J. Physiol. (1955) 127, 352–357

A COMPARISON OF THYROXINE AND TRIIODOTHYRONINE AS INHIBITORS OF PITUITARY THYROTROPHIC HORMONE SECRETION IN THE RABBIT

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(*Received* 30 *July* 1954)

The compound 3:5:3′-triiodothyronine has been identified as a normal constituent of plasma and of the thyroid gland, and in many tests has been found to be markedly more active than thyroxine (Gross & Pitt-Rivers, 1953). The majority of investigations to date have been made on rats or man, and extension to other species seems desirable. In the present work, the ability of these compounds to reduce the rate of release of ^{131}I-labelled hormone from the thyroid gland of the normal rabbit has been investigated. In effect this is a measure of the ability to inhibit the secretion of thyrotrophic hormone by the anterior pituitary gland.

In addition, a technique is described which affords a convenient, rapid and repeatable method for the determination of thyroid activity in an individual rabbit in terms of the amount of thyroxine secreted per day, without the necessity for the administration of goitrogenic drugs.

EXPERIMENTAL METHODS

Adult female Chinchilla rabbits (weight 2·2–3·3 kg) were used. The animals were kept in a constant-temperature room (28° C) and fed a standard pellet diet (M.R.C. diet no. 18) and tap water *ad lib.* Thyroid 'release curves' were determined by twice daily counts over the thyroid region starting 48 hr after the subcutaneous injection of a tracer dose of ^{131}I, according to the technique previously described (Brown-Grant, von Euler, Harris & Reichlin, 1954). The only modifications were that a standard dose of 3 μc carrier-free ^{131}I was used, and that the initial count, 48 hr after injection, was taken as 100%, and subsequent counts, after correction for isotope decay, were expressed as a percentage of the initial value when plotting the release curves. The reliability of a 'neck count' with this technique is $\pm$2·5% (S.E. of mean of four 2 min counts) and includes variation due both to positioning of the animal and statistical errors of counting. For details of the apparatus and method the paper referred to above should be consulted.

Pure synthetic sodium L-thyroxine (THY.) and sodium L-triiodothyronine (T.I.T.) (prepared by Glaxo Laboratories Ltd., Greenford, Middlesex) were dissolved in 0·9% NaCl solution adjusted to pH 8·5 by the addition of 0·1 N-NaOH and administered by subcutaneous injection in the

dosage indicated in the text in volumes of 0·2–1·0 ml. Fresh solutions were prepared every second or third day during the experiments. In control experiments, 0·9% NaCl solution (pH 8·5) and KI dissolved in 0·9% NaCl solution were injected subcutaneously in volumes of 0·25–1·0 ml.

RESULTS

Administration of repeated daily doses of THY. and T.I.T.

Seven rabbits were used in fourteen experiments. After an initial control period, increasing daily doses of THY. or T.I.T. were given; the initial dose was usually 8 or 10 μg/day. The result was a progressive slowing in the rate of release of thyroidal radio-iodine, as the dose was increased by steps of

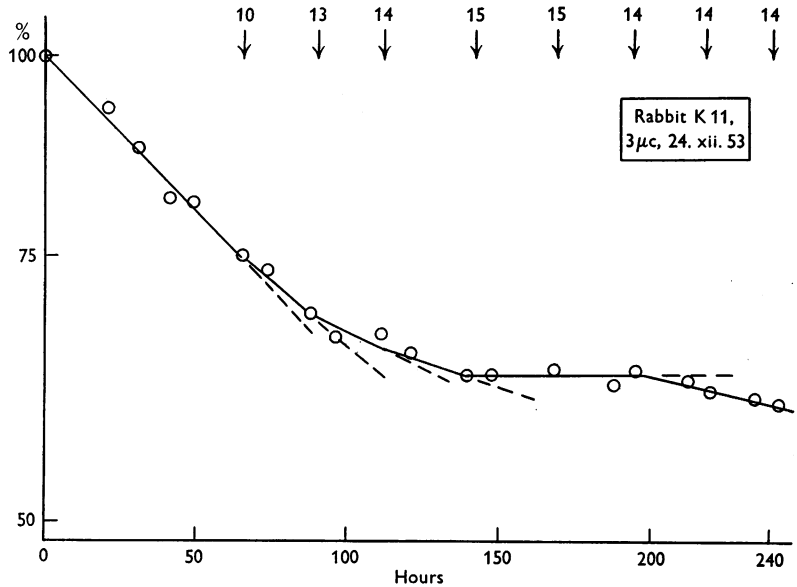

Fig. 1. The result of an experiment to determine the 'inhibitory dose' of thyroxine for a rabbit. Ordinate: thyroid counts as percentage of counting rate at 0 hr. Abscissa: time in hr. 0 hr is 48 hr after injection of ^{131}I. ↓ indicates subcutaneous injection of thyroxine. Figures refer to dose in μg injected.

2 or 4 μg/day. Complete inhibition was produced by doses of between 9 and 16 μg/day of T.I.T. and between 14 and 20 μg/day of THY. and was maintained by further daily injections of this dose for at least 48 hr. The daily dose was then reduced by steps of 1 or 2 μg/day until the release of radio-iodine was resumed (Fig. 1). In about half the experiments the daily dose was then increased again and a second period of complete inhibition produced.

It seems unlikely that the progressive slowing was related to a lag in effect of the increasing doses. The lag in onset of the effect of thyroxine after injection is only about 3 hr (Brown-Grant *et al.* 1954), and more important, the resumption of release when the dose was reduced was apparent within 12 or

24 hr in the present series of experiments, and the onset of the second period of inhibition was equally prompt when the dose was raised again.

In recording the results, the following method was adopted. In each experiment, a dose of THY. or T.I.T. was found that would produce complete inhibition of the release of ^{131}I from the thyroid, a dose 2 or 4 μg/day less having failed to produce this effect. After two or more days at this dose level, the amount injected daily was reduced by 1 or 2 μg when the release of ^{131}I began again. The inhibitory dose was taken as the average of these two doses, i.e. when 16 but not 14 μg/day produced complete inhibition the dose was recorded as 15 μg, and when 10 but not 9 μg/day produced inhibition, 9·5 μg was taken as the inhibiting dose.

TABLE 1. The 'inhibitory dose' in μg/day of THY. and T.I.T. for seven rabbits. The calculated daily secretion rate as μg THY./day/kg body wt. is also shown

Rabbit no.	Inhibitory dose (μg/day)		Secretion rate (μg THY./kg/day)
	T.I.T.	THY.	
1	13·5	19·0	6·45
2	13·5	15·0	6·50
3	13·5	19·0	6·33
4	14·0	15·5	5·96
5	13·5	15·5	6·33
6	9·5	14·5	5·18
7	13·5	17·5	5·47

Four rabbits were first given THY. and in a second experiment, T.I.T.; in the other three the order was reversed. The results of all experiments are given in Table 1. Calculation of the ratio of the daily dose of THY. and T.I.T. necessary just completely to inhibit each rabbit in the series gave a value of $1:1{\cdot}29 \pm 0{\cdot}06$ (S.E. of seven determinations) for the relative activities of THY. and T.I.T. on a weight basis and $1:1{\cdot}09 \pm 0{\cdot}05$ (S.E. of seven determinations) on a molar basis.

Control experiments

In a second group of experiments, the seven rabbits used in the first experiment were given control injections of 0·9% NaCl solution at pH 8·5 (1 ml. twice a day), two successive daily injections of 13 μg iodide and one injection of 26 μg iodide (as KI in 0·9% NaCl solution) during a release curve. The amounts of iodide injected were equivalent to and double the iodine content of the largest daily dose of thyroxine or triiodothyronine used (20 μg THY. = 13 μg I'). In no case was any effect on the rate of release of thyroidal ^{131}I observed following the injections of saline or of potassium iodide at this dosage.

DISCUSSION

The method of 'release curves' has been shown to give a reliable measurement of thyroid activity in the rabbit. The temporary complete inhibition of release of ^{131}I that follows the administration of thyroxine or triiodothyronine

is principally, if not entirely, due to a suppression of pituitary thyrotrophic hormone secretion.

By the method of repeated daily doses described, the 'inhibitory' dose level for THY. and T.I.T. has been determined in seven rabbits. The relative potencies by this technique were 1:1·29 (weight for weight) THY.:T.I.T.

From the data of these experiments, the thyroid activity of the rabbits (in μg of THY. secreted per day) was from 14·5 to 19·0 (Table 1). This range is in good agreement with the results of Maqsood (1950), who used the goitre prevention method and found a secretion rate of about 18 μg THY./day in the adult male rabbit.

While T.I.T. appears to be rather more potent than THY. in the experiments described in this paper, the difference in activity is far less than that reported by many other workers. Pitt-Rivers (1953) found that the ratio of THY.:T.I.T. activity was 1:5 in the rat-goitre prevention test, 1:4 in producing changes in the blood cholesterol level in human myxoedema, and 1:5 in restoring the growth rate in young thyroidectomized rats. Tomich & Woollet (1953) found the ratio 1:7·4 (rat-goitre prevention) and 1:5 (rat B.M.R.). Heming & Holtkamp (1953) found a 1:3·5 ratio (goitre prevention in rats), 1:3·5 (B.M.R. in thyroidectomized rats). In man, Lerman (1953) reports that T.I.T. is 4–5 times as effective as THY. in the treatment of human myxoedema, and Starr & Liebhold-Shoeck (1953) found it to be 10 times as effective in depressing thyroidal ^{131}I uptake in normal men.

In contrast to these findings, T.I.T. was observed to be only slightly more effective than THY. in the experiments on rabbits described in this paper. In agreement with the present findings, however, T.I.T. was found to be no more active than THY. in its action on the B.M.R. of normal, as opposed to *thyroidectomized*, rats by Heming & Holtkamp (1953). Gemmill (1953) found a slightly higher activity only (on a weight basis) for T.I.T. in the mouse anoxia test and equal activity in the *intact* mouse with respect to stimulation of the B.M.R. Bartlett, Burt, Folley & Rowland (1954) found that oral T.I.T. was relatively inactive when tested for its galactopoietic effect in lactating cows, and that injected subcutaneously T.I.T. was only slightly more effective, on a weight basis, than THY. Gilliland & Strudwick (1953) compared the effects of equimolecular doses of THY. and T.I.T. on the release of ^{131}I-labelled hormone from the thyroid gland of the chick. From their published results, the ratio of activity was not more than 1:2, THY.:T.I.T. and possibly as low as 1:1·3.

It appears, therefore, that when the relative activities of THY. and T.I.T. in maintaining a euthyroid state, as judged by various criteria (goitre prevention, B.M.R., restoration of growth, blood cholesterol changes) in the thyroid-deficient animal (thiouracil treated, thyroidectomized or myxoedematous), are compared, T.I.T. is strikingly more active. In the euthyroid

animal, on the other hand, T.I.T. is apparently only slightly more active as judged by its effects on O_2 consumption (rat and mouse), anoxia survival test (mouse), galactopoiesis (cow) and inhibition of the release of ^{131}I-labelled hormone (chick and rabbit). Starr & Liebhold-Shoeck (1953), however, found T.I.T. much more effective in depressing the ^{131}I uptake of the thyroid gland in man.

The distribution and metabolism of injected T.I.T. differs markedly from that of THY. (Deiss, Albright & Larson, 1953; Keating & Albert, 1953). Roche, Michel, Michel & Tata (1954) have shown that T.I.T. disappears from the blood more rapidly than THY., and that after ligature of the bile duct T.I.T. may be found as a glucuronide in the plasma earlier than THY. in the rat. Thyroxine glucuronide, which is formed in the liver, was found by Briggs, Taurog & Chaikoff (1953) to be less well absorbed from the gastro-intestinal tract than thyroxine in the rat. As the formation of a glucuronide is more marked after administration of a large unphysiological dose of thyroxine in the rat than after a tracer dose, these workers have suggested that this conjugation may represent a mechanism whereby the body can deal with an excess of thyroid hormone. The work of Roche *et al.* (1954) suggests that an excess of T.I.T. may be more effectively dealt with in this way than THY. Similar mechanisms may exist in other species, and this difference in dealing with large pharmacological doses may also apply to the metabolism of less excessive but still supernormal amounts of exogenous thyroxine or triiodothyronine. If this is so, an excess of T.I.T. might be more effectively dealt with than an excess of THY. This may account for the failure of experiments on normal euthyroid animals, dependent on the presence of an excess of thyroid hormone, to demonstrate the markedly higher activity of T.I.T. as compared with THY. which has been found after the administration of these compounds to hypothyroid or potentially thyroid-deficient animals in experiments where the restoration or maintenance of the euthyroid state is taken as an index of their activity.

SUMMARY

1. The relative abilities of thyroxine and triiodothyronine to depress pituitary thyrotrophic hormone secretion in the rabbit have been determined, using the rate of release of ^{131}I-labelled hormone from the thyroid gland as an index of thyroid activity. A simple method for the determination of the daily thyroid secretion rate in the rabbit is described. The thyroid secretion rate of adult, non-pregnant female rabbits at 28° C is approximately 6 μg thyroxine/kg body wt./day.

2. With repeated daily doses, a ratio of 1:1·29 (weight for weight) is found for the relative activity of thyroxine:triiodothyronine.

3. The possible significance of these findings is discussed.

My thanks are due to Prof. G. W. Harris, F.R.S., in whose department this work was performed, for laboratory facilities, and for valuable advice in the preparation of this paper. Thyroxine and triiodothyronine were generously supplied by Messrs Glaxo Laboratories Ltd., through the courtesy of Dr B. A. Hems. This work was performed during the tenure of a Medical Research Council Studentship which is gratefully acknowledged.

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