EFFECT OF THYROTROPHIN RELEASING HORMONE AND THYROID-STIMULATING HORMONE ON SERUM PROTEIN-BOUND $^{131}$I

By

Egil Haug, Harald Frey and Terje Sand

ABSTRACT

Seventeen subjects without any clinical or laboratory evidence of thyroidal or pituitary disease were given 1.0 mg thyrotrophin-releasing hormone (TRH) as a rapid iv injection 48 hours after an oral dose of 50 $\mu$Ci $^{131}$I. In all subjects there was a clear rise in serum PB$^{131}$I. The elevation in the mean serum PB$^{131}$I was significant ($P < 0.01$) one hour after TRH, and the mean peak response was noted at 4 hours. It is suggested that this elevation in serum PB$^{131}$I following TRH administration reflects the effect of the TSH released. In order to find the most suitable method of administration, 1.0 mg TRH was given iv, im, or as a 1 hour infusion. The maximal responses seemed to be independent of the mode of administration. Six subjects were given 3.0 mg TRH iv and 4 others 6.0 mg TRH iv. It was not possible to demonstrate a clear dose-response relationship. In five subjects the serum PB$^{127}$I and the serum PB$^{131}$I were measured at the same times following administration of TRH. This showed that the serum PB$^{131}$I was a more sensitive index of TSH release than the serum PB$^{127}$I. Twenty-four hours after the TRH injection the same subjects were given 5 IU TSH as a rapid iv or im injection. All subjects responded with a significant rise in serum PB$^{131}$I. In the subjects who did not respond to TRH the response to TSH allows the differentiation between pituitary and thyroid disease.

Intravenous injections of synthetic thyrotrophin-releasing hormone (TRH) have been shown to produce a significant rise in serum thyroid-stimulating hormone (TSH) in normal subjects (Bowers et al. 1970; Hall et al. 1970; Fleischer et al.)
1970; Hershman & Pittman 1970; von zur Mühlen et al. 1970). At present only a few laboratories use the radioimmunoassay for human TSH, and therefore the TRH tests described (Hall et al. 1970; Ormston et al. 1971; Hershman & Pittman 1971; Karlberg et al. 1971) are available only to a limited number of hospitals. The increase in serum protein-bound \(^{125}\)I (PBI) following TRH injections are too small and unreliable to be used as an index of TSH release (Hershman & Pittman 1971; von zur Mühlen et al. 1971; Karlberg et al. 1971).

We have developed a method for studying the effect of exogenous TRH in man by measuring the increase of protein-bound \(^{131}\)I (PB\(^{131}\)I) in the serum. This indirect test is simple and available to most hospital laboratories, and is more sensitive than the measurements of PBI.

**MATERIALS AND METHODS**

Volunteer patients hospitalized in the Medical Department B, Aker Hospital were studied. All the subjects were without clinical and laboratory evidence of any pituitary or thyroidal disease. The subjects were not required to undergo any preparation before the test.

*Thyrotrophin-releasing hormone*

Synthetic TRH (pyroglutamyl-histidyl-proline-amide) supplied by Farbwereke Hoechst AG was used in this study. The TRH was delivered in ampoules containing 0.5 mg as dry powder. Immediately before use the powder was dissolved in distilled water. The TSH preparation used was Actyron\(^{\text{®}}\) (Ferring).

**Determination of serum protein-bound \(^{131}\)I**

Two different methods have been used for the determination of PB\(^{131}\)I. For both methods 10 ml of venous blood is sufficient.

1. 4 ml of 12.5 % trichloracetic acid (TCA) was added to 4 ml of serum during careful mixing. After centrifugation (15000 \(\times g\)) for 5 min the supernatant fluid was removed and the precipitate counted for 30 min in a NUKAB autogamma scintillation counter. (The PB\(^{131}\)I fraction is found in the precipitate).

2. 180 mg dried Dowex exchange resin (Dowex 1,\(\times 10\)) was added to 4 ml of serum and rotated for 2 min in a Hetomix rotator. After rotation the resin was allowed to precipitate and the supernatant fluid (3.5 ml) was counted for 30 min in the same scintillation counter. (The PB\(^{131}\)I fraction is found in the supernatant).

In order to compare the two different methods for the determination of PB\(^{131}\)I, known amounts of \(^{131}\)I- or \(^{125}\)I-thyroxine were added to the serum samples. The results are shown in Table 1. The PB\(^{131}\)I fraction separated by the TCA precipitation is composed of 96 % of the organic bound \(^{131}\)I- and 40 to 45 % of the inorganic bound \(^{131}\)I- in the serum. With the resin method 85 % of the protein-bound \(^{131}\)I and 3 % of the inorganic \(^{131}\)I- remains in the (supernatant) PB\(^{131}\)I fraction.

Thus, the second method seemed to be more suitable for the present purpose, and all the results given refer to the Dowex resin method.
**Table 1.**
Serum $^{131}$I- and serum $^{125}$I-thyroxine precipitated by TCA and Dowex ion exchange resin.

<table>
<thead>
<tr>
<th>Method</th>
<th>$^{131}$I-</th>
<th>$^{125}$I-thyroxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCA</td>
<td>40-45%</td>
<td>96%</td>
</tr>
<tr>
<td>Dowex 1,X10</td>
<td>97%</td>
<td>15%</td>
</tr>
</tbody>
</table>

**TRH and TSH stimulation test**

An oral dose of 50 μCi $^{131}$I- was given at 9 a.m. An indwelling plastic cannula was inserted into a forarm vein 48 hours later. Blood samples were drawn at zero time and then 1, 2, 3, 4, 6 and 24 hours respectively after the TRH injection. The dose of TRH was 1, 3 or 6 mg given as a rapid iv injection. One mg of TRH was also given im to other subjects, or infused in 500 ml of saline over a period of 60 min. The response is expressed as counts/4 ml serum/30 min.

Five IU of TSH was given iv or im 72 hours following the oral dose of $^{131}$I-. Blood samples were drawn at zero time and 2, 4, 6, 10 and 24 hours respectively after the TSH injection.

**RESULTS**

**The PB$^{131}$I response to TRH**

Seventeen subjects (11 females and 6 males) were given 1.0 mg of TRH as a rapid iv injection. In all subjects there was a rise in serum PB$^{131}$I. The response curve is shown in Fig. 1. The peak values occurred at 2 hours in 3, at 3 hours in 7, at 4 hours in 4, at 6 hours in 2 and at 24 hours in 1 subject. The magnitude of the zero values was in the order of 500 to 2500 counts/4 ml serum/30 min. The peak responses varied from 288 to 2016 $\Delta$ counts/4 ml serum/30 min. All the values are corrected for back-ground activity. The peak values were from 118 % to 204 % of the zero values. The mean percentage response was 154 %.

The rise in the mean serum PB$^{131}$I was significant ($P < 0.01$) one hour after the TRH injection. The mean peak response was noted at 4 hours. Thereafter the mean values gradually declined over the next 20 hours without quite reaching the baseline again.

In order to find the most suitable method of administration, 1 mg of TRH was given im, iv, or as a 1 hour infusion. The results are presented in Fig. 2. The means ($\Delta$ counts/4 ml serum/30 min) for the maximal increments over zero values were: Im 634 (sem = 260), iv 647 (sem = 119), and for infusions
Euthyroid subjects

Fig. 1.
Response of serum PB$^{131}$I to intravenous TRH (1.0 mg).

753 (sem = 222). The mean peak response occurred at 4 hours following iv administration and infusions, and at 3 hours following im injections.

Six subjects were given 3.0 mg of TRH iv and 4 others 6.0 mg of TRH iv. It was not possible to demonstrate clear dose-response relationship. The highest doses of TRH, however, had a tendency to produce the highest response in serum PB$^{131}$I.

The PB$^{131}$ response to TSH

The results are illustrated in Fig. 3. Eleven subjects (6 females and 5 males) were given 5 IU TSH iv, and in all subjects there was a rise in serum PB$^{131}$I. The mean increments were significant (P < 0.01) at 2 hours. The mean values gradually increased over the next 22 hours with mean peak value at 24 hours following the injection. Nine subjects (5 females and 4 males) were given 5 IU im. All the subjects responded with a rise in serum PB$^{131}$I. The rise was significant (P < 0.01) 4 hours after the administration of the drug. The mean peak response occurred at 10 hours with a slight decrease over the next 14 hours.
Euthyroid subjects

![Graph showing response of serum PB¹³¹I to intravenous, intramuscular, or infused TRH (1.0 mg).]

Response of serum PB¹³¹I to intravenous, intramuscular, or infused TRH (1.0 mg).

**Side effects**

The side effects were mild and transient and occurred in about one half of the subjects. Most common were mild nausea, a flushing sensation, and a peculiar taste. A few subjects described a temporary desire to micturate. The onset of the side effects was rapid, and they lasted for only 5 min. The side effects seemed to be more frequent with the highest doses of TRH.

**The PBI response to TRH**

In 5 subjects the PBI and PB¹³¹I were measured at the same times following 1 mg of TRH given intravenously. The results are shown in Table 2. There was a clear rise in serum PB¹³¹I in all the subjects. Three subjects showed a rise in serum PBI, while two had a fall in serum PBI after TRH.

**DISCUSSION**

It is well established that synthetic TRH given intravenously produces an immediate and consistent rise in serum TSH in normal subjects (Bowers et al. 1970; Hall et al. 1970; Fleischer et al. 1970; Hershman & Pittman 1970; von
Euthyroid subjects

Response of serum PB\(^{131}\)I to intravenous or intramuscular TSH (5 IU).

*zur Mühlen et al. 1970*. The peak value occurred between 15 and 60 min after TRH administration (*Bowers et al. 1970; Hershman & Pittman 1970; Ormston et al. 1971; Karlberg et al. 1971*) and the rise was detectable within 10 min (*Karlberg et al. 1971*). *Einhorn & Larsson* (1959) measured the PB\(^{131}\)I after a single intramuscular injection of TSH. The rate of thyroid hormone release was significantly increased after 90 min and did not return to the pre-TSH rate for at least 24 hours. The release of hormone was greatest between 2 and 4 hours following the TSH injection.

In this study the rise in serum PB\(^{131}\)I was significant 1 hour after the TRH administration, and the peak value occurred at 4 hours. This time course of the PB\(^{131}\)I response agrees well with the above mentioned findings. It is also known from the work of *Einhorn & Larsson* (1959) that the release of thyroid hormone increases with increasing doses of TSH. We suggest therefore that the PB\(^{131}\)I elevation after TRH administration reflects the effect of the TSH released from the anterior pituitary.

TSH administration leads to a prompt release of inorganic iodide as well as hormonal iodine from the thyroid gland (*Rosenberg et al. 1961*). The
Response of serum PB131I and serum PBI to intravenously administered TRH (1.0 mg).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>TRH (mg)</th>
<th>PB131I (Δ counts)</th>
<th>PBI (μg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Hours) 0 2 3 4</td>
<td>(Hours) 0 2 3 4</td>
</tr>
<tr>
<td>A. W.</td>
<td>F</td>
<td>1.0</td>
<td>– 436 772 994</td>
<td>5.6 6.7 6.7 6.4</td>
</tr>
<tr>
<td>E. A.</td>
<td>F</td>
<td>1.0</td>
<td>– 231 279 207</td>
<td>7.8 7.1 3.5 8.4</td>
</tr>
<tr>
<td>L. E.</td>
<td>F</td>
<td>1.0</td>
<td>– 289 140 252</td>
<td>3.2 4.4 2.9 2.4</td>
</tr>
<tr>
<td>E. M.</td>
<td>M</td>
<td>1.0</td>
<td>– 341 500 587</td>
<td>8.6 6.7 6.7 6.4</td>
</tr>
<tr>
<td>H. M.</td>
<td>M</td>
<td>1.0</td>
<td>– 1291 1655 2016</td>
<td>7.9 7.1 7.0 7.6</td>
</tr>
</tbody>
</table>

rate of release of 131I- from the gland exceeded the entry rate from the plasma, and consequently masked the familiar increased disappearance of 131I- in the plasma for the first 12 to 24 hours following TSH administration (Deiss et al. 1958; Halmi et al. 1961).

Since there are fluctuations in the plasma concentration of 113I-, the TCA precipitation method can give false results because of the varying amounts of co-precipitated 113I-. The Dowex resin method, however, is not influenced by these fluctuations since only 3% of the plasma 131I- appears in the PB131I fraction.

Elevated values of serum PB131I after TRH injections confirm the integrity of the anterior pituitary-thyroid axis. For this reason the TRH test may be helpful in distinguishing between hypothalamic and pituitary lesions. Non-responders, however, may have primary as well as secondary hypothyroidism. In order to differentiate between pituitary and thyroid disease, an injection of 5 IU TSH is given 24 hours after the administration of TRH (Fig. 3).

The commonly used thyrotrophin stimulation tests are time consuming (Fore & Wynn 1966; Taunton et al. 1965; Burke 1968). Our method using 5 IU TSH in one injection and serum PB131I at zero time and also 24 hours after the injection, is probably just as sensitive and reliable. We have therefore adopted this procedure as our test for thyrotrophin stimulation.

We found an increase in serum PBI in 3 out of 5 normal subjects, while the 2 other subjects showed a fall in PBI. Karlberg et al. (1971) and von zur Mühlen et al. (1971) reported a small rise in the serum PBI and the serum thyroxine in some normal subjects tested, while in others there was no rise following TRH administration. Hershman & Pittman (1971) found no rise in serum PBI or thyroxine. The doses used were between 0.1 to 1.0 mg given intravenously. With these doses of TRH the serum PBI and thyroxine are
not of value as an index of the TSH release following the administration of TRH.

In all the subjects tested we found a rise in the serum PB\textsuperscript{131}I after TRH administration. Included in this group were 5 subjects who were also tested for rise in the serum PBI. These results seem to demonstrate that the serum PB\textsuperscript{131}I is a more sensitive parameter of thyroidal hormone release than serum PBI.

On the basis of our experience with the TRH test described, we have adopted the following test procedure:

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Draw serum for the thyroxine determination and the T\textsubscript{3} test. Give 50 μCi \textsuperscript{131}I.</td>
</tr>
<tr>
<td>1</td>
<td>Thyroid uptake determination (if required).</td>
</tr>
<tr>
<td>24</td>
<td>Thyroid uptake determination (if required).</td>
</tr>
<tr>
<td>48</td>
<td>Thyroid uptake determination (if required). Draw serum for PB\textsuperscript{131}I determination, and give TRH 1.0 mg iv.</td>
</tr>
<tr>
<td>51</td>
<td>Draw serum for PB\textsuperscript{131}I determination.</td>
</tr>
<tr>
<td>52</td>
<td>Draw serum for PB\textsuperscript{131}I determination.</td>
</tr>
<tr>
<td>72</td>
<td>Draw serum for PB\textsuperscript{131}I determination, and give 5 IU TSH iv or im.</td>
</tr>
<tr>
<td>96</td>
<td>Draw serum for PB\textsuperscript{131}I determination.</td>
</tr>
</tbody>
</table>

This simple procedure is available in most hospital laboratories, and may be performed on out-patients.

ACKNOWLEDGMENTS

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REFERENCES


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