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Research Article

CALORIGENIC EFFECTS OF SINGLE INTRAVENOUS DOSES OF L-TRIIODOTHYRONINE AND L-THYROXINE IN MYXEDEMATOUS PERSONS

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In 1950 and 1951 two unknown radioiodine-bearing compounds were observed (1–3) on chromatograms of plasma obtained from animals treated with I^{131}. Gross and Pitt-Rivers (4) identified one of these as 3,5,3′-l-triiodothyronine, which they had previously isolated (5) from the plasma of euthyroid and hyperthyroid individuals treated with I^{131}. They excluded the possibility of analytical or radiation artefact and considered 3,5,3′-l-triiodothyronine to be a normal constituent of the organic iodine moiety of plasma. Concomitantly, Roche, Lissitzky, and Michel (6) reported the conversion *in vitro* of diiodothyronine to triiodothyronine and crystallization of the latter compound. On the basis of its ability to prevent goiter in rats treated with thiouracil, Gross and Pitt-Rivers (7) found triiodothyronine three times as active as thyroxine. They also demonstrated its effectiveness (8) in raising the metabolic rate and correcting the symptoms of myxedema in two patients. In this respect they considered it to be at least as calorigenic as l-thyroxine and probably more so, and suggested that it might prove to be the tissue form of the thyroid hormone.

Since 3,5,3′-l-triiodothyronine is the only other amino acid to be found thus far which possesses hormonal properties comparable in intensity to those of thyroxine, it appeared desirable to make qualitative and quantitative comparison of the calorigenic properties of the two substances in human subjects.

METHOD

Eight persons with untreated myxedema who served as volunteers were hospitalized throughout the study. Duplicate determinations of the basal metabolic rate using the closed-chamber method were made daily except Sunday. Before the tests were made, the basal metabolic rate was determined daily for 6 to 16 days to establish a satisfactory base line. Serum-protein-bound iodine was estimated by the method of Barker (9).

Triiodothyronine and thyroxine [2] were administered in a small volume of sterile physiologic saline solution in which the substance was dissolved with the aid of sufficient sodium hydroxide. A single injection of the amino acid to be tested was given intravenously after the morning metabolic rate had been determined. Daily observations were then continued for as long as possible, in most instances until the curve depicting the basal metabolic rate had returned to a level within 1 or 2 calories per hour of the original base line. Careful record was made of clinical reaction and symptoms following the injections. Serum-protein-bound iodine was also determined at several intervals after injection.

In accordance with a procedure previously employed by other investigators (10, 11), mean daily metabolic rates were expressed as calories per day rather than as calories per hour. For each day following injection the mean base line obtained during the initial control period was then substracted from each daily value. The difference was considered to represent the increment in calories produced by the hormone being studied.

Each subject underwent two to six individual studies. In all, the effects of 18 doses of triiodothyronine and 11 doses of thyroxine were studied, involving a total of 1,213 determinations of basal metabolic rate. The data are summarized in Table I.

RESULTS

Following injection of either substance, the basal metabolic rate responded initially by rising to a peak value and subsequently by declining gradually toward the original base line (Figure 1). The decline of metabolic rate following injection of either drug could be fitted to a straight line when plotted on semilogarithmic paper versus time.

[1] The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

[2] These substances were provided through the courtesy of Smith, Kline & French Laboratories, who obtained the former from Glaxo Laboratories where it was synthesized.

TABLE I

Summary of data on patients to whom triiodothyronine and thyroxine were administered *

Subject	Age, sex	Type of myxedema	Base line BMR	Base line PBI	Injection	Body weight, Kg.	Drug	Dose, μg.	T_{max}	C_{max}	$T_{\frac{1}{2}}$	C_{tot}	PBI 2 hr. after injection
1	35 M	Spontaneous	−34	0.5	1	56.5	T_4	333	7	80	—	1700	3.8
					2	57.1	T_3	333	2	220	7.2	1600	2.6
					3	57.6	T_3	160	3	112	9.7	1300	1.4
					4	58.8	T_4	3000	11	330	—	6700	14.3
2	45 F	Post I^{131} therapy	−26	1.9	1	72.2	T_3	200	2	300	7.0	2100	3.1
					2	72.4	T_4	300	7	145	10.8	2400	5.3
3	49 M	Spontaneous	−38	0.5	1	78.8	T_3	100	2	210	7.6	2100	0.7
					2	78.3	T_4	300	6	100	—	2100	1.7
					3	78.5	T_3	600	2	500	8.0	4600	2.6
					4	77.5	T_3	600	2	480	7.4	4100	—
					5	78.5	T_4	720	12	290	11.0	5000	5.6
					6	77.4	T_3	250	2	360	7.8	3300	0.9
4	58 M	Spontaneous	−33	0.6	1	81.5	T_3	100	2	195	7.7	1800	0.4
					2	79.4	T_3	100	1	200	7.7	1800	0.7
					3	79.8	T_3	600	3	320	8.2	3200	2.4
					4	79.0	T_3	400	2	290	7.7	2800	—
5	43 F	Spontaneous	−34	7.2†	1	60.9	T_3	100	2	190	7.9	1700	15.7
					2	61.1	T_3	600	2	375	7.7	2900	9.9
					3	60.5	T_3	100	2	190	7.8	1600	—
					4	61.3	T_4	800	13	164	11.4	4500	7.4
6	67 F	Spontaneous	−29	0.6	1	66.4	T_4	600	6	100	10.8	1900	3.5
					2	65.5	T_4	1800	9	240	—	5100	12.3
7	29 F	Spontaneous	−40		1	38.4	T_4	400	11	160	12.0	3300	—
					2	38.0	T_3	325	2	240	7.6	2100	—
					3	37.8	T_3	1000	3	270	7.6	2700	—
					4	37.6	T_4	1200	14	295	—	6600	—
8	52 F	Spontaneous	−27	Out of range‡	1	94.0	T_3	500	2	520	4.2	3300	Out of range‡
					2	92.8	T_3	1000	3	480	4.0	3100	—
					3	90.0	T_4	680	6	120	9.0	2100	—

* Abbreviations: BMR, basal metabolic rate in per cent; PBI, protein-bound iodine in micrograms per 100 cc.; T_{max}, time of maximal response in days; C_{max}, maximal caloric response in calories per day; $T_{\frac{1}{2}}$, half-value time in days; C_{tot}, total metabolic response in calories; T_3, triiodothyronine; and T_4, thyroxine.

† Recent cholecystogram.

‡ Recent excretory urogram.

With doses in excess of 4 micrograms per kilogram of body weight, triiodothyronine invariably produced subjective discomfort including malaise, restlessness, generalized aching, muscular and joint pain, loss of appetite, and headache. When the dose exceeded 10 micrograms per kilogram, these symptoms became intense. The symptoms accompanied the period of maximal metabolic response and, in most instances, subsided by the third or fourth day. These symptoms were identical with those often encountered in myxedematous patients whose metabolic rate is being rapidly elevated by administration of desiccated thyroid.

In contrast to the striking subjective discomfort following injection of triiodothyronine, similar symptoms did not occur after the administration of thyroxine in the doses here employed, although such symptoms have previously been observed to follow larger doses (10 mg. dl-thyroxine) given intravenously (10).

The curves depicting the effect of intravenous doses of each substance upon the metabolic rate were compared from four standpoints: (1) the time of maximal response (T_{max}), defined as the time in days elapsing between injection and greatest observed elevation of metabolic rate, (2) the half-value time ($T_{1/2}$), defined as the time in days required for the fitted curve of diminishing metabolic response (second limb) to reach half its value, (3) the maximal caloric response (C_{max}),

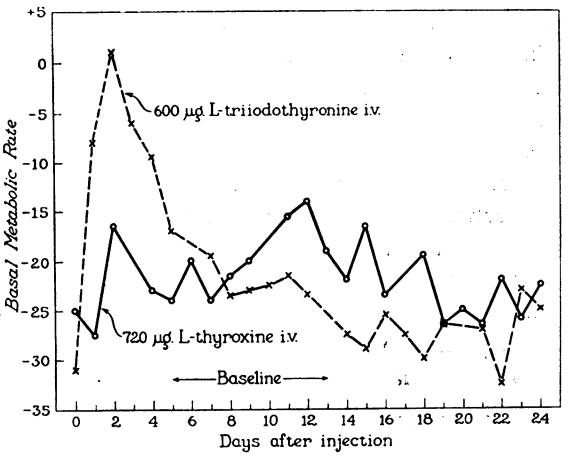

FIG. 1. THE BASAL METABOLIC RATES AFTER INJECTION OF EQUIMOLAR AMOUNTS OF TRIIODOTHYRONINE AND THYROXINE (SUBJECT 3)

defined as the maximal metabolic rate expressed in calories per day produced by a given injection, and (4) the total metabolic response (C_{tot}), defined as the total increment of increased energy production in calories throughout the time of action of the compound.

Following injection of triiodothyronine, the metabolic rate invariably rose more rapidly and to a higher maximal level than following equimolar doses of thyroxine (Table II). Maximal effects were always observed within 48 or 72 hours, the mean value for T_{max} being 2.2 ± 0.1 days. In contrast, thyroxine produced a slower and more sustained rise in metabolic rate, the maximal level being reached only after an interval of 6 to 13 days. The mean T_{max} for thyroxine was 9.4 ± 0.9 days. There was no correlation between dose and time of maximal response in the case of either drug.

The magnitude of the maximal response (C_{max}) reflected dosage and was always higher after triiodothyronine than after comparable doses of thyroxine. The dose-response relationships of the two substances in terms of this measurement are shown in Table II and Figure 2. Triiodothyronine was consistently more potent by a factor of 3 to 4.

The subsequent dissipation of calorigenic effect (thyroxine-decay curve of Boothby) was much more rapid after injection of triiodothyronine than after injection of thyroxine. Since in the case of either substance caloric response diminished exponentially, this rate could be expressed quantitatively either as a rate constant or as half-value time. Observed half-value times for triiodothyronine varied from 4.0 to 9.7 days (mean 7.4 ± 0.6 days) compared with corresponding values for thyroxine varying from 9.0 to 12.4 days (mean 11.9 ± 0.4 days). The latter values may be compared with those obtained in studies with thyroglobulin ("thyroiodin") made by Magnus-Levy in 1904 (12) which are calculated to have a half-value time of 15.6 days, and with the curves for dl-thyroxine published by Boothby and Baldes (13) and by other investigators (11) which are calculated to have half-value times of 10.8 days and 13.8 days, respectively. This difference in disappearance rate means that dose for dose the meta-

TABLE II

Comparison of peak values (maximal calories per day) and total calories resulting from similar doses of l-thyroxine and l-triiodothyronine

Subject	Thyroxine			Triiodothyronine		
	Dose, millimols/Kg. (×10⁶)	Effect		Dose, millimols/Kg. (×10⁶)	Effect	
		Maximal cal./day	Total calories		Maximal cal./day	Total calories
1	7.6	90	1727	9.0	220	1585
2	5.4	145	2352	4.3	300	2120
3	5.0	100	2120	5.0	400	3300
	11.8	290	5020	11.8	480	4568
5	17.0	164	4800	15.1	375	2860
7	13.0	160	3300	13.0	270	2100
	40.0	295	6600	40.0	360	2700
8	8.0	120	2100	8.0	520	3300

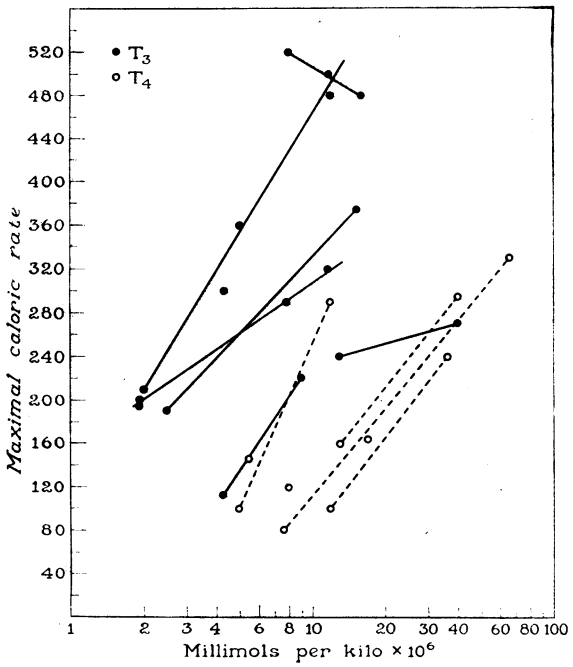

FIG. 2. DOSE-RESPONSE CURVES IN TERMS OF MAXIMAL CALORIES PER DAY FOR L-TRIIODOTHYRONINE AND L-THYROXINE

Solid lines connect points representing separate doses of triiodothyronine (T_3) given the same subject, and dotted lines connect points representing separate doses of l-thyroxine (T_4) given the same subject.

bolic effect of thyroxine long outlasts that of triiodothyronine. Seventy to 90 days are required for the dissipation of 99 per cent of the effect of a single injection of thyroxine compared to 45 to 55 days for the dissipation of 99 per cent of the effect of a single injection of triiodothyronine.

The half-value times, like the T_{max} values, were not correlated with dosage. There was some indication that variations in these values represented real differences in biologic response between individuals. If a given subject had a relatively short $T_{1/2}$ value for an initial study with triiodothyronine, subsequent studies also yielded low values, and more significantly, studies with thyroxine in the same subject disclosed low values for $T_{1/2}$ for this hormone also (see Subject 8, Table I).

Because of the difference in temporal relations observed with the two substances, the most significant basis for comparing their over-all effects appeared likely to be the total integrated caloric response (C_{tot}), represented by the total area under each curve of caloric response versus time. The area of the initial limb and the observed portion of the descending limb could be calculated by application of the trapezoidal rule. Since the descending limb of each such curve fitted a simple exponential function, it was possible to estimate a correction for the small residue of calorigenic activity remaining at the end of the period of observation and, in addition, for the residue of calorigenic activity remaining at the onset from any previous injection. Times of observation were arranged so that such corrections with few exceptions represented less than 10 per cent of the total area under each curve.

A dose-response curve constructed from such observations (Figure 3) indicated that when the dose was small the total caloric response resulting from an injection of l-triiodothyronine exceeded the response produced by an equimolar amount of l-thyroxine. However, with larger doses the reverse was true, that is, the total caloric response produced by thyroxine was greater.

The effect of the injections on protein-bound iodine can be judged from Table I. Thyroxine invariably produced a greater and more sustained elevation of protein-bound iodine than a comparable dose of triiodothyronine.

COMMENT

Since several successive injections were studied in each subject, it was necessary to be sure that

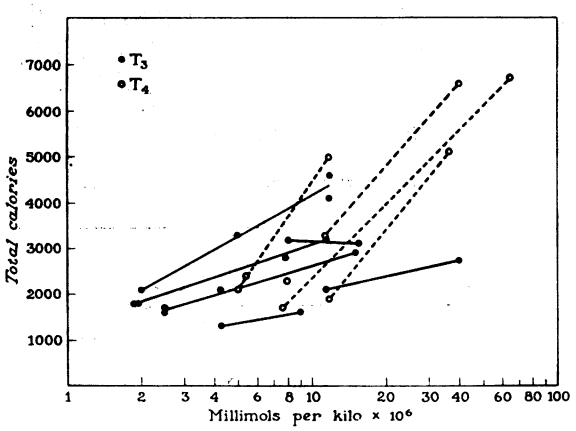

FIG. 3. DOSE-RESPONSE CURVES IN TERMS OF TOTAL CALORIES FOR L-TRIIODOTHYRONINE AND L-THYROXINE

Solid lines connect points representing separate doses of triiodothyronine (T_3) given the same subject, and dotted lines connect points representing separate doses of l-thyroxine (T_4) given the same subject.

subsequent responses were not essentially different from previous ones. For this reason duplicate studies were carried out in three subjects as shown in Table III and Figure 4. These studies indicate that results obtained with this method are reproducible and that successive doses may be evaluated accurately in the same subject providing sufficient time has elapsed between them. Investigators (11), in 1929, found a striking difference in the effectiveness of 10 mg. of intravenously administered racemic thyroxine at a basal metabolic level of − 40 per cent compared with the biologic effectiveness of the same dose at a metabolic level of − 4 per cent. However, they were comparing metabolic levels which differed by 20 or more calories per hour whereas in our study most comparisons were made at metabolic levels which did not differ by more than 1 calorie per hour.

In comparable doses, triiodothyronine was found to have an early and large maximal effect which disappeared rapidly whereas thyroxine exhibited a more delayed and less intense maximal effect which persisted for a longer interval. The more intense maximal effect of triiodothyronine frequently produced annoying subjective symptoms. These were not encountered with thyroxine except in doses much larger than those employed in this study. These qualitative differences posed practical obstacles to the bio-assay in man of small doses of thyroxine or large doses of triiodothyronine. Thyroxine could not be tested in doses of less than 300 micrograms because such doses did not produce sufficient elevation of the basal metabolic rate. Large doses of triiodothyronine could not be evaluated with safety because of the disturbing subjective symptoms. Therefore, single doses of the two drugs could be compared directly only in the dose range of 0.3 to 1.0 mg. Within this range of dosage the dose-response relationships of single injections of the two hormones have been compared in two ways. On the basis of maximal caloric response, triiodothyronine was found to be three to four times as potent as thyroxine. On the basis of total calories produced, the relative potency depended upon dose; with small doses, triiodothyronine was consistently more effective than thyroxine; with larger doses, thyroxine was more calorigenic.

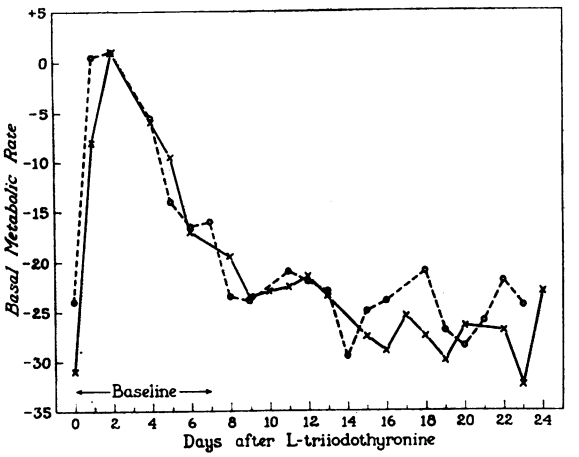

FIG. 4. METABOLIC RESPONSE TO INJECTION OF IDENTICAL AMOUNTS OF TRIIODOTHYRONINE ON TWO SEPARATE OCCASIONS (SUBJECT 3)

Valid comparison of the calorigenic potency of thyroidal substances may also be made on the basis of multiple doses, either by parenteral or oral routes. Salter, Lerman, and Means (14, 15) have employed an assay method based upon the administration of daily doses of the test substance over a period of two weeks or longer, accompanied by observation of the ascending limb of the resulting metabolic-response curve. This method could be subject to error if substances having major temporal differences in their responses were to be compared. A more secure comparison is one based upon the dose of each substance necessary to maintain a myxedematous subject in a steady euthyroid state over a period of time. Preliminary studies employing this technic would indicate triiodothyronine to be approximately two to three times more potent than thyroxine. The dose levels required for maintenance of a myxedematous subject are in the range 50 to 100 micrograms for triiodothyronine and 200 to 300 micrograms for thyroxine. This relationship agrees satisfactorily with that deduced from comparison of C_{max} values, and can also be inferred by extrapolation of the graph (Figure 3).

TABLE III

Duplicate observations with triiodothyronine

Subject	Dose, μg.	Days between injections	Injection 1	Injection 2	Difference	
			Total calories		*Calories*	*Per cent*
3	600	26	4568	4100	468	10.2
4	100	34	1790	1838	48	2.5
5	100	42	1673	1595	78	4.4

The changes which were observed in protein-bound iodine following injections of triiodothyronine and thyroxine could reflect more rapid destruction of triiodothyronine, a larger volume of distribution, or both, than in the case of thyroxine. Preliminary studies with radioactive triiodothyronine suggest that both phenomena are involved. A wider distribution of triiodothyronine could easily explain its more immediate calorigenic effect. If one accepts the premise that a hormone is metabolized in the course of exerting its effect, more rapid destruction would be expected, which might also be compatible with a shorter duration of action. These comparisons are compatible with the suggestion of Gross, Pitt-Rivers and Trotter (8) that triiodothyronine may be the tissue form of thyroid hormone, but do not provide any direct proof of such a theory.

SUMMARY

The calorigenic effect of single intravenous doses of l-triiodothyronine and l-thyroxine has been compared in eight subjects having myxedema. Following administration of l-triiodothyronine the basal metabolism rose more quickly to a maximal value, and subsequently fell toward the base line more rapidly than following administration of l-thyroxine. The mean time elapsing between injection and maximal metabolic rate was 2.2 ± 0.1 days in the case of the former and 9.4 ± 0.9 days in the case of the latter. After a maximal value was reached, both curves decreased exponentially, the half-value time for triiodothyronine being 7.4 ± 0.6 and for thyroxine 11.9 ± 0.4 days. When compared on the basis of the maximal metabolic rate resulting from a given injection, triiodothyronine appeared to be three or four times as potent as thyroxine. On the basis of total calories added to the base line throughout the duration of action of each injection, the dose-response curves of the two substances were not parallel. On this basis, with small doses, triiodothyronine was more calorigenic than thyroxine but with larger doses (above about 10 micrograms per kilogram of body weight) thyroxine was more effective than triiodothyronine.

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