hormone = ●——●. Each value is the mean of three experiments. A hormone (10 µg/ml) or vehicle was added 15 min prior to the addition of indoleacetaldehyde. Whole rat brain homogenate, 20 mg/ml; reaction time, 20 min.

In order to define further the locus of thyroid activity, the effect of T_3 and T_4 was studied on whole brain homogenates using indoleacetaldehyde instead of tryptamine. Figure 3 shows no effect of either hormone on IAA formation. Thus, it may be concluded that the thyroid hormones influence the primary stage of tryptamine oxidation through modulation of MAO activity.

When either T_3 or T_4 was added to brain homogenate at the same time as tryptamine, no increase in indoleacetic acid formation resulted. Figure 4 shows clearly that pre-incubation of the hormones with the homogenate, prior to substrate addition, is an absolute requirement for increased deamination.

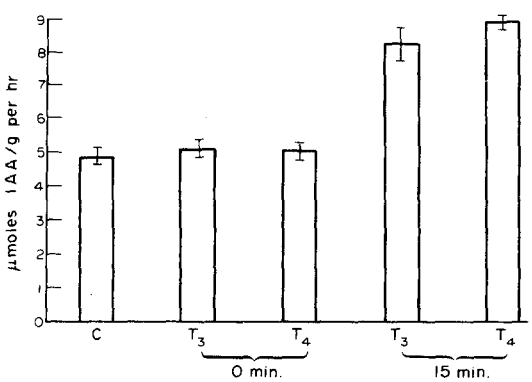

Fig. 4. Effect of pre-incubation on thyroid-induced stimulation of indoleacetic acid (IAA) formation from tryptamine. Shown are mean values ± S. E. M. for at least three experiments. The hormone (10 µg/ml) or vehicle was added either with tryptamine (0 min) or 15 min prior to the addition of tryptamine. Pre-incubation for 15 min with the vehicle did not change control values. Key: C = control, no hormone; T_3 = L-triiodothyronine; T_4 = L-thyroxine. Whole rat brain homogenate, 20 mg/ml; reaction time, 20 min.

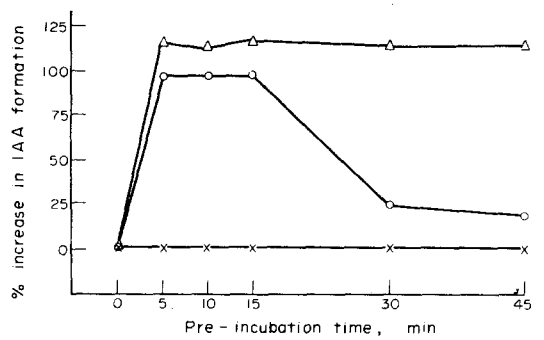

Fig. 5. Effect of varying pre-incubation times of L-triiodothyronine (O——O) or L-thyroxine (Δ——Δ) at 37° and at 4° (×——×) (both hormones combined) upon indoleacetic acid (IAA) formation from tryptamine. Each value is the mean of two determinations. A hormone (10 µg/ml) or vehicle was added at the various times shown prior to the addition of tryptamine. In the experiment made at 4°, pre-incubation was made in an ice-water mixture, and tryptamine was added immediately prior to incubation at 37°. Whole rat brain homogenate, 20 mg/ml; reaction time, 20 min.

Figure 5 shows the effect of varying the pre-incubation time. It can be seen that both T_3 and T_4 (10 µg/ml) produced maximal stimulation of IAA production after only 5 min. The effect of T_4 remained constant over the various times tested but T_3 exhibited a marked decline in effectiveness at 30 and 45 min. This decline was not due to heat instability since T_3 incubated for 45 min in the absence of tissue was found to retain full activity when subsequently added to a homogenate 5 min prior to tryptamine. The figure also shows the marked temperature dependence for the effect of both T_3 and T_4 upon IAA formation. Pre-incubation at 4° completely inhibited the action of the hormones. Figure 6 shows that the

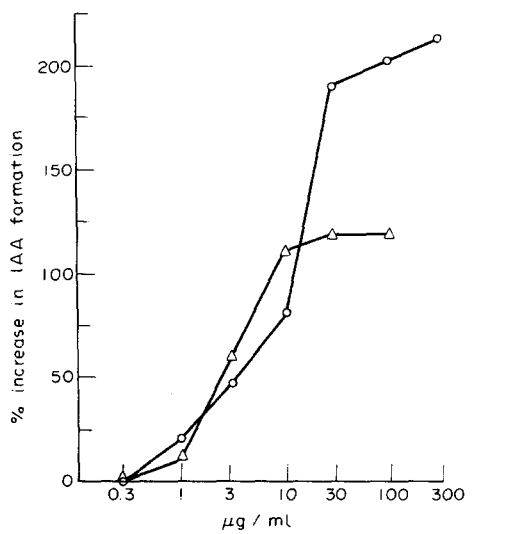

Fig. 6. Effect of various concentrations of L-triiodothyronine (O——O) or L-thyroxine (Δ——Δ) upon indoleacetic acid (IAA) formation from tryptamine. Each value is the mean of three determinations. The control specific activities (µmoles/g/hr) were: 2.4 for L-triiodothyronine and 4.9 for L-thyroxine. Whole rat brain homogenate, 20 mg/ml; reaction time, 20 min.

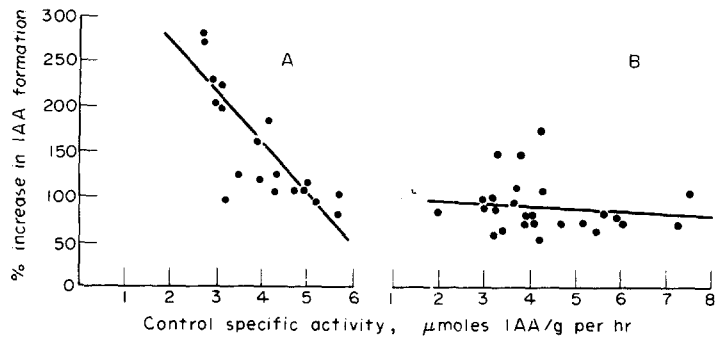

Fig. 7. Correlation between the control specific activity of monoamine oxidase in whole rat brain homogenates (20 mg/ml) and the percentage increase in indoleacetic acid (IAA) formation from tryptamine with L-thyroxine (panel A, N = 20) or L-triiodothyronine (panel B, N = 31). Each value is the mean of at least two determinations. A hormone (10 μg/ml) or vehicle was added 15 min prior to the addition of tryptamine. Correlation coefficients are as follows: panel A, 0.88, and panel B, −0.10.

effect of both T_3 and T_4 is concentration related. However, the differences between the two curves can vary according to the specific activity of the particular homogenate under test (Fig. 7). A good correlation exists between the specific activity of differing homogenates and the percentage increase in IAA formation with T_4 (10 μg/ml). No such correlation was found with T_3 (10 μg/ml). Within any particular homogenate, variation in the effect of the two hormones was small as was day-to-day variation when assaying from the same homogenate.

Table 2 shows a comparison of the effect of the thyroid hormones on tryptamine deamination from various rat tissues. IAA production was increased in all organs but the effect was most marked in the brain and liver compared with the kidney. In the heart, only a very marginal increase was found. The difference between the brain and kidney is especially interesting since the mean specific activity of MAO in control homogenates was virtually identical. Overall, however, no correlation exists between control specific activities of MAO and the effect of the thyroid hormones.

DISCUSSION

The present study has revealed a new effect of the thyroid hormones in that both T_3 and T_4 can increase IAA formation from tryptamine using whole rat brain homogenate *in vitro*. MAO activity appears to be modulated since the hormones failed to enhance IAA production from the intermediate aldehyde (Fig. 3).

After the deamination of tryptamine, formed indoleacetaldehyde may be reduced by aldehyde reductases [20–22] to form tryptophol. This pathway will predominate if sufficient aldehyde dehydrogenase is lacking and/or if reductase activity is stimulated [23, 24]. Additionally, the high reactivity of biogenic aldehydes leads to significant non-specific protein binding [22, 25]. Thus, the possibility existed that the thyroid hormones might either (1) divert metabolism away from tryptophol formation, or (2) compete with non-specific binding sites to allow more IAA production. The experiments utilizing excess aldehyde dehydrogenase (Table 1) and indoleacetaldehyde (Fig. 3) eliminated these possibilities. Aldehyde dehydrogenase activity was shown to be neither rate-limiting nor activated by the thyroid hormones. Likewise, IAA formation from indoleacetaldehyde was unaffected by T_3 and T_4 in a concentration (10 μg/ml) which produced marked increases in IAA production when using tryptamine as the substrate. The exclusion of tryptophol formation as a factor in the present experiments is consistent with the observations of Tabakoff and Erwin [20] that indoleacetaldehyde, unlike certain other biogenic aldehydes, is only a poor substrate for rat brain aldehyde reductases.

Tryptamine may be *N*-methylated by brain tissue and can be metabolized to tryptolines (1,2,3,4-tetrahydro-beta-carbolines) [26–29]. It was possible, therefore, that the thyroid hormones might inhibit these metabolic routes, thereby shunting more tryptamine through the MAO pathway. However, the extent of the thyroid-induced changes was not critically depen-

Table 2. Effect of L-triiodothyronine (T_3) and L-thyroxine (T_4) (both 10 μg/ml) on indoleacetic acid (IAA) formation from tryptamine in various organs of the rat*

Organ†	Control specific activity (μmoles IAA/g/hr)	Per cent increase in IAA formation	
		T_3	T_4
Brain	4.37 ± 0.22 (31)	87.6 ± 5.5 (31)	163.5 ± 14.1 (20)
Liver	15.40 ± 2.00 (3)	79.9 ± 15.9 (3)	101.3 ± 6.2 (3)
Kidney	4.5 ± 0.70 (4)	19.8 ± 7.1 (4)	20.2 ± 4.0 (4)
Heart	7.10 ± 0.90 (4)	9.3 ± 4.9 (3)	10.4 ± 1.5 (3)

* Mean values ± S. E. M. for (N) experiments.
† Tissue concentrations were: 10 mg/ml for liver and 20 mg/ml for the other organs.

dent upon substrate concentration. When the concentration of tryptamine was decreased by 10-fold, product formation and the effect of T_3 were unaltered. Two further conclusions may be drawn from this latter result. First, it suggests that altered access of the substrate to MAO is not a critical factor, and this contention is supported by the fact that homogenization in progressively harsher media failed to alter IAA formation (see Experimental). Second, it excludes the possibility that endogenous amine substrates functioned as competitive inhibitors of tryptamine metabolism. Additionally, the use of water and Triton X-100 as media for homogenization would be expected to destroy organelle compartmentalization in the homogenate, thus detracting from the possibility that the thyroid hormones functioned to prevent the release of modulating factors.

Although the influence of the thyroid hormones can be placed at the level of MAO, their effect upon the enzyme is not a simple one. This complexity relates to the fact that MAO activity itself appears to be governed by modulating influences present in the whole rat brain homogenates. Figure 1 shows that, as the concentration of whole rat brain homogenate is increased, there is a progressive deviation from linearity with regard to product formation. One explanation for this phenomenon is that MAO activity is being regulated by an endogenous substance or substances and that this regulatory inhibitory influence increases with increased homogenate concentration. This postulated inhibitory influence is unlikely to result from formed metabolites of tryptamine since the rate of formation of IAA in control homogenates was linear with time (Fig. 2). For instance, certain tetrahydro-beta-carbolines have been shown to exert a weak reversible inhibitory action on MAO [30]. Thus, the rate experiments demonstrate that the postulated inhibitor must be preformed or made available maximally (within 5 min; see Fig. 5) prior to the addition of tryptamine. Both T_3 and T_4 maintained the linear relationship with time but allowed IAA to be formed at a faster rate. This shows that the effect of the hormones was exerted maximally within the 15-min pre-incubation period, a contention verified by the data illustrated in Fig. 5. Thus, the experimental results are consistent with the postulate of a preformed endogenous inhibitor of MAO in whole rat brain homogenates and with the notion that the thyroid hormones and/or their metabolites function to remove the inhibition. An alternative explanation is that progressive enzyme or tissue aggregation occurred with increasing homogenate concentrations so as to reduce MAO activity. Under these circumstances the thyroid hormones might induce dissociation to increase product formation. Alterations in the properties of MAO dependent upon enzyme concentration have been postulated previously [31].

A direct action of the thyroid hormones upon MAO itself is not tenable. If this were the case, then an increased MAO activity would be expected at the low homogenate concentrations. Indeed, any increase here might be predicted to be greater than at higher homogenate concentrations since the ratio of hormone to enzyme would be highest. The absence of a direct influence upon MAO is consistent with previous reports [3, 10, 12] which employed low enzyme concentrations, different substrates for MAO and different preparative forms of the enzyme.

L-Triiodothyronine and T_4 produced concentration-related increases in IAA formation but differences exist between the two relationships (see Fig. 6). The correlation data suggest that the mechanism of action of T_3 on MAO activity may differ, at least in part, from that of T_4 and additional experimentation is required to further investigate this aspect. However, the effect of both thyroid hormones is clearly temperature dependent (Fig. 5). The lack of effect after pre-incubation at 4° suggests that metabolic processes may be involved and/or that temperature-dependent conformational changes are required. Ongoing metabolism of the hormones themselves seems likely and would explain the decline in effectiveness of T_3 after 30 and 45 min of pre-incubation at 37°. In fact, the shorter duration of action of T_3, compared with T_4, is consistent with their respective susceptibilities to de-iodination [32]. Additionally, the results illustrated in Fig. 5 demonstrate that the nature of the thyroid-induced effect is a reversible one. Besides the obvious reversibility of T_3, further evidence stems from the inability of both hormones to produce enhanced IAA formation with increasing pre-incubation time. This result suggests a rapid equilibrium of both T_3 and T_4 with their active site(s) and a lack of progressive binding. Such reversible modulation of MAO activity may well preclude the observation of rapid effects on MAO activity after administration *in vivo*. Tissue homogenization and dilution for assay *in vitro* would favor rapid dissociation and loss of the effect, unless the hormones and/or their metabolites were highly concentrated in the organ under test (see Ref. 13). Another common prerequisite for both hormones is pre-incubation with the homogenate prior to substrate addition. The hormones and/or their formed metabolites are unable to increase MAO activity when added together with tryptamine. This points to a high affinity of tryptamine for the postulated inhibitor or the aggregated enzyme so as to preclude thyroid intervention. It is pertinent to note that a 10-fold decrease in the tryptamine concentration failed to alter the augmentation of IAA production by T_3; thus competition between tryptamine and T_3 for a common site seems unlikely.

The effect of T_3 and T_4 on IAA production was not restricted to the brain. High activity was found in the liver, less in the kidney, and only marginal changes occurred in the heart. This selectivity argues against non-specific mechanisms, such as enzyme aggregation and the notion that some ubiquitous cellular constituent became liberated by homogenization to exert inhibitory influences. However, no conclusive interpretations can be drawn since the heterogeneity of MAO, both within and between organs [33, 34], could also explain this differential effect. Clearly, further experimentation is required to elucidate the precise mechanism or mechanisms involved, and to establish whether the presently described effect has relevance to intact cellular systems. However, the present experiments do point to the fact that thyroid hormones can modulate MAO activity by a mechanism which cannot be explained adequately upon the basis of mitochondrial biogenesis [14], increased

enzyme [11, 12] or co-factor synthesis [15], nor does it relate to a direct effect upon MAO itself.

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