A MODIFIED BIOASSAY FOR THYROID-STIMULATING HORMONE AND LONG-ACTING THYROID STIMULATOR

J. NUTT, K. HUMPHREYS AND F. CLARK

Department of Medicine, Wellcome Research Laboratories, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP

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SUMMARY

After iodine depletion of mice, the time needed for the bioassay of long-acting thyroid stimulator (LATS) and thyroid-stimulating hormone (TSH) can be shortened to allow the completion of two assays per week. Suppression of mouse TSH with 0.1 µg tri-iodothyronine was necessary in the TSH assay and with 5 µg thyroxine in the LATS assay. These can be administered as a single i.p. injection with the ¹³¹I. The response of the assay mice to TSH can be improved by giving theophylline (0.5 mg) just before, with and after the stimulus.

INTRODUCTION

The long-acting thyroid stimulator (LATS) in serum is usually measured by the McKenzie bioassay (McKenzie, 1958a, b) which can also be used to detect raised levels of thyroid-stimulating hormone (TSH). The original method involved feeding mice on a low-iodine diet for 1 to 3 weeks and then treating them with thyroid hormone for 3 to 4 days before the assay. Shortened procedures have been reported by Bowers, Lee & Schally (1968) and Bowers & Schally (1970) for TSH and by Furth, Rathbun & Posillico (1969) for LATS. This report also describes modified, quicker bioassays for TSH and LATS and attempts to improve the sensitivity for TSH by the administration of theophylline.

METHODS

Female weanling mice, S.P.F. Ash Porton strain, were fed a low iodine diet (40 µg/kg) for 2 weeks before use. Initially the mice were given tap water, but in later experiments distilled water was used. The mouse thyroids were labelled by i.p. administration of 5 µCi carrier-free ¹³¹I (Radiochemical Centre, Amersham).

Suppression of endogenous TSH was assessed by recording whole blood radioactivity in groups of five mice for 72 h after the i.p. injection of 5 µCi ¹³¹I and s.c. injection of varying amounts of thyroxine (T₄ 0.2–10 µg) or tri-iodothyronine (T₃ 0.1–5 µg). The response of the mice to LATS and TSH was also assessed.

The influence of changes in the timing of the stimulus relative to that of thyroid labelling and the effect of treatment with T₄ or potassium iodide on responses to TSH or LATS were measured.
Thyroidal uptake of $^{131}$I was determined after the injection of 5 $\mu$Ci $^{131}$I and 5 $\mu$g T$_4$ or 0·1 $\mu$g T$_3$. Thyroidal radioactivity as a percentage of the total counts administered was calculated. The effect of administration of $^{131}$I and thyroid hormone i.p. as a single injection was compared with separate i.p. and s.c. injections.

**Assay procedure**

Carrier-free $^{131}$I was diluted with distilled water to a concentration of 50 $\mu$Ci/ml. Thyroxine (5 mg; Koch-Light) was dissolved in 20 ml distilled water, together with a few drops of 1 m-sodium hydroxide, and the volume was made up to 100 ml with distilled water. Tri-iodothyronine was prepared similarly except that 0·1 $\%$ bovine serum albumin (BSA) was also added, and the solution was diluted with 0·9 $\%$ NaCl solution to a concentration of 1 $\mu$g T$_3$/ml before mixing with an equal volume of $^{131}$I diluted as above.

Mice were injected with 0·1 ml $^{131}$I (5 $\mu$Ci) i.p. and 0·1 ml T$_4$ (5 $\mu$g) s.c. or 0·2 ml of a combination of T$_3$ and $^{131}$I (5 $\mu$Ci $^{131}$I and 0·1 $\mu$g T$_3$) i.p.

After 24 h, 100 $\mu$l venous blood samples were withdrawn. Standard solutions of the stimulators were given by i.p. injection. Normal human serum (deactivated for 1 h at 56 $^\circ$C) was used as a control injection. The LATS standards were heat-deactivated serum samples from patients with pretibial myxoedema. The TSH standards were prepared from the M.R.C. Standard Bovine TSH in saline containing 1 $\%$ normal human serum. Five mice were used for each sample.

For measurement of the responses to TSH and LATS, mice were bled between 2 and 3 h and 24 h respectively, after injection. All blood samples were then counted for $^{131}$I content and the response expressed as:

$$\frac{\text{radioactive count in blood at 2 or 24 h}}{\text{radioactive count in blood at 0 h}} \times 100 \%.$$  

**Effect of theophylline**

Theophylline (as aminophylline) was dissolved in 0·9 $\%$ NaCl solution to give 5 or 10 mg/ml for intraperitoneal injection. Theophylline at a concentration of 11 mmol/l could not be administered orally. Injections of theophylline were given either alone (0·1 ml, i.p.) or with TSH (0·2 ml). The effect of the number (2 to 4), dosage (0·5 mg or 1 mg) and timing of the injections of theophylline was investigated.

**RESULTS**

**Effect of administration of thyroid hormones on bioassay of TSH and LATS**

In the McKenzie bioassay of TSH and LATS, endogenous TSH is suppressed by administration of thyroid hormone during the period between the administration of $^{131}$I and the test stimulus. Without this suppression there was a spontaneous rise in blood radioactivity within 24 h (Fig. 1), and a high ‘zero’ blood count so that the subsequent assay response to TSH and LATS was diminished (TSH from 653 to 137 $\%$ and LATS from 3520 to 143 $\%$). Most of the blood radioactivity present at 6 and 48 h after injection of $^{131}$I was protein bound (85 and 97 $\%$, respectively) indicating incorporation into thyroid hormones.
After the injection of 10 µg thyroxine, the blood radioactivity fell to a low stable level within 24 h and remained static for 72 h. (Fig. 1). Identical results were obtained with 5 µg thyroxine. It is, therefore, possible to inject TSH or LATS within 24 h of ¹³¹I and T₄, and still measure the 24 h response to LATS. After suppression with 5 µg thyroxine, an improved response to TSH was obtained (P < 0.05) when it was injected at 24 h as compared with 72 h. When LATS was administered 24 or 72 h after T₄, no significant difference in response was found (Fig. 2). When this shortened

![Graph showing blood radioactivity in mice after injection of ¹³¹I alone or with thyroxine and the subsequent effect of injecting normal serum, TSH, or LATS on blood ¹³¹I levels. Figures in parentheses show time after administration of stimulus. The vertical lines indicate the s.e.m. Horizontal bar indicates time of injection of normal serum, TSH or LATS.](image)

method using 5 µg T₄ was compared with the technique of Major & Munro (1962) which involves two s.c. injections of 10 µg T₄ at an interval of 48 h, similar responses to TSH and LATS were obtained in both systems. The responses obtained in the short and long assays with 1 mu. TSH were 428.8 ± 102.4% (s.e.m.) and 227.8 ± 36.6% respectively and with LATS (0.1 ml) were 811.6 ± 280.1% and 452.3 ± 99.8% respectively.

The radioactivity in mouse blood after smaller doses of thyroid hormones (0.2 µg, 1.0 µg and 5.0 µg) also decreased to a minimum by 24 h. The responses to control serum, TSH and LATS are shown in Table 1. The poor response to the low dose of T₄ is due to inadequate suppression and the apparently superior response to LATS using the low dose of T₃ is due to a spontaneous rise in blood radioactivity. Subse-
sequently the smallest dose of thyroid hormone adequate to cause suppression (0.1 µg T₃) was used for TSH (Table 2).

The effect of administration of potassium iodide (3 µg) at the same time as ¹³¹I was examined: raised basal blood radioactivity levels and diminished responses to TSH and LATS were found (Fig. 3).

Thyroid suppression by separate injections of ¹³¹I (i.p.) and T₃ or T₄ (s.c.) was identical to the combined intraperitoneal injection. No significant difference in responses to TSH or LATS was detected (Table 2).

Effect of theophylline on bioassay of TSH

No effect on the response to TSH was obtained when four injections of 1 mg theophylline were given at 12-h intervals, the final injection being given with T₃ and ¹³¹I. Similarly, no enhancement in response was obtained when 0.5 mg theophylline was administered simultaneously with the TSH after pretreatment with theophylline. However, the iodine uptake in the mouse thyroid was decreased in this

![Graph](image-url)
instance from 50 to 22%. A single dose of theophylline (1 or 2 mg) given with TSH also had no effect on the response.

When three injections of 1 mg theophylline were given to the assay mice, 30 min before TSH, simultaneously with TSH, and 30 min after TSH, an increased response was frequently obtained particularly with doses of above 0-05 mu. TSH (results with 0-1 mu. TSH were significant: $P < 0-02$). A similar response was also obtained with three doses of 0-5 mg theophylline as shown in Fig. 4, and also when the third injection of theophylline was omitted.

Table 1. Percentage response (means ± s.e.m.) at 2 and 24 h to normal serum, bovine thyroid-stimulating hormone (TSH) or long-acting thyroid stimulator (LATS) administered 24 h after injection of $^{131}$I and various doses of tri-iodothyronine ($T_3$) and thyroxine ($T_4$)

| Stimulus and period between its administration and sampling (h) | Dose of thyroid hormone |
|---|---|---|---|---|---|
| | 0.2 µg $T_4$ | 0.2 µg $T_3$ | 1 µg $T_4$ | 1 µg $T_3$ | 5 µg $T_4$ | 5 µg $T_3$ |
| Normal serum 2 | 105.7 ± 12.5 | 79.1 ± 6.1 | 89.5 ± 6.3 | 94.8 ± 8.2 | 87.2 ± 9.9 | 85.5 ± 7.2 |
| 24 | 255.5 ± 21.0 | 892.8 ± 224.3 | 157.8 ± 25.3 | 126.4 ± 16.4 | 78.7 ± 12.6 | 108.6 ± 11.7 |
| 5 mu. TSH 2 | 281.2 ± 16.2 | 688.1 ± 176.1 | 1293.1 ± 131.9 | 1231.5 ± 261.1 | 1113.5 ± 113.4 | 1092.7 ± 76.3 |
| 24 | 131.6 ± 24.9 | 299.3 ± 55.2 | 411.5 ± 48.0 | 315.4 ± 41.1 | 303.4 ± 27.6 | 218.5 ± 12.6 |
| 0.2 ml. LATS 2 | 202.6 ± 57.2 | 393.9 ± 55.2 | 355.9 ± 80.0 | 293.4 ± 51.3 | 317.5 ± 52.0 | 486.2 ± 119.7 |
| 24 | 765.4 ± 333.6 | 3064.2 ± 655.0 | 2634.2 ± 660.8 | 1929.4 ± 388.7 | 1776.1 ± 148.7 | 2260.9 ± 202.9 |

Table 2. Response to normal serum and thyroid-stimulating hormone (TSH) (0.5 mu.) given 24 h after injection of $^{131}$I (i.p.) and 0.1 µg tri-iodothyronine ($T_3$) (s.c.) or as a single i.p. injection, and responses to long-acting thyroid stimulator (LATS) and normal serum after injection of $^{131}$I and 5 µg thyroxine ($T_4$)

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Preparation and route of injection</th>
<th>Period of response (h)</th>
<th>Response (%) (means ± s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum</td>
<td>$T_p$, s.c.; $^{131}$I, i.p.</td>
<td>2</td>
<td>78.6 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>$T_p + ^{131}$I, i.p.</td>
<td>2</td>
<td>109.1 ± 7.8</td>
</tr>
<tr>
<td>TSH</td>
<td>$T_p$, s.c.; $^{131}$I, i.p.</td>
<td>2</td>
<td>437.4 ± 69.5</td>
</tr>
<tr>
<td></td>
<td>$T_p + ^{131}$I, i.p.</td>
<td>2</td>
<td>384.0 ± 81.0</td>
</tr>
<tr>
<td>Normal serum</td>
<td>$T_p$, s.c.; $^{131}$I, i.p.</td>
<td>24</td>
<td>95.3 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>$T_p + ^{131}$I, i.p.</td>
<td>24</td>
<td>46.9 ± 1.6</td>
</tr>
<tr>
<td>LATS</td>
<td>$T_p$, s.c.; $^{131}$I, i.p.</td>
<td>24</td>
<td>1645.8 ± 157.7</td>
</tr>
<tr>
<td></td>
<td>$T_p + ^{131}$I, i.p.</td>
<td>24</td>
<td>1253.2 ± 169.3</td>
</tr>
</tbody>
</table>
Fig. 3. Radioactivity in mouse blood, at various times (h) (a) after administration of $^{131}$I and 5 μg thyroxine (hatched bars) or 3 μg potassium iodide (white bars) showing the effect of normal serum (control), thyroid-stimulating hormone (TSH) and long-acting thyroid stimulator (LATS). (b) Figures show the times (h) after administration of the stimulus that percentage response was determined. Vertical lines indicate ±S.E.M. Figures above bars are percentage response of zero bleed (means ± S.E.M.).
DISCUSSION

Theoretically, maximum labelling of the mouse thyroid gland with radioactive iodine to increase the specific activity of stored hormones should produce greater responses to TSH and LATS. Using $^{125}$I there is some evidence that this is so (McKenzie & Williamson, 1966). In the present study, thyroidal uptake of $^{131}$I increased from $8.8 \pm 1.7\%$ before iodine depletion to $27.2 \pm 2.9\%$ after a week on the low iodine diet. A higher mean uptake (65%) was obtained in more recent TSH assays after 3 weeks on the low iodine diet.

Administration of lower doses of thyroid hormone produced inadequately prolonged suppression for the LATS assay but was suitable for the shorter TSH assay. Bowers et al. (1968) found 0.1 $\mu$g T$_3$ to be satisfactory. Florsheim, Williams & Schönbaum (1970) gave T$_4$ in drinking water for 4 days before assay; at the lowest level studied (0.67 $\mu$g T$_4$/ml), sensitivity to TSH was impaired, whereas at the highest level (18 $\mu$g T$_4$/ml) responses to LATS were reduced. Sharard, Purves & Cague (1970) reported that 1.0 $\mu$g T$_4$/day for 3 days was the optimum dose for the assay of both LATS and TSH. These authors suggested that the impaired response seen with higher doses of T$_4$ may be due to liberated iodide producing a Wolff-Chaikoff effect.
In the present experiments the use of iodide impaired the responses. Ochi & De Groot (1969) also found inhibition of release by pretreatment with potassium iodide for 24 h.

A delay in administration of the stimulus after injection of $^{131}$I and thyroid hormone appeared to be unnecessary, and with TSH a better response was obtained the sooner the stimulus injection was administered. No significant difference in responses to TSH or LATS was found when the thyroid hormone and $^{131}$I were given as a single i.p. injection and this method of preparation of the assay mice was adopted.

The average index of precision ($\lambda$) obtained by McKenzie (1958b) in the bioassay of TSH was 0.24. In the shortened TSH assay a mean (10 values) of 0.28 was obtained, with a range from 0.084 to 0.462, and for LATS a mean (11 values) of 0.27 with a range 0.125 to 0.462.

Bastomsky & McKenzie (1967) reported that simultaneous administration of TSH and theophylline increased thyroidal uptake of radioactive iodine and also increased blood radioactivity after the administration of TSH and theophylline. Further work by Bastomsky & McKenzie (1968) and Kapitola, Schreiberova & Schullerova (1970) has shown potentiation by theophylline of TSH and LATS on thyroidal uptake of radioactive iodine. In our modified McKenzie bioassay, three injections of theophylline given at 30-min intervals, TSH being given with the second injection, led to a significant increase in response.

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REFERENCES


