Effect of Thyrotropin-Releasing Hormone on Rat Myometrium

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Abstract

The effect of TRH in vitro was observed on electromyograms and isometric tension changes in the uterine horn isolated from the rat. TRH induced transient prolongation of the duration of spike bursts in the electromyogram and an increased tension in contraction of diestrous uterine horns. No distinct response to TRH was elicited in preparations from rats during other estrous stages. TRH produced a contraction associated with a burst of spike potentials in the quiescent horn from the estrogen-primed ovariectomized rat. Priming with progesterone was not a prerequisite for responsiveness to TRH. In a medium with a high Ca concentration, diestrous uteri were quiescent but a transient contraction associated with a burst of spike potentials was induced by TRH. In a Ca-free medium, TRH failed to elicit any response in the diestrous uterus but acetylcholine induced a contraction without associated spike potentials. It appears that TRH stimulates Ca-influx into the uterine muscle in which responsiveness is dependent on estrogen priming.

Many neuropeptides were originally discovered because of their action on smooth muscle and, when applied to smooth muscle, cause either contraction or relaxation of the tissues (Stewart and Channabasabaiah, 1979). Though TRH was first isolated and identified for its action on pituitary cells, it has been shown to act directly on the smooth muscle of rat duodenum and to induce relaxation (Tenoue, et al., 1979). Included in the extrahypothalamic distribution of TRH, the presence of TRH in the placenta (Gibbons, et al., 1975; Shambaugh, et al., 1978) or aminotic fluid (Morley, et al., 1979) has been reported. This seems to imply a possible effect of TRH on the smooth muscle of the reproductive organs. Recently we proposed that TRH action is closely related with transmembrane calcium flux in the reacting tissue since the generation of calcium spikes is either inhibited by TRH in the rat duodenum (Tonoue, et al., 1979; 1980) or stimulated in the pituitary cells (Kidokoro, 1975; Ozawa and Kimura, 1979; Dufy, et al., 1979). If receptors for TRH are involved in the regulation of transmembrane calcium flux, a calcium-dependent reaction may be influenced by TRH where TRH-receptivity exists. Since the importance of calcium in the regulation of mechanical activity of the uterus has been established (Abe, 1970), the influence of TRH on myometrial activity is expected.

This study reports that TRH potentiates the contractile activity of the uterus of diestrous rat; the myometrial response to TRH is dependent on a preliminary ex-
posure to estrogen and TRH exerts its effect by stimulating calcium influx into the smooth muscle cells.

Materials and Methods

Virgin female rats of Wistar-Imamichi strain weighing 270-320g were used. The animals were maintained in a temperature- and humidity-controlled room on a 14 h light, 10 h dark cycle (light onset, 0600h). Pellet food and water were supplied ad libitum. All animals were assessed for estrous cycle by daily vaginal smears and were used after demonstrating at least two consecutive 4 day estrous cycles. After the vaginal smears were examined at 0930h, the animals were sacrificed between 1000 h and 1500 h by a blow on the head and bleeding from the carotid artery. The uterine horns were removed, washed with Krebs solution and cut at a distance of 3.5 cm from the cervical end. In most experiments, left horns were used though essentially no difference was observed between right and left horns. Uterine horn segments were suspended in a horizontal position in an organ bath with the cervical end fixed to a rod fitted to the wall of the bath and with the opposite end connected by a thread to an isometric force transducer (Nihonkohden, SB-1T). A resting tension of 0.5 g was applied to each tissue. The organ bath contained 80 ml Krebs solution of the following composition: mm: NaCl 122.0, NaHCO₃ 15.47, KCl 4.69, CaCl₂ 2.64, KH₂PO₄ 1.18, MgCl₂ 11.52. The solution was continuously bubbled with a 95% O₂-5% CO₂ gas mixture and maintained at a temperature of 36°C throughout the experiment. The tip of a suction electrode was positioned at about 3 mm from the cervical end and the electromyographic activity was conducted to an amplifier (Nihonkohden, AB620G, time constant set at 0.03 sec). Details of the suction electrode were described previously (Tonoue and Nomoto, 1979). An Ag-AgCl reference electrode was placed in the organ bath. Tension changes and electromyograms were recorded simultaneously with a pen recorder.

Following preliminary recording for 20 min, TRH (pyroglutamyl histidyl prolineamide, supplied by Takeda Chem. Ind.) dissolved in 0.8 ml saline was added to the organ bath immediately after a spontaneous contraction returned to the resting level. Other drugs used were luteinizing hormone releasing hormone (LHRH, Protein Research Foundation), methionine⁵-enkephalin (ENK, Protein Research Foundation) and acetylcholine chloride (Sankyo).

In some experiments ovariecimized rats were used. Ovariectomy under nembutal anesthesia was performed 25 days or more prior to experiments. Estrogen or progesterone priming of ovarietomized rats was accomplished by a single sc injection (0.1 ml) of estradiol benzoate (20 μg suspension in saline) or progesterone (5 mg in cotton-seed oil).

For parameters of statistical analysis the duration (sec) of bursts of spike potentials in electromyograms and the amplitude (g) of tension changes in contractions were chosen. Three consecutive spontaneous contractions before TRH addition (control) and 3 contractions after TRH were compared. After confirming that there was no statistically significant difference among 3 control contractions by a variance analysis, the same analysis was applied to a group consisting of 3 control contractions and a contraction after TRH.
Table 1. Duration of burst of spike potentials of electromyogram of isolated uterine horns from estrus, metestrus, diestrus and ovarietomized rats before and after addition of TRH.

<table>
<thead>
<tr>
<th></th>
<th>TRH (M)</th>
<th>Duration (Mean±S.E., sec)</th>
<th>Frequency cycles/30min</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>-3</td>
<td>-2</td>
</tr>
<tr>
<td>Estrus</td>
<td>$10^{-6}$ (7)</td>
<td>24.0±0.60</td>
<td>23.4±0.42</td>
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<tr>
<td>Metestrus</td>
<td>$10^{-6}$ (9)</td>
<td>33.0±3.30</td>
<td>35.0±3.84</td>
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<tr>
<td>Diestrus</td>
<td>$10^{-6}$ (9)</td>
<td>24.5±1.62</td>
<td>21.4±1.80</td>
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<tr>
<td>Diestrus</td>
<td>$10^{-6}$ (6)</td>
<td>29.6±2.88</td>
<td>29.3±3.06</td>
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<tr>
<td>Diestrus</td>
<td>$10^{-5}$ (6)</td>
<td>25.7±1.56</td>
<td>25.2±1.56</td>
</tr>
<tr>
<td>Spayed</td>
<td>$10^{-6}$ (4)</td>
<td>13.5±0.75</td>
<td>15.8±4.20</td>
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</tbody>
</table>

( ), No. of animals. **, *, difference from duration before TRH is statistically significant at 1 and 5% levels, respectively.

Table 2. Amplitude of tension change in mechanical activity of uterine horns from estrus, metestrus, diestrus and ovarietomized rats before and after addition of TRH.

<table>
<thead>
<tr>
<th></th>
<th>TRH (M)</th>
<th>Amplitude (Mean±S.E., g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-3</td>
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<tr>
<td>Estrus</td>
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<td>4.4±0.38</td>
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<tr>
<td>Metestrus</td>
<td>$10^{-6}$ (9)</td>
<td>2.7±0.11</td>
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<tr>
<td>Diestrus</td>
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<td>2.2±0.17</td>
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<tr>
<td>Diestrus</td>
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<td>2.6±0.34</td>
</tr>
<tr>
<td>Diestrus</td>
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<td>2.1±0.10</td>
</tr>
<tr>
<td>Spayed</td>
<td>$10^{-6}$ (4)</td>
<td>1.4±0.31</td>
</tr>
</tbody>
</table>

( ), No. of animals. **, *, difference from tension change before TRH is statistically significant at 1% level.

Results

The mechanical activity of isolated uterine horns varied extensively during the estrous cycle. As shown in Figure 1, uterine horns from proestrous rats were quiescent except for occasional weak contractions, while uterine horns from estrous, metestrous or diestrus rats contracted rhythmically. The frequency was lower but amplitude was greater in estrous uteri than in metestrous and diestrus uteri (Table 1 and 2). In estrus, metestrus and diestrus, each contraction was associated with a burst of electrical activity. The onset of a burst of spike potentials either initiated or followed the onset of contractions indicating that electrical and contractile activity initiated at any point was conducted throughout the uterine horn.

TRH added at a final concentration of $10^{-6}$ M did not produce any statistically significant changes in the myometrial activity of estrous and metestrous rats (Fig. 1, Table 1 and 2). A minor deflection of tension tracing observed in some preparations from proestrous rats was not distinguishable from the occasional spontaneous contractions (Fig. 1). On the other hand, myometrium of the diestrous rat consistently responded to TRH with a prolongation of the duration of bursts of spike potentials and an increase in the amplitude of contractions (Fig. 1, Table 1 and 2). Because of the difficulty of expressing the extent of the response to TRH, a precise dose-response relationship was not observed. However, TRH at a dose of $10^{-8}$ M did not elicit any response while a dose of $10^{-7}$ M produced the response comparable with that to $10^{-6}$ M TRH, suggesting that the effective concentration of TRH is of the order of $10^{-8}$ M (Table 1 and 2, Fig. 3).

LHRH or ENK at a dose of $10^{-6}$ M did not produce any change in the uterine activity of diestrus rats (not shown), indi-
cating that the effect of TRH was not that of non-specific peptides. TRH added 5 to 10 min after the previous dose of TRH did not produce a response, showing a clear tachyphylaxis (not shown).

The variation in the response of estrous cycle uterus to TRH seemed to indicate the dependence of the response on circulating sex steroids. This was examined in uteri from ovariectomized rats treated with estradiol benzoate, progesterone or both. Myometrium from ovariectomized rats exhibited very strong electrical activity, but some bursts of the spike potentials were not associated with appreciable mechanical activity (Fig. 2). The irregularity in mechanical and electrical activity in the spayed uterus made it difficult to quantitate the effect of TRH. However, 4 preparations out of 8 showed relatively good rhythmicity and association between two parameters, so that it was apparent that TRH (10^-6 M) did not induce any appreciable change in the uterine activity of ovariectomized rats (Table 1 and 2, Fig. 2).

Progesterone priming 72 h prior to experiments improved the regularity of spontaneous activity and the association between mechanical and electrical activity. But, the response to TRH (10^-6 M) was not produced in preparations from progesterone-primed rats (n=6) (Fig. 2). On the contrary, estradiol priming almost completely inhibited the spontaneous activity of isolated uteri. And TRH (10^-6 M) produced a sharp contraction associated with a burst of spike potentials in the preparation from rats treated with estradiol benzoate 72 h prior to experiments (n=6) (Fig. 2). Uterine horns from rats treated with estradiol plus progesterone were also quiescent. And TRH (10^-6 M) produced a contraction associated with a burst of spike potentials in uteri of spayed rats treated with both steroids 72 h prior to experiments (n=8) (Fig. 2). The response profile was similar to that observed in the diestrous uterus. The response to TRH was induced in some preparations (2 out of 6) 48 h after estradiol treatment but was not observable 24 h after treatment (n=6). Again ENK did not elicit any response and a complete desensitization by TRH itself was demonstrated (Fig. 2). Thus it was indicated that estrogen priming is required for the development of uterine responsiveness to TRH while circulating
estrogen has an inhibitory effect on spontaneous activity.

It should be noted that a 1 min delay existed between TRH addition and the onset of response in estradiol-primed preparations. The same type of delay was reproduced in the following experiment. Spontaneous rhythmic contractions associated with bursts of spike potentials in diestrous uteri were abolished immediately after the calcium concentration in the medium was tripled (Fig. 3). In the absence of spontaneous activity in the high calcium medium, TRH (10^{-7} M) induced a contraction with a burst of spike potentials of long duration after 1 min delay (n=6) (Fig. 3). Quiescence of uterine horns in a high calcium medium was ascribed to muscle membrane hyperpolarization (Csapo and Kuriyama, 1963; Marshall, 1965). Thus TRH might be assumed to induce a contraction by depolarizing a muscle membrane or by stimulating calcium influx into muscle cells even in the hyperpolarized state.

This was examined by comparing the effect of TRH and acetylcholine which is known to depolarize muscle membrane by increasing potassium, sodium and calcium fluxes (Setkleiv, 1970). In a calcium-free medium (30 min preincubation), diestrous uteri did not show any spontaneous activity and TRH (10^{-7} M) did not induce any response, but acetylcholine (2.4 \times 10^{-6} M) elicited an immediate rise in tension without associated spike potentials. By bringing the calcium level back to normal, Krebs solution promptly restored the spontaneous mechanical and electrical activity (n=6) (Fig. 3). The finding indicated that TRH exerted its effect by stimulating calcium influx into myometrial cells.

**Discussion**

The observed variation in mechanical and electrical activity of rat myometrium during the estrous cycle and the effect of sex steroids on spontaneous activity in this study are generally consistent with recent in vivo studies (Ishikawa and Fuchs, 1978; Downing, et al., 1978) in the following points: Uterine horns from proestrous rats show the least activity; myometrial activity in ovariectomized rats is very strong and estrogen inhibits this activity, progesterone does not show any strong inhibitory in vivo effect on the spontaneous activity. Because of this similarity we may conclude that the present experimental system could reproduce physiological activity of rat uterine horns in general.

It is demonstrated that uteri from diestrous rats are responsive to TRH while pre-
parations from other estrous stages fail to show any distinct response. In diestrous uteri, TRH seems to augment the spontaneous contractions or to induce a contraction by itself. The result that TRH induces a contraction even in the preparations which were made quiescent by an elevated calcium concentration or by estradiol-treatment, demonstrates a potency of TRH to produce a myometrial contraction. Augmentation of spontaneous contraction by TRH is suggested by the finding that not only the first contraction after TRH but also second and third contractions were potentiated.

The effective minimum concentration of TRH in the diestrous uterus seems to be of the order of $10^{-8}$ M which is comparable with the range of dissociation constant of TRH-binding to pituitary and brain receptors (Burt and Snyder, 1975) or the effective concentration in the relaxation response in the rat duodenum (Tonoue, et al., 1979).

From what we know of the endocrine profile of the 4-day estrous rat, circulating sex steroids increase during proestrus (Butcher, et al., 1974). Thus the responsiveness of the uterus to TRH seems to occur about 3 days after exposure to high circulating sex steroids. This assumption is supported by the fact that preparations from ovariectomized rats treated with estradiol 72 h prior to experiments consistently responded to TRH while those from rats 24 h after treatment did not. Progesterone priming seems not to be indispensable for the development of responsiveness to TRH.

This modification of uterine reaction to TRH by estradiol should be noted since the stimulatory effect of TRH on the pituitary secretion of TSH or prolactin is augmented by estradiol also 4 days after the treatment (DeLean, et al., 1977; Labrie, et al., 1978). This coincidence is likely to be more than a fortuitous one since the effect of the estrogen does not become apparent within 2 days of treatment in either case.

There is currently a consensus of opinion that smooth muscle contraction is the result of an increase in intracellular Ca++ availability which originates from either transmembrane Ca++ influx or the release from intracellular sequestered sites (Kuriyama, et al., 1977). The latter is elicited by a membrane depolarization (Kuriyama et al., 1977). The present finding that TRH fails to elicit a contraction of the diestrous uterus in a calcium-free medium but that acetylcholine, a depolarizing agent, is capable of inducing a contraction, indicates a stimulation by TRH of Ca++ influx, and little possibility of inducing membrane depolarization. Acetylcholine-induced contraction in a calcium-free medium may be ascribed to the release of intracellular stored Ca++. The rat myometrial sensitivity to oxytocin has been shown to be increased by estrogen treatment (Berger and Marshall, 1961). However, the myometrial response to oxytocin of the estrogen-treated rat was a sustained type and not abolished in a calcium-free medium (Berger and Marshall, 1961), suggesting a difference in the mode of action on the rat myometrium between TRH and oxytocin.

It has been shown that TRH induces a relaxation associated with the blocking of spike potentials in the rat duodenal smooth muscle indicating a suppression of Ca++ influx (Tonoue, et al., 1978; 1980). Thus, differential action of TRH on the Ca++ in the smooth muscle of different tissues is demonstrated. These findings support our hypothesis that the TRH-effect might be related with the Ca++ channel, either stimulatory or inhibitory, depending on the nature of the response of the tissues (Tonoue, et al., 1980).

With respect to these effects of TRH on calcium flux, the response of electrical activity of pituitary clonal GH3 cells to TRH appears interesting. Ozawa and Kimura (1979) have observed three types of TRH
effects on the calcium spike generation in the pituitary cells; 1) an enhancement of spike generation after a delay; 2) an immediate blocking of spike generation followed by an enhancement; and 3) an immediate blocking without facilitation. A similar delay of onset of response to TRH in the pituitary cells has been recorded by Dufy et al. (1979). It should be remembered that TRH-blocking of calcium influx was observed immediately after addition in the duodenal smooth muscle (Tonoue, et al., 1979; 1980) while about a 1min delay occurs in the onset of the stimulatory effect of TRH on the uterine muscle in the present study. Thus it appeared so far that the facilitatory action of TRH on calcium influx is manifested after a delay of several ten seconds while suppressive effects are elicited without such a delay. The mechanism underlying this difference between facilitatory and inhibitory action seems to be an important topic to be studied in order to elucidate the mode of action of TRH. The smooth muscle seems to be a useful model for this study.

The physiological role of TRH in uterine activity is unclear at present. Studies on the pregnant uterus are required for the understanding of the peripheral, physiological and pharmacological significance of TRH in reproduction.

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References


