EFFECT OF SYNTHETIC THYROTROPHIN RELEASING FACTOR ON PROLACTIN AND LUTEINIZING HORMONE SECRETION IN MALE AND FEMALE RATS DURING VARIOUS REPRODUCTIVE STATES

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(Received 27 March 1975)

SUMMARY

The effect of synthetic thyrotrophin releasing factor (TRF) on serum prolactin and LH concentrations was determined by radioimmunoassay in male, cyclic and pseudopregnant female rats. A solution of TRF (0.1, 0.25, 0.5 and 1 μg/rat) was injected i.v. at 17.00 h into rats pretreated with sodium pentobarbitone at 13.00 h. A group of male rats was also treated with TRF at 11.00 h after pretreatment with sodium pentobarbitone at 07.00 h. Fifteen minutes after TRF administration, blood samples were obtained by heart puncture. Doses of 0.25, 0.5 and 1 μg TRF significantly increased the serum prolactin concentration in pro-oestrous rats. The mean serum prolactin level after the injection of 0.5 and 1 μg into oestrous rats and 0.5 μg TRF into dioestrous day 2 rats, was significantly greater than the control values. Injection of TRF on day 1 of dioestrus had no effect. Serum LH concentration was not significantly modified by the various doses of TRF administered. On day 3 of pseudopregnancy a significant increase of serum prolactin values was obtained with 0.5 and 1 μg TRF. On day 7 of pseudopregnancy a dose of 0.5 μg produced the same effect, but on day 10 of pseudopregnancy only 1 μg TRF significantly increased serum prolactin levels when compared with the control rats. In male rats serum prolactin concentration was significantly greater than the control values after TRF treatment either in the morning or the afternoon. The response was similar to that obtained in pro-oestrous rats. The results suggest that the ability of synthetic TRF to stimulate prolactin release exists in both female and male rats and that TRF does not affect LH secretion.

INTRODUCTION

It is now well established that synthetic thyrotrophin releasing factor (TRF) affects the release of prolactin in several species, man (Jacobs, Snyder, Wilber, Utiger & Daughaday, 1971), lactating cows (Convey, Tucker, Smith & Zolman, 1972), lambs (Davis & Borger, 1973) and sheep (Davis & Borger, 1972). Deis & Alonso (1973) demonstrated an effect of TRF on prolactin release in pro-oestrous rats confirming the in-vitro findings of Tashjian, Barowsky & Jensen (1971). The present study was undertaken to assess the effect of TRF on serum prolactin and luteinizing hormone (LH) concentrations in cyclic and pseudopregnant female rats and male rats. The results were briefly reported at the XI Latin American Congress of Physiological Sciences, Mendoza, Argentina (Alonso & Deis, 1973).
Virgin white rats weighing between 200 and 250 g were used. The rats were kept in a constant temperature room (24 °C) with a lighting schedule of 14 h light (06.00–20.00 h): 10 h darkness. Serum prolactin and LH were determined in normal female rats at various stages of the oestrous cycle after following their cycle by means of vaginal smears for two or more regular 4 day cycles. Serum prolactin, but not LH, was also measured in normal male rats and in pseudopregnant rats on days 3, 7 and 10 of pseudopregnancy. Vaginal stimulation was carried out for 1 min between 10.00 and 10.15 h on the first day of oestrus with a glass rod connected to a dental drill.

On the day of the experiment 20 µl of physiological saline containing various concentrations of TRF (kindly supplied by Hoechst, Argentina) were injected into the jugular vein at 17.00 h. Control rats were injected with the same volume of physiological saline. As in the previous study (Deis & Alonso, 1973) the females and a group of male rats were treated with 4 mg sodium pentobarbitone (Nembutal; Abbott, Argentina)/100 g body wt, i.p. at 13.00 h in order to prevent any spontaneous release of prolactin and LH (especially in pro-oestrous rats). At the time of TRF or saline administration, light ether anaesthesia was induced in order to allow rapid injection of the substances into the jugular vein; this operation took less than 30 s. Fifteen minutes after the injection (17.15 h), blood samples were obtained by heart puncture. Another group of male rats was injected with saline (control rats) or TRF at 11.00 h after being treated with sodium pentobarbitone at 07.00 h. Blood samples were obtained at 11.15 h. Serum prolactin and LH from individual rats were assayed at two dose levels by a double antibody radioimmunoassay (Monroe, Rebar, Gay & Midgley, 1969; Niswender, Chen, Midgley, Meites & Ellis, 1969). The sensitivity of the assay for LH ranged from 7·9 to 125 ng/sample. The results are expressed in terms of the NIAMDD-rat-prolactin RP-1 standard (biological potency equivalent to 11 i.u./mg; see Vermouth & Deis, 1974) and the NIAMDD-rat-LH RP-1 standard (biological potency equivalent to 0·03 x NIH-LH-S1). All serum samples were assayed in a single radioimmunoassay to eliminate assay to assay variation. Student’s t-test was used to assess the level of significance.

RESULTS

Effect of TRF on serum prolactin and LH levels in cyclic rats (Fig. 1)

A significant increase in serum prolactin concentration was observed in the pro-oestrous rats treated with 0·25 (P < 0·01), 0·5 (P < 0·02) or 1 µg (P < 0·001) TRF. The lowest dose of 0·1 µg did not induce prolactin release. In oestrous rats both doses of 0·25 and 0·5 µg TRF induced a significant (P < 0·01) increase in serum prolactin levels. In dioestrous day 2 rats, 0·5 µg TRF significantly increased prolactin values (P < 0·05) but in dioestrous day 1 rats the same dose had no effect.

As shown in Table 1, TRF had no significant effect on serum LH concentrations in any of the treated groups.

Effect of TRF on serum prolactin levels in male rats (Fig. 2)

Significant increases in serum prolactin levels were observed 15 min after administration of 0·5 (P < 0·05) or 1 µg (P < 0·01) TRF to male rats at 11.00 h. The same effect was observed when TRF was administered in the afternoon (17.00 h). The mean serum prolactin concentration after injection of 0·25, 0·5 or 1 µg TRF was significantly greater than the control values (P < 0·05; P < 0·001 and P < 0·001 respectively).
Table 1. Serum LH concentration (ng/ml) in normal cyclic female rats after thyrotrophin releasing factor (TRF) treatment (means ± S.E.M.)

<table>
<thead>
<tr>
<th>Day of treatment</th>
<th>Control (saline-treated)</th>
<th>Dose of TRF (µg)</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-oestrus</td>
<td>118.3±9.6 (6)</td>
<td>95.8±5.3 (9)</td>
<td>110.2±11.7 (6)</td>
<td>106.1±3.2 (7)</td>
<td></td>
</tr>
<tr>
<td>Oestrus</td>
<td>101.3±9.5 (9)</td>
<td>—</td>
<td>98.8±2.4 (5)</td>
<td>115.0±14.7 (5)</td>
<td></td>
</tr>
<tr>
<td>Dioestrus day 1</td>
<td>90.2±2.8 (5)</td>
<td>—</td>
<td>86.8±7.3 (5)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Dioestrus day 2</td>
<td>101.1±10.8 (6)</td>
<td>—</td>
<td>98.3±3.0 (6)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Effect of thyrotrophin releasing factor (TRF) on prolactin secretion in female rats during the oestrous cycle. Each column and horizontal line represent the mean ± S.E.M. Shaded columns indicate saline-treated control values. Number of rats injected in parentheses.

Fig. 2. Effect of thyrotrophin releasing factor (TRF) on the mean serum prolactin concentration in male rats. Each column and horizontal line represent the mean ± S.E.M. Shaded columns indicate saline-treated control values. Number of rats treated in parentheses.
Serum prolactin levels in pseudopregnant rats treated with TRF (Fig. 3)

The effect of TRF on prolactin release was determined on days 3, 7 and 10 of pseudopregnancy. On day 3 a significant increase in the mean level of prolactin was observed with doses of 0·5 (P < 0·005) or 1 µg (P < 0·025) TRF. On day 7 of pseudopregnancy 0·5 µg TRF significantly increased the mean serum prolactin concentration (P < 0·01). However on day 10 of pseudopregnancy only 1 µg TRF induced a significant increase in the prolactin levels (P < 0·01) when compared with the control values.

![Fig. 3. Serum prolactin concentration on days 3, 7 and 10 of pseudopregnancy after treatment with physiological saline only (shaded columns) or thyrotrophin releasing factor (TRF; open columns). Each column and the horizontal line represent the mean ± S.E.M. Number of rats treated in parentheses.](image-url)

DISCUSSION

The present results suggest that the ability of synthetic TRF to stimulate prolactin secretion exists both in female and male rats. No effect on LH release was obtained with the doses of TRF used in this study. Bowers, Friesen, Hwang, Guyda & Folkers (1971) reported that TRF had no effect on LH release in man. In lambs, Davis & Borger (1973) obtained an indirect demonstration that TRF may not stimulate release of LH, and in sheep (Fell, Findlay, Cumming & Goding, 1973) the serum LH concentration is not altered by TRF.

In pseudopregnant rats, TRF was capable of stimulating prolactin release. Thyrotrophin releasing factor was more effective on days 3 and 7 than on day 10 of pseudopregnancy since, in the latter group, 0·5 µg TRF did not modify serum prolactin values. In male rats a stimulatory action of TRF on prolactin release was obtained in the morning and in the afternoon. Lu, Shaar, Kortnight & Meites (1972) reported that TRF does not stimulate prolactin release from normal male rat pituitary (in vivo or in vitro) or from rat pituitary tumour cells in vitro. However Mueller, Chen & Meites (1973) recently reported an effect of TRF on male and pro-oestrous rats. Stimulation of prolactin and growth hormone release by TRF infused into a male rat hypophysial portal vessel has been observed by Takahara, Arimura & Schally (1974).

The response of the pituitary to the action of TRF on prolactin release seems to be related in female rats to the level of oestrogen in the circulation. No response to 0·5 µg TRF was observed in dioestrous day 1 rats and on day 10 of pseudopregnancy. According to Hori, Ide & Miyake (1968) and Brown-Grant, Exley & Naftolin (1970) the levels of oestrogen in peripheral blood at metoestrus are very low when compared with those at other stages of the cycle. Shaikh & Abraham (1969) have described a significant increase in blood...
oestrogen on day 4 of pseudopregnancy (day 3 in our group) in the rat. It is probable that this oestrogen surge facilitates the response of the pituitary to TRF. In man, TRF has been reported to release more thyroid-stimulating hormone and prolactin in women than men (Bowers et al. 1971). However, our results show a good response in male rats and notably the effect of TRF was similar to that obtained in pro-oestrous rats. Further studies on ovariectomized rats are necessary to elucidate the role of the ovarian hormones on the effect of TRF on prolactin release.

Thyrotrophin releasing factor stimulates the release of prolactin in cyclic, pseudopregnant, male and also in lactating rats (Blacke, 1974) but the physiological importance of TRF with respect to prolactin release is unknown. Recently Vale, Blackwell, Grant & Guillemin (1973) have proposed that TRF receptors in prolactin or thyrotrophin-secreting cells are similar. Thus the implication of TRF in the mechanism controlling the release of prolactin may be considered.

R.P.D. is a career scientist and N.A. is a postdoctoral fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (C.O.N.I.C.E.T.). This work was supported by a Grant from the C.O.N.I.C.E.T. We are grateful to the National Institute of Arthritis, Metabolic and Digestive Diseases, rat pituitary hormone distribution programme for the radioimmunoassay kits for prolactin and LH.

REFERENCES


