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Thyrotropin-releasing hormone facilitates spinal nociceptive responses by potentiating NMDA receptor-mediated transmission

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Abstract

The interaction of thyrotropin-releasing hormone (TRH) with NMDA receptor-mediated responses has been investigated in α-chloralose-anaesthetized spinalized rats with respect to its relevance to spinal nociceptive transmission. The effects of TRH and of the uncompetitive NMDA antagonist ketamine were tested on responses of dorsal horn wide dynamic range neurones to noxious pinch, heat and electrical stimuli in parallel with those to iontophoretically applied *N*-methyl-D-aspartate (NMDA) and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid). Tests with NMDA blocking doses of ketamine (4 mg/kg i.v.) demonstrated a variable NMDA receptor-mediated component of all synaptic responses. TRH (0.5–1 mg/kg i.v.) enhanced the responses to NMDA (but not AMPA) in parallel with an increase of responses to all noxious stimuli and the 'wind-up' component of the responses to repeated electrical stimulation. This potentiation was completely reversed by a subsequent administration of ketamine (4 mg/kg i.v.). The results indicate that TRH facilitates nociceptive transmission in the spinal dorsal horn via a selective positive modulation of NMDA receptor-mediated transmission.

Keywords: TRH (thyrotropin-releasing hormone); NMDA receptor; Spinal cord; Nociception; 'Wind-up'

1. Introduction

The widespread distribution of thyrotropin-releasing hormone (TRH) and its binding sites in the central nervous system (Manaker et al., 1985; Mantyh and Hunt, 1985), and its co-localization with several neurotransmitters (Arvidsson et al., 1990) suggest a neuromodulatory role for this peptide separate from its hormonal activity. Recent findings indicate that TRH modulates excitatory amino acid-mediated transmission in the brain, in particular that involving NMDA receptors (Kharkevich et al., 1991; Kasparov and Chizh, 1992; Rekling, 1992; Kasparov et al., 1994; Stocca and Nistri, 1995; see also Jackson and White, 1988). In the spinal cord, TRH selectively potentiated the responses of dorsal horn wide dynamic range neurones to iontophoretic *N*-methyl-D-aspartate (NMDA) (Chizh and Headley, 1994a). TRH is also known to facilitate spinal dorsal horn neurone and reflex responses evoked by noxious stimulation (Clarke et al., 1988; Behbehani and Zemlan, 1990; Chizh and Headley, 1994a). However, the TRH-induced potentiation of NMDA receptors and the modulation of spinal nociceptive processing have not been related directly. In the present study we have recorded dorsal horn neurone responses to microelectrophoretically administered amino acids in parallel with responses evoked synaptically by noxious natural and electrical stimuli. We have compared the actions of TRH with those of ketamine, an uncompetitive NMDA antagonist that was used to define the NMDA receptor-mediated component of the responses. We conclude that enhancement of NMDA receptor-mediated neurotransmission is the main mechanism of the facilitation of nociceptive responses caused by TRH. Some of the results have been published in abstract form (Chizh and Headley, 1994b).

2. Materials and methods

Experiments were performed on 19 male Wistar rats (300–350 g) using methods detailed elsewhere (Cumberbatch et al., 1995). Under halothane anaesthesia, tracheal,

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carotid and jugular cannulae were inserted and a lumbothoracic laminectomy was performed. The spinal cord was cut at T9–T11 and the animal was prepared for recording from lumbar dorsal horn neurones. Anaesthesia after surgery was maintained with α-chloralose (50 mg/kg i.v. initially followed by i.v. infusion at 10–15 mg/kg/h). In some cases animals were artificially ventilated after paralysis with pancuronium (1.5 mg/kg/h); in these animals end-tidal CO_2 was monitored and kept close to pre-paralysis levels and the adequacy of anaesthesia was verified by maintaining the same anaesthetic regimen and by checking that there were only minimal cardiovascular responses to noxious stimuli applied rostral to the level of spinalization. Arterial blood pressure was monitored continuously; systolic pressure remained above 100 mm Hg. Fluid therapy (isotonic saline or Haemaccel, Hoechst) was provided throughout experiments at approximately 3 ml/kg/h. Core temperature was maintained close to 37°C. Extracellular recordings of single dorsal horn neurone action potentials were made with multi-barrel glass microelectrodes filled with 3.5 M NaCl (recording barrel); the sodium salts of NMDA (100 mM in 100 mM NaCl) and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; 10 mM in 200 mM NaCl), both at pH 7.5–8; and 200 mM NaCl for current balancing. Spikes were monitored continuously on a digital oscilloscope in order to ensure that spike configuration remained constant.

Wide dynamic range (or convergent) neurones were selected on the basis of their receptive field characteristics. Cell position within the dorsal horn was estimated from micromanipulator readings. Cells were activated in cycles (3.5–4 min) by iontophoretic excitatory amino acid ejections and by adequate sensory stimuli applied to the peripheral receptive field on a hind paw. Two stimulus protocols were used: the first was iontophoretic applications of NMDA and AMPA (both 40–45 s, 2–85 nA) and noxious heat (15 s; 46.5–49.7°C, mean 48.5°C, applied by a contact thermode); the second was iontophoretic NMDA, noxious pinch (15 s; 0.6–1.5 N, mean 1.1 N, applied over 12.5 mm^2 by pneumatically controlled jaws) and electrical stimuli (1–2 ms, 10–15 × threshold for eliciting a response (T_r) in the neurone under study, mean 14 T_r, 16 stimuli at 1 Hz applied by percutaneous needles within the receptive field). The electrical stimuli apparently activated A- and C-fibres, as the responses were clearly divisible into two components, with a latency for the second of around 200 ms (see Hartell and Headley, 1991). Cell firing rates were monitored on a chart recorder and counts of action potentials were made in epochs related to the stimuli. With heat and pinch stimuli, only the last 10 s of the response was analysed so as to select for the non-adapting phase of nociceptive responses. With electrical stimuli, the response was subdivided into 5 epochs corresponding to stimulus Nos. 1, 2–4, 5–8, 9–12 and 13–16 of the train, so as to permit analysis of progressively increasing responses; this 'wind-up' (Mendell, 1966) was quantified as: $WU = R_{tot} - 16\ R_1$, where R_{tot} was the spike count of the response to the whole train and R_1 was the response to the first stimulus of the train. Between stimulus cycles a 60 s interval was used for assessing the level of ongoing (background) activity, which was used to compensate all counts for ongoing activity levels. This interval was in all cases sufficient for full recovery from stimulations.

TRH (pGlu-His-Pro amide, Sigma, 0.5–1.0 mg/kg) and ketamine hydrochloride (Vetalar, Parke-Davis, 4 mg/kg) were administered i.v. Ketamine was selected as the most appropriate NMDA antagonist because of its relatively short action in vivo (time to half-recovery of 10–15 min) allowing for multiple drug tests on the same cell (see Headley et al., 1987). Drug effects are expressed as percentages of control, which was taken as the mean of the corresponding counts in the last 3 pre-drug cycles; means ± S.E.M. are indicated. In the first protocol described above, ketamine was tested on 10 of the 16 cells examined with TRH. With the second protocol ketamine was tested on all cells first; following full recovery TRH was injected and then ketamine was administered a second time 2 cycles (7–8 min) after the TRH injection; in this case the pre-TRH counts were taken as the control for the second ketamine test. Neither ketamine nor TRH consistently affected spike amplitude. Statistical analysis was performed using Wilcoxon matched pairs test and repeated measures analysis of variance (ANOVA) followed by Student-Newman-Keuls post-hoc tests (for multiple comparisons). These tests were performed on spike count data. Correlation was estimated on percentage data using linear regression and Spearman rank analysis.

3. Results

This study is of 25 wide dynamic range cells located in the deep dorsal horn (580–1390 μm, mean 1000 μm from the dorsal pial surface).

In our previous work (Chizh and Headley, 1994a), we found that a dose of 0.5 mg/kg TRH potentiated responses to iontophoretic NMDA whilst producing only minimal changes in blood pressure and respiration. In the present study TRH was mainly used at this dose, but with 6 cells an additional dose of 0.5 mg/kg TRH was given 2 cycles (7–8 min) after the first injection so as to test for dose-response relationships. The effects of the cumulative dose of 1 mg/kg were virtually no different from those of 0.5 mg/kg (e.g. NMDA responses 181 ± 14% and 176 ± 28% control, respectively, $n = 6$). The effects of the total TRH dose tested on each cell were therefore used for the analysis.

With 16 of the 18 cells tested with the first protocol, TRH selectively enhanced responses to NMDA (as compared with AMPA) and also increased those to noxious heat. With the remaining 2 cells, non-selective reduction of all responses occurred after TRH injection (NMDA 19 and

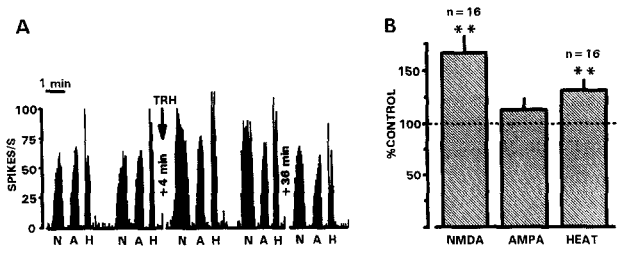

Fig. 1. The effects of TRH (thyrotropin-releasing hormone; 0.5 mg/kg i.v.) on responses of spinal dorsal horn wide dynamic range neurones to iontophoretically administered NMDA and AMPA and to noxious heat in α-chloralose-anaesthetized, spinalized rats. A: An original ratemeter record of the spike discharge of a spinal wide dynamic range neurone in the deep dorsal horn responding to regular cycles of iontophoretic NMDA (N, 43 nA, 40 s), AMPA (A, 12 nA, 40 s) and to noxious heat stimuli (H, 49.3°C, 15 s) applied to the peripheral receptive field on one hind paw by a contact thermode. B: Pooled data from 16 wide dynamic range neurones in the dorsal horn. Significant difference from control: ** $P < 0.005$ (Wilcoxon matched pairs test).

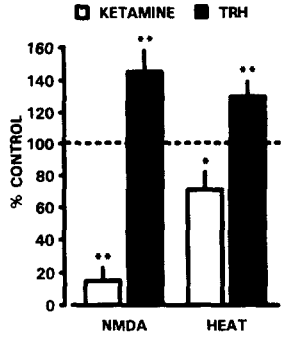

Fig. 2. Reciprocal effects of the NMDA antagonist ketamine (4 mg/kg i.v.) and TRH (0.5 mg/kg i.v.) on responses of spinal wide dynamic range neurones to iontophoretic NMDA and to noxious heat stimuli (mean 48.4°C). All data from the same 10 cells. Significant difference from control: * $P < 0.05$; ** $P < 0.005$ (Wilcoxon matched pairs test).

30%, AMPA 28% and 51%, heat 59 and 21% control); these cells were excluded from the subsequent analysis.

Fig. 1A shows a ratemeter record of one neurone with which TRH was tested on responses to NMDA, AMPA and noxious heat. With this cell, responses (spike counts) to heat were increased to 179% control and those to NMDA to 210%, whereas those to AMPA remained at 128% control. The pooled data from the 16 cells showing such enhancements were similar (Fig. 1B); the enhancements of responses to heat and NMDA were significant ($P < 0.005$) whereas those to AMPA were not. Linear regression analysis indicated that there was a significant correlation between the effects of TRH on responses to NMDA and those to noxious heat ($r = 0.67$, $P < 0.005$). The time-course of the TRH effects (half-recovery time of 15–20 min) was similar to that observed previously (Chizh and Headley, 1994a).

With 10 of these 16 experiments, ketamine (4 mg/kg) was used to determine the magnitude of the NMDA receptor-mediated component of the responses to noxious heat stimuli. This i.v. dose has been shown to be selective for responses to NMDA on spinal dorsal horn cells (Anis et al., 1983; Headley et al., 1987). Fig. 2 compares the effects of ketamine and TRH on these 10 cells. Ketamine decreased responses to NMDA to 15 ± 8% control ($P <$ 0.005) and those to heat to 71 ± 11% control ($P < 0.05$), indicating that these nociceptive responses had a component mediated by NMDA receptors. On these cells, TRH significantly potentiated both NMDA and heat responses (to 145 ± 13% and 130 ± 9% control, respectively; $P <$ 0.005 vs. control). AMPA was tested on 5 of these 10 cells; ketamine reduced responses somewhat, to 74 ± 13% control (not significant), but TRH did not affect the responses (104 ± 18% control).

In another group of 9 wide dynamic range neurones, TRH was tested using the second protocol, that is upon cycles of cell responses to NMDA, noxious pinch and trains of electrical stimulation at 1 Hz. Fig. 3 shows that all three kinds of response were reduced by ketamine and enhanced by TRH. The actions of TRH on NMDA-evoked firing correlated positively with those on responses to pinch ($r = 0.86$, $P < 0.05$) and electrical stimulation ($r =$ 0.71, $P < 0.05$, based on the spike counts of the complete train).

With electrical stimulation, wind-up was observed in all 9 cells tested, such that the number of spikes evoked by each stimulus was increased progressively after the first stimulus (Fig. 4). Following the last stimulus of the train there was 5–10 s of 'afterdischarge'. Fig. 3 indicates that under these conditions the NMDA receptor-mediated component was little altered during the train (similar effects of TRH and ketamine on responses to the first stimulus and the last 4 stimuli). The wind-up and especially the afterdis-

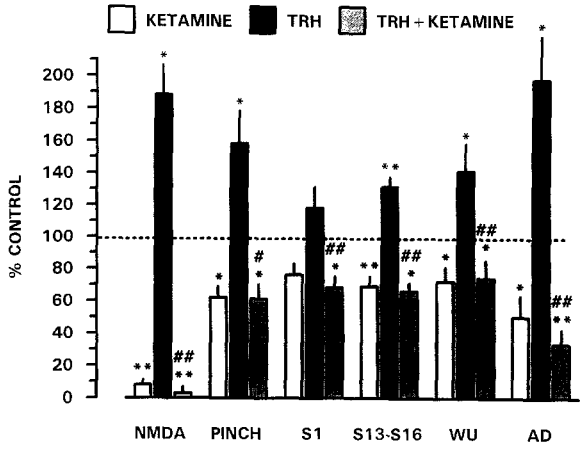

Fig. 3. Effects of ketamine (4 mg/kg i.v.), TRH (0.5–1 mg/kg i.v.) and of ketamine after TRH on responses of 9 dorsal horn neurones to iontophoretic NMDA, noxious pinch ($n = 7$) and on different phases of the response to a train of electrical stimuli at 1 Hz: the first (S1) and the last 4 (S13–S16) stimuli, the 'wind-up' (WU; see Materials and methods), and the afterdischarge following the last stimulus (AD). Significant difference from pre-drug control: * $P < 0.05$; ** $P < 0.005$; significant difference from TRH effect: # $P < 0.05$; ## $P < 0.005$ (Wilcoxon matched pairs test).

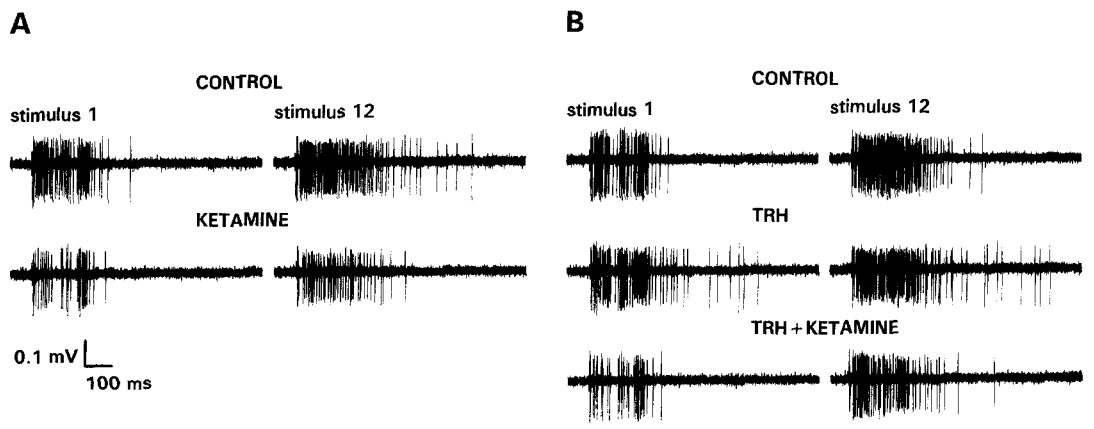

Fig. 4. Wind-up of responses to electrical stimulation and effects (A) of ketamine (4 mg/kg i.v.), and (B) of TRH (0.5 mg/kg i.v.) followed by ketamine (4 mg/kg i.v.) given at the time of the peak TRH effect. Original traces of a deep dorsal horn wide dynamic range neurone responding to trains of 16 electrical stimuli (1 Hz, 15 T_t) applied to the cutaneous receptive field in an α-chloralose-anaesthetized, spinalized rat.

charge had NMDA receptor-mediated components (Fig. 3, Fig. 4A and Fig. 5A) and TRH increased these, particularly the afterdischarge.

As indicated above, ketamine was tested at the same dose after as well as before TRH on these cells. The second test with ketamine completely abolished the TRH-induced potentiations of all responses; in each case ketamine brought the responses down to values almost identical to those observed when the NMDA antagonist was tested prior to TRH (Fig. 3; Fig. 4B and Fig. 5B vs. Fig. 4A and Fig. 5A).

When counts obtained at the peak of TRH effect were taken as control for the second ketamine test, the effect of ketamine appeared to be greater than in the first test, indicating a higher level of NMDA receptor involvement. The first ketamine administration reduced pinch responses from 274 ± 42 to 170 ± 34 spikes (to 62 ± 7% control), whereas injected 5-7 min after TRH it depressed the response from 423 ± 80 to 169 ± 38 spikes (to 42 ± 7% control, a significantly greater reduction than the first test, $P < 0.05$). Similar results were observed with responses to electrical stimuli and wind-up.

TRH also increased the spontaneous firing significantly (control 5.6 ± 1.7 spikes/s; test 8.3 ± 2.0 spikes/s, $P < 0.005$, $n = 24$). This TRH-enhanced background discharge was depressed by ketamine. On the 9 cells tested with ketamine both before and after TRH, the first ketamine test reduced firing to 1.5 ± 1.0 (from 4.1 ± 2.2) spikes/s; after TRH, ketamine brought it back to the same level (1.5 ± 1.2 spikes/s, from a TRH-enhanced level of 5.1 ± 2.5 spikes/s).

No significant correlation was found between the location of the cell in the dorsal horn and the magnitude of the TRH effects on heat, pinch or NMDA responses, though these effects tended to be somewhat greater with the deeper cells.

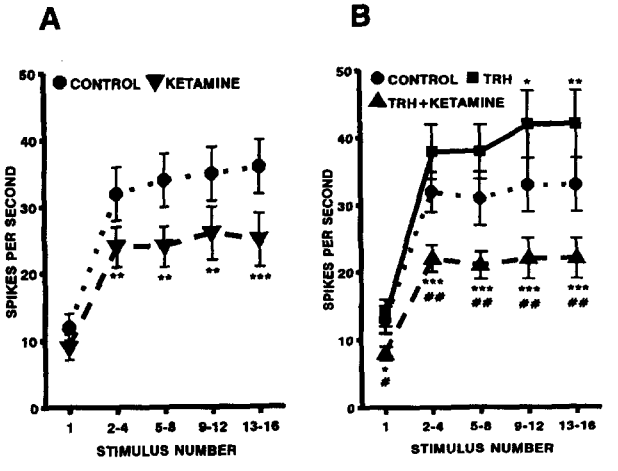

Fig. 5. Effects (A) of ketamine (4 mg/kg i.v.), and (B) of TRH (0.5–1 mg/kg i.v.) and of ketamine (4 mg/kg i.v.) given at the time of the peak TRH effect, on different stages of the response to trains of 16 electrical stimuli at 1 Hz. All data from the same 9 dorsal horn wide dynamic range neurones. Significance marks as in Fig. 3 (repeated measures ANOVA followed by Student-Newman-Keuls post-hoc test).

4. Discussion

In the dorsal horn of the spinal cord there are TRH binding sites (Manaker et al., 1985; Mantyh and Hunt, 1985), pituitary-like TRH receptor mRNA (Calzá et al., 1992) and TRH-like immunoreactivity (Arvidsson et al., 1990). This suggests a modulatory role of endogenous TRH in spinal sensory transmission, and indeed, facilitation of spinal nociceptive transmission by TRH has been reported by several authors (e.g. Clarke et al., 1988; Behbehani and Zemlan, 1990). Equally, NMDA receptors are present in the spinal cord (e.g. Franklin et al., 1993; Petralia et al., 1994) and are widely considered to be involved in some forms of nociceptive transmission (Headley and Grillner, 1990; Dougherty et al., 1993; Meller and

Gebhart, 1994). They are thought to be of particular importance in the induction of hyperalgesia and sensitization (Meller and Gebhart, 1994), whilst their role in responses to acute stimuli may be more limited (Headley et al., 1987; Headley and Grillner, 1990).

In the present experiments, acute nociceptive responses of dorsal horn wide dynamic range neurones (evoked by heat or pinch applied to the peripheral receptive field) had an NMDA receptor-mediated component, as revealed by the fact that they were reduced by ketamine given at a dose that selectively reduced responses to exogenous NMDA. Conversely, TRH potentiated the responses of the same cells to these noxious stimuli. Ketamine also reduced, and TRH enhanced, the 'wind-up' and especially the afterdischarges of the responses evoked by repetitive electrical stimulation. Wind-up is regarded as being largely NMDA receptor-mediated (Davies and Lodge, 1987; Dickenson and Sullivan, 1987). In the present experiments the NMDA antagonist did not abolish the wind-up; this would be compatible with a role for NMDA receptors plus, under these conditions, an additional involvement of voltage-dependent Ca^{2+} channels, which can also mediate wind-up (Russo and Hounsgaard, 1994).

As observed in previous studies (Jackson and White, 1988; Behbehani and Zemlan, 1990; Chizh and Headley, 1994a, b), TRH also increased spontaneous activity. This may reflect a potentiation of ongoing NMDA receptor-mediated activity, which is consistent with previous reports that the spontaneous activity of dorsal horn neurones is sensitive to NMDA antagonists both in vivo (Headley et al., 1987) and in vitro (Alford et al., 1990).

All these findings, like our previous work (Chizh and Headley, 1994a), suggested that the TRH effect might be due to enhanced NMDA receptor-mediated transmission. Three aspects of the present results support this suggestion. Firstly, the enhancement of nociceptive responses by TRH was positively correlated with a selective increase of the firing evoked by exogenous NMDA (but not AMPA). Secondly, in every case when the NMDA antagonist ketamine reduced the responses, TRH had the opposite effect. Thirdly, and most importantly, ketamine administered after TRH reduced responses to the same level as ketamine administered before TRH, indicating that all of the TRH-induced enhancement was mediated via NMDA receptors.

These considerations therefore indicate that TRH potentiates nociceptive responses of dorsal horn neurones via NMDA receptor-dependent mechanisms. However, the process by which TRH may cause this modulation remains unclear. Although facilitation of presynaptic glutamate release has been suggested (Lacey et al., 1989; Behbehani et al., 1990), the potentiation of dorsal horn cell responses to iontophoretic NMDA in this study, and the lack of effect on responses to AMPA, strongly support a principally postsynaptic interaction. Results indicating postsynaptic mechanisms have also been obtained with neocortical and hippocampal neurones, where TRH enhanced the depolarisations evoked by iontophoretic NMDA (Kasparov et al., 1994) and NMDA receptor-mediated EPSPs (Stocca and Nistri, 1995).

One mechanism for TRH enhancement of responses would be an increase in cell input resistance. This was suggested for hypoglossal motoneurones (Rekling, 1992), and in spinal motoneurones TRH presumably blocks a K^+ conductance, leading to a rise in input resistance and depolarization (Fisher and Nistri, 1993). Such a mechanism cannot, however, explain the lack of effect of TRH on non-NMDA receptor-mediated responses in this and other studies (Chizh and Headley, 1994a; Kasparov et al., 1994; Stocca and Nistri, 1995), since an increase in cell input resistance would be expected to cause a non-selective potentiation of all responses. One could argue that if an increase in input resistance was accompanied by a tonic depolarization, NMDA receptor-mediated responses could be preferentially promoted due to their voltage dependence. However, since non-NMDA receptor-mediated effects would also be enhanced, such direct and indirect consequences of increased input resistance cannot account for the block of *all* TRH effects by the NMDA antagonist ketamine. Because the NMDA antagonist tested after TRH brought the responses back to the same values as those observed when ketamine was tested alone, NMDA receptor potentiation must have been the primary mechanism underlying the enhancements of synaptic responses elicited by TRH.

Interactions between TRH and NMDA mechanisms are likely to occur at an intracellular level. TRH receptors in the pituitary are known to be linked to phospholipase C via G proteins, leading to the formation of IP_3 (inositol 1,4,5-triphosphate) and 1,2-diacylglycerol (Gershengorn, 1989). The resulting increase of cytosolic Ca^{2+} concentration could lead to positive modulation of NMDA receptors via activation of Ca^{2+}-dependent protein kinases (Raymond et al., 1993) and perhaps relief of magnesium block. Ca^{2+}-independent pathways of TRH signal transduction have also been demonstrated (Yajima et al., 1990), allowing for other intracellular mechanisms of NMDA receptor regulation. Specific interactions of TRH with NMDA-induced intracellular events were shown in cultured cerebellar granule cells, where TRH stimulated basal phosphoinositol hydrolysis only in the absence of Mg^{2+} in the medium, and selectively potentiated inositol monophosphate formation induced by NMDA, but not by quisqualate or carbamylcholine (Casabona et al., 1992). The relatively long duration of TRH effects in the present work (half-recovery time of approximately 15–20 min) as compared to its short plasma half-life (4–5 min, Bassiri and Utiger, 1973) also indirectly supports the idea of metabotropic intracellular interactions.

The variability of TRH effects on dorsal horn cells observed in this and previous work (Jackson and White, 1988; Behbehani and Zemlan, 1990; Chizh and Headley, 1994a) may reflect TRH interactions with more than one

receptor type. Two TRH receptor cDNAs have recently been isolated from the rat pituitary; both isoforms are also expressed in the central nervous system, including the spinal cord (Satoh et al., 1993). The existence of several functionally distinct TRH receptors in the brain has been suggested by some authors (Toledo-Aral et al., 1993). In addition, biological activity of TRH metabolites (Toledo-Aral et al., 1993), and the presence of specific TRH-catabolizing enzymes in the spinal cord matching the distribution of TRH receptors (Vargas et al., 1992), suggest that TRH metabolites could mediate at least some of the diverse effects of TRH.

In conclusion, TRH administered systemically enhances spinal nociceptive responses by augmenting NMDA receptor-mediated processes. This enhancement is reminiscent of that reported for neurokinins, but appears to be both greater in magnitude and considerably more selective for NMDA mechanisms (cf. Cumberbatch et al., 1995). The presence of TRH and its receptors in the spinal cord implies that there may be a physiological modulatory role for TRH in spinal sensory processing. Further progress in elucidating this possibility will depend largely on the developement of selective TRH receptor antagonists.

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