ENZYMIC DEIODINATION OF L-THYROXINE AND 3:5:3'-TRIIODO-L-THYRONINE

INTRACELLULAR LOCALIZATION OF "DEIODINASE" IN RAT BRAIN AND SKELETAL MUSCLE

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Incubation of L-thyroxine and 3:5:3'-triiodo-L-thyronine** in the presence of slices or suspensions of brain and skeletal muscle led to their deiodination[1,2]. In diluted brain suspensions a small amount of deaminated analogues of the two hormones were also formed under certain conditions. Deiodination and deamination of mono- and diiodo-L-tyrosine, L-thyroxine and triiodo-L-thyronine with liver and kidney preparations are also known to occur[3-8]. Whereas inorganic iodide is the major or the only product of incubation of L-thyroxine and 3:5:3'-triiodo-L-thyronine with brain and muscle preparations, incubation with liver and kidney slices or suspensions gave rise to as many as 7 or 8 (some of which are unidentified) iodinated compounds in varying amounts[9]. Hence, for a quantitative study of "deiodinase" the use of brain and skeletal muscle was preferred although the enzyme content in these two tissues is lower than in liver or kidney.

Before undertaking the extraction and purification of the deiodinating enzyme or enzyme system, it was thought to be of interest to determine its morphological localization in brain and skeletal muscle. In this communication, the gross distribution of deiodinating activity in the principal regions of the rat brain is described, together with experiments on its intracellular localization in this tissue and in skeletal muscle. While deiodination of L-thyroxine and triiodo-L-thyronine was the principal object of study, estimation of the rate of formation of deaminated products in the presence of subcellular fractions of the brain was also attempted.

METHODS AND MATERIALS

Male rats, 100–125 g in weight, of the Sprague-Dawley and hooded (National Institute for Medical Research, London) strains were sacrificed by decapitation. Whole brain and fragments of thigh muscle (1–4 g), were rapidly removed and chilled in ice. All subsequent procedures leading to incubation were carried out at 0–5°. For studies on the distribution of "deiodinase" in the main areas of the brain, the following regions were dissected out: cerebrum, mid-brain, medulla, cerebellum and cerebral cortex. Each of these portions was homogenized in 10 volumes of an isotonic bicarbonate medium as described previously[1]. The subcellular fractionation of brain and muscle was achieved by differential centrifugation of the tissue suspension in 0.25 *M* sucrose. Procedure II

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** The abbreviations T_4, T_3 and I^- will be employed for L-thyroxine, 3:5:3'-triiodo-L-thyronine and inorganic iodide respectively.

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of BRODY AND BAIN[10] was employed for obtaining the different particulate and the "soluble" fractions of brain cells, while for skeletal muscle the method of KITIYAKARA AND HARMAN[11] was used. The following intracellular fractions of the two tissues were assayed for deiodination: (a) nuclear, with some whole cells, stroma and erythrocytes; (b) mitochondrial, with some nuclei, and (c) supernatant or "soluble" fraction, containing microsomes. Deamination was also assayed in the above fractions obtained from the brain. In some experiments a microsomal fraction obtained by centrifugation of the supernatant at 60,000 *g* for 60 min was also tested for enzyme activity. An aliquot of each particulate fraction was washed twice with 0.25 *M* sucrose and 0.15 *M* KCl while the unwashed part served as a control to determine the effect of washing. The final volume of suspension of the particulate fractions in bicarbonate-buffered medium was equal to half the volume of tissue homogenate used for fractionation. In some experiments, tissue was homogenized in the bicarbonate-containing medium[1]; the resulting supernatant fraction was compared for enzyme activity against the supernatant fraction in 0.25 *M* sucrose.

Enzyme content of each sample was determined by incubation with ^{131}I-labelled L-thyroxine* and 3:5:3′-triiodo-L-thyronine* at concentrations varying between 10^{-8} *M*–10^{-7} *M*. Each reaction vessel contained 3.0 ml of tissue preparation with the added substrate, and incubations were carried out at 37° under atmospheric air. Heat-denatured samples corresponding to each preparation (100° for 10 min) served to give control values. The measurement of deiodination and deamination of the substrate was performed by paper-chromatographic and electrophoretic analyses of the reaction products in serial samples obtained during the first 60 min of incubation. All analytical techniques employed for this purpose have been described previously[1]. Initial velocity rates were calculated from values obtained after 10 min of incubation. Each incubation sample was analyzed by the micro-Kjeldahl method for protein-nitrogen content; results are expressed as relative specific enzyme activity using the specific activity of the unfractionated homogenate as the standard of comparison. Tissue samples from 6 to 10 animals were pooled per batch and each experiment was performed in duplicate or triplicate. Average values derived from different series of experiments are reported here.

RESULTS

When incubated with whole brain and muscle homogenates, 1:10 w/v in bicarbonate-buffered medium or 0.25 *M* sucrose, the average initial rates of degradation of L-thyroxine and 3:5:3′-triiodo-L-thyronine were as follows:

	Brain	Muscle
	(In mμmoles/10 min/reaction vessel)	
L-thryoxine	$1.0–1.6 \cdot 10^{-2}$	$1.5–2.3 \cdot 10^{-2}$
3:5:3′-triiodo-L-thyronine	$0.6–0.8 \cdot 10^{-2}$	$0.8–1.3 \cdot 10^{-2}$

These figures were calculated from the percentage of total ^{131}I left as T_4 or T_3 at any time, assuming that specific radioactivity remained constant. These rates are comparable to those observed previously[1,2] and once again it will be seen that the "deiodinase" seems to have a greater affinity for T_4 than for T_3 under similar conditions. At least 90% of the ^{131}I lost from T_4 or T_3 could be accounted for in the inorganic iodide fraction in all cases; deaminated products of the two substrates did not make up more than 8–10% of total radioactivity at any time during incubation with whole-brain homogenates.

Distribution of "deiodinase" in different areas of rat brain

The relative amounts of deiodinating enzyme in bicarbonate-buffered homogenates of the main areas of rat brain are compared in Table I on the basis of their protein content.

The figures are derived from the rate of appearance of ^{131}I in the inorganic iodide

* Supplied by Abbot Laboratories, Oak Ridge, Ten. L-Thyroxine and 3:5:3′-triiodo-L-thyronine were labelled in 3′-5′- and 3′-positions respectively.

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fraction, any ^{131}I of either substrate converted to the deaminated analogues being excluded. A large part of the "deiodinase" of the central nervous system is seen to be concentrated in the cerebral cortex with activity in other areas distributed as: cerebrum > cerebellum > medulla > mid-brain.

TABLE I

DEIODINATING ACTIVITY IN DIFFERENT REGIONS OF RAT BRAIN

Region	*Relative specific activity*	
	T_4	T_3
Whole brain	100	100
Cerebrum	158	172
Cerebral cortex	229	240
Cerebellum	97	82
Medulla	28	37
Midbrain	21	13

Substrate concentration: $3.4 \cdot 10^{-8}$ *M* for T_4; $4.5 \cdot 10^{-8}$ *M* for T_3. Specific activity = Δ% Total ^{131}I as I^-/mg protein/min.

Intracellular localization of deiodinating and deaminating activities

Enzyme activities as measured by the incorporation of radioactivity into iodide or deaminated analogues with the particulate and "soluble" fraction obtained from the brain are compared in Table II.

TABLE II

INTRACELLULAR DISTRIBUTION OF DEIODINATING AND DEAMINATING ACTIVITIES IN RAT-BRAIN CELLS

Fraction	*Relative specific activity*			
	Deiodination		*Deamination*	
	T_4	T_3	T_4	T_3
Homogenate	100	100	100	100
Nuclear	26	20	0.0	5.0
Unwashed mitochondrial	63	71	22.0	28.0
Washed mitochondrial	20	13	—	—
Microsomal	80	58	—	—
Supernatant	380	339	10.0	6.0

Substrate concentration: $5.5 \cdot 10^{-8}$ *M* for T_4; $5.8 \cdot 10^{-8}$ *M* for T_3. Specific activity = ΔI^- or Tetrac or Triac as % total ^{131}I/mg protein/min. Tetrac = 3:5:3′:5′-tetraiodothyroacetic acid. Triac = 3:5:3′-triiodothyroacetic acid.

Specific enzyme activities for both L-thyroxine and triiodo-L-thyronine as substrates gave almost identical values throughout all the experiments. A 3–4-fold enrichment in concentration of "deiodinase" resulted from the separation of the "soluble" cytoplasmic fraction. There was relatively little enzyme activity in the particulate fractions, and practically none in the nuclear fraction. Washing with 0.25 *M* sucrose or the bicarbonate-buffered medium had no effect on nuclei or microsomes but a net loss of enzyme was observed with the mitochondrial fraction as seen in Table II. "Deiodinase" activity in supernatant fractions of bicarbonate medium

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was not very different from that seen in 0.25 M sucrose supernatant samples. The deaminating activity in whole brain homogenate, which was only a fraction of the deiodinating activity, was almost totally lost on fractionation of the tissue suspension. It was only in the mitochondrial fraction incubates that deaminated analogues of T_4 or T_3 were observed. No evidence was obtained of deamination of the substrates by any of the muscle preparations, but a similar pattern of intracellular distribution of "deiodinase" was noted as with brain preparations. This is shown in Table III.

TABLE III

INTRACELLULAR DISTRIBUTION OF "DEIODINASE" IN RAT SKELETAL MUSCLE

Fraction	*Relative specific activity*	
	T_4	T_3
Homogenate	100	100
Nuclear	31	17
Mitochondrial*	25	36
Supernatant	585	449

Substrate concentration = 10^{-7} M for T_4; $1.6 \cdot 10^{-7}$ for T_3. Specific activity = $\triangle I^-$ as % total ^{131}I/mg protein/min.

* Unwashed mitochondrial fraction.

DISCUSSION

Enzymes or enzyme systems which catalyze the degradation of L-thryoxine and triiodo-L-thyronine show a definite pattern of distribution in the different regions of brain and in the intracellular fractions of brain and muscle. The "deiodinase" was observed to be by far more prominent than the deaminating enzyme. In fact, inorganic iodide accounted for nearly 80–95% of radioiodinated reaction products with brain preparations while this figure was above 95% with muscle preparations.

The relatively high concentration of "deiodinase" in the cerebral cortex (Table I) is not surprising. This area of the central nervous system, and to some extent the cerebellar region, has the largest proportion of most other enzymes of the brain. In this connection, it is of interest to consider the results of ALLEVA AND MARIÑONI[12] in which these workers described a higher concentration of ^{131}I in frontal lobes and in the cerebellum after injection of labelled thyroxine into the rat.

In the cell, the "deiodinase" has the highest concentration in the "soluble" cytoplasmic fraction in both brain and muscle (Tables II and III). The disappearance of deiodinating activity from mitochondria on washing suggests an adsorption of enzyme or co-factors on this particulate fraction. The fraction of deiodinating activity in microsomes in proportion to that in whole brain homogenate was not comparable to the value ascribed to liver microsomes by MACLAGAN AND REID[13]. In the latter work, the "deiodinase" content was found to be most elevated in the microsomal fraction although only slightly higher than in the "soluble" fraction. The difference in analytical techniques used for estimating the amount of deiodinated products between their studies and those reported in this communication might account for this variance. On the other hand, the "soluble" cytoplasmic fraction of the liver was found to be most active of all the sub-cellular fractions in the deiodination of L-diiodo-

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tyrosine[14]. The significance of the localization of "deiodinase" in brain and muscle is limited by the fact that no information is yet available on the intracellular distribution of L-thyroxine and L-triiodothyronine in these tissues. In liver, however, where such studies were made with ^{14}C-labelled thyroxine, the major part (58%) of radioactivity was localized in the non-particulate cytoplasmic fraction[15]. Although this localization was not strikingly selective, it has been described as an active process. In any case, it is still an open question whether only that fraction of the thyroid hormones which is localized or "bound" to the non-particulate cytoplasmic fraction is susceptible to deiodination.

Sub-cellular fractionation of the cell led to the loss, to a large extent, of deaminating activity observed in brain slices or homogenates. It was only in the mitochondrial fraction that this activity was recovered to some significant degree.

Once again, as in previous studies[1,2], 3:5:3'-triiodo-L-thyronine was not a major product of deiodination of L-thyroxine. In most instances iodide was the only product observed; this is at variance with work reported elsewhere[3,4].

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SUMMARY

1. The distribution of L-thyroxine- and 3:5:3'-triiodo-L-thyronine-"deiodinase" in principal regions of the central nervous system and in centrifugally prepared fraction of whole brain and skeletal muscle of the rat is reported. The intracellular distribution in the CNS of deaminating activity on the two hormones was also studied.

2. The cerebral cortex was found to have the highest concentration of "deiodinase" activity in the brain.

3. In the cell, the deiodinating activity was chiefly localized in the "soluble" cytoplasmic fraction of both brain and muscle. Some activity was also detected in the microsomal fraction of brain.

4. Centrifugal fractionation of brain homogenates led to a virtual loss of deaminating activity except for small recoveries in the mitochondrial fraction.

5. The significance of these distribution patterns is discussed.

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