EFFECT OF THYROID HORMONE ANALOGUES ON THE DISPLACEMENT OF ^{125}I-L-TRIIODOTHYRONINE FROM HEPATIC AND HEART NUCLEI IN VIVO: POSSIBLE RELATIONSHIP TO HORMONAL ACTIVITY.

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SUMMARY. The capacity of iodotyrosines and iodothyronine analogues to displace tracer [^{125}I] L-3,5,3' triiodothyronine from specific nuclear binding sites in rat liver and heart was related to the displacement capacity of nonradioactive triiodothyronine. Iodotyrosines and L-3,3',5' triiodothyronine ("reverse T_3") were devoid of displacement activity. Analogues with 3,5 substitution in the "inner" ring and single "bulk" substitution in the 3' position in the phenolic ring exhibited the strongest displacement activity. When the distribution, fractional removal rates and metabolic conversion of the analogues were taken into account, displacement activity appeared to correlate well with the reported thyromimetic activity. These results support the biologic relevance of the nuclear sites.

With the use of *in vivo* displacement techniques we have recently reported limited capacity high affinity binding sites for L-T_3 in rat liver and kidney (1). These sites have been identified as nonhistone nucleoproteins with a probable molecular weight of 60-70,000 (2). L-T_3 bound to these sites rapidly exchanges with cytoplasmic hormone (3). Other tissues responsive to thyroid hormones such as heart have also been shown to contain nuclear binding sites. These sites, however, are not absolutely specific for L-T_3 since they appear to crossreact minimally with T_4. In order to obtain a better understanding of the structural requirements for the binding of various analogues of L-T_3 we initiated experiments to assess the ability of various thyroid hormone analogues to displace radioactively labeled tracer L-T_3 from liver and heart nuclei. We were particularly interested in correlating displacement potency of a given analogue and its reported thyromimetic activity.

METHODS. Male Sprague-Dawley rats (100-150 g) were injected through the tail vein with tracer doses of [^{125}I] L-T_3 (Abbott, specific activity 30-40 μCi/μg) along

ABBREVIATIONS USED. T_4, L-thyroxine; L-T_3, L-3,5,3' triiodothyronine; reverse T_3, L-3,3',5'-triiodothyronine; tetrac, L-3,5,3',5' tetraiodothyroacetic acid; isopropyl-T_2, 3'-isopropyl-3,5-diiodothyronine; MIT, monoiodotyrosine; DIT, diiodotyrosine.

with various doses of nonradioactive compounds. Animals were killed at a time when the L-T_3 concentration was maximal in the nuclei of the organ studied, one-half hour in the liver and one hour in the heart. The concentration of [^{125}I] L-T_3 in plasma was determined after trichloroacetic acid precipitation in order to remove $^{125}I^-$, the only significant source of radioactivity other than L-T_3. Chromatographic studies indicated that over 95% of nuclear radioactivity was in the form of L-T_3. The [^{125}I] L-T_3 nuclear/plasma concentration ratio per mg nuclear DNA was determined under tracer conditions as well as the percentage depression of this ratio by increasing doses of nonradioactive L-T_3 and the test substance. In each experiment 4-5 dose levels of the test substance and nonradioactive L-T_3 were assayed. In studies with liver nuclei, the response at each dose level was determined by the mean nuclear/plasma/mg DNA value of four animals; in studies with heart nuclei, similar values were determined in nuclei derived from pools of three hearts. Experiments with each test substance for liver and for heart were repeated at least once and the data combined for graphic analyses. The molar dose of L-T_3 and the test substance required to reduce the nuclear/plasma concentration ratio to one-half the tracer value was assessed from plots of the nuclear/plasma ratio as a function of the log of the dose per 100 g body weight. The ratio of the one-half displacement dose of L-T_3 to the corresponding dose of the analogue was defined as the relative displacement potency. A representative study is illustrated in Fig. 1.

Hepatic nuclei were isolated as previously described (1). Hearts were pooled in groups of three (approximately 1.5 g) and homogenized in five volumes of 0.32 M sucrose-0.003 M $MgCl_2$ in a Potter-Elvhejem homogenizer. The homogenate was diluted to 10 volumes in the same solution and subjected to preliminary centrifugation for 15 minutes at 700 x g. The pellet was resuspended in 20 ml of homogenizing solution containing 0.25% Triton. This suspension was further homogenized with a Virtis "45" mixer for 1 minute at 20,000 RPM and centrifuged for 10 minutes at 700 x g. The pellet was washed twice by resuspending it in 25 ml of homogenizing solution without Triton. The final pellet

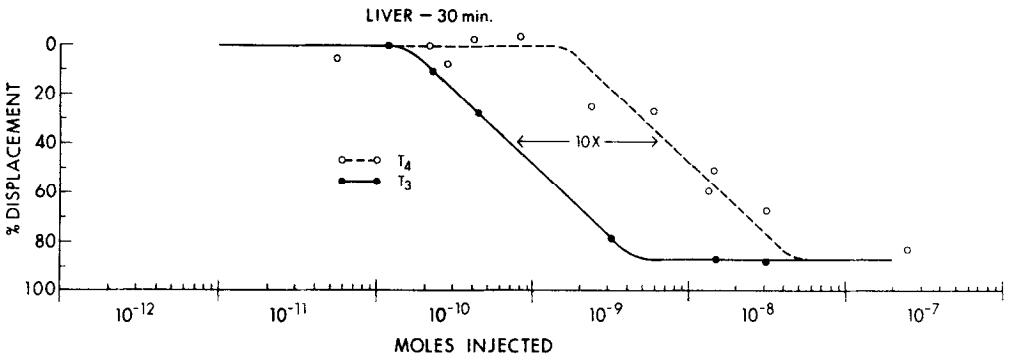


Figure 1. Percent displacement of ^{125}I-T_3 bound to hepatic nuclei by nonradioactive T_3 and T_4. Each point represents the average of 4 animals.

was resuspended in 28 ml of 2.4 M sucrose-0.003 M $MgCl_2$ and centrifuged in a Spinco SW 25.1 rotor for one hour at 50,000 x g. Nuclear radioactivity was related to DNA which was determined by the method of Burton (4).

RESULTS. The relative displacement potency of the iodothyronine analogues and iodotyrosines tested is summarized in Table 1. Inspection of these results support the following generalizations: 1. Displacement appears to require the diphenyl-ether structure since MIT and DIT are without effect. 2. Compounds containing a single bulk substitution in the 3' position (L-T_3, D-T_3, triac, isopropyl T_2) have a markedly greater displacement potency than compounds which contained substitutions in both the 3' and 5' positions (T_4 and tetrac). Iodine is not an essential in the 3' position since substitution of the isopropyl group in this position yielded a compound as potent as L-T_3 in the liver and more potent than L-T_3 in the heart. 3. Since reverse T_3, lacking one iodine in the inner ring, is far less potent than T_4 in liver and in heart, substitutions in both the 3 and 5 positions are probably important for displacement. 4. Certain differences in the relative displacement potency between the heart and liver are apparent. Thus, D-T_3 appears to be more potent in liver than in heart and the converse situation obtains in the case of isopropyl T_2.

Although the relative displacement potency of triac was equal to that of L-T_3 both in heart and in liver it is known that triac has substantially less

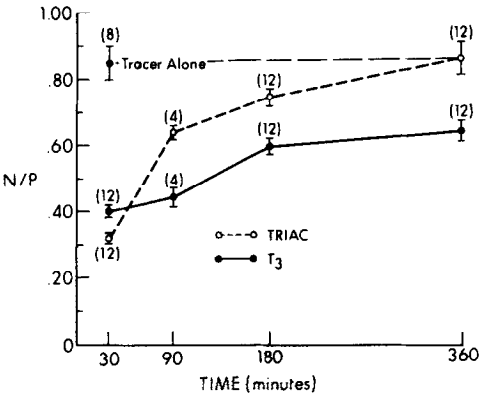
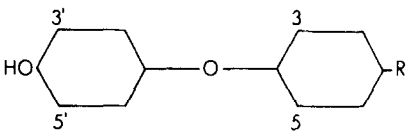


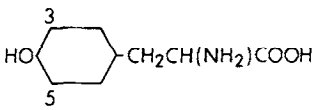
Figure 2. Nuclear/plasma ratio of ^{125}I-T_3 (N/P) as a function of time after injection of 6.8 x 10^{-10} moles of L-T_3 and triac per 100 g body weight. Numbers in parentheses indicate number of animals averaged at each point. Bars indicate ±SEM. N/P returns to tracer level more rapidly after injecting triac than T_3.

thyromimetic activity than T_3 in most conventional bioassays (5). In order to determine whether or not this discrepancy could arise from a more rapid fractional metabolism of triac, serial measurements of the nuclear/plasma ratio of [^{125}I] L-T_3 were made in groups of animals injected with equimolar doses of nonradioactive L-T_3 and triac. The results, illustrated in Fig. 2, indicate that indeed displacement by triac was more short-lived than that induced by L-T_3. This observation is thus compatible with the concept that the shorter residence time of triac on the receptor sites is responsible for the lesser biologic potency of this analogue.

DISCUSSION. The capacity to displace tracer doses of [^{125}I] L-T_3 by various iodothyronines is clearly dependent on multiple factors. In addition to the conformational characteristics of the nuclear receptor sites, the distribution and metabolism of L-T_3 and the analogues must be taken into account. Thus, one cannot automatically relate the relative displacement potency to the chemical characteristics of the receptor site. Nevertheless, there are striking correlations between the requirements for displacement and those previously observed for hormonal activity. Thus, MIT, DIT and reverse T_3 lack both displacement and hormonal effects, whereas triac and isopropyl T_2 are active with respect to both parameters (5,6). In fact, results of the sequential displacement experi-



	ANALOGUE	3'	5'	3	5	R	RELATIVE POTENCY RATIO LIVER	HEART
IODOTHYRONINES	L-T_3	I	–	I	I	$CH_2CH(NH_2)COOH$	1.0	1.0
	L-THYROXINE	I	I	I	I	$CH_2CH(NH_2)COOH$	0.1	0.04
	TETRAC	I	I	I	I	CH_2COOH	0.05	0.02
	REVERSE T_3	I	I	I	–	$CH_2CH(NH_2)COOH$	0	0
	TRIAC	I	–	I	I	CH_2COOH	1.0	1.0
	D-T_3	I	–	I	I	$CH_2CH(NH_2)COOH$	0.7	0.3
	ISOPROPYL-T_2	CH_2 CH CH_2	–	I	I	$CH_2CH(NH_2)COOH$	1.0	2.5



IODOTYROSINES	MIT		I	–	0	0
	DIT		I	I	0	0

Table 1. Relative displacement potency of iodotyrosines and analogues of iodothyronines. Relative displacement potency is defined as the ratio of the molar dose of T_3 to produce ½ displacement of nuclear $[^{125}I]T_3$ to the molar dose of the analogue necessary to produce a comparable displacement of nuclear $[^{125}I]T_3$.

ment appeared to provide an explanation for the quantitative discrepancies between the biologic and displacement activities of T_3 and triac. The more rapid fractional disappearance of triac from nuclei suggested a shorter net residence time of the analogue and consequently a lesser thyromimetic effect. D-T_3 exhibits more displacement and thyromimetic activity in liver than in heart. If the behavior of the heart nuclei with respect to D-T_3 is representative of extrahepatic tissues, this may provide an explanation for the overall lesser hormonal effectiveness of D-T_3 as compared to L-T_3 (7). Although L-T_4 has major metabolic activity this may in large part be derived from the peripheral conversion of T_4 to T_3 (8,9). On the basis of previous metabolic studies in the rat (8), we estimate that at one-half hour 0.3% and at one hour 0.7% injected T_4 has been converted to T_3. These considerations account for the relatively low displacement potency of T_4 in heart and liver.

Of parenthetic interest is that reverse T_3 has been shown to antagonize the calorigenic effects of T_4 (10). Since reverse T_3 and T_4 do not interact at the nuclear site, consideration should be given to the possibility that reverse T_3 exerts anti-T_4 effect by some mechanism such as an inhibition of T_4 to T_3 conversion at extranuclear sites.

Attention should be directed to the striking resemblance between the thyroid hormone receptor site postulated by Jorgensen in 1962 on the basis of functional activity studies of thyroid hormone analogues (11), and the requirements of thyroid hormone displacement as suggested by the present studies. Both models require single bulk substitution, not necessarily iodine, in the 3' position of the phenolic ring and substituents in the 3 and 5 positions of the inner ring.

A definitive analysis of the relationship between the structure of a thyroid hormone analogue and its functional effects at the nuclear site requires a detailed description of the concentration and time-course of the analogue at these sites and accurate data with respect to the dose responsivity of an individual tissue to a given analogue delivered in a comparable fashion. Since such information is not available the results of the present set of experiments must be interpreted simply as a first step in the analysis. Nevertheless, in light of available information about the distribution, metabolism, and functional activity of the individual analogues administered, a good correlation was observed between displacement and hormonal potency. This correlation supports the concept that thyroid hormone activity is initiated by the occupancy of the previously described high affinity limited capacity nuclear sites, either by the analogue injected or by its active metabolic derivatives.

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