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Effects of Thyrotropin-releasing Hormone on GABAergic Synaptic Transmission of the Rat Hippocampus

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Abstract

The effect of thyrotropin-releasing hormone (TRH), a neuropeptide physiologically present in the mammalian hippocampus, on spontaneous, miniature and evoked GABAergic postsynaptic currents was investigated using whole-cell patch-clamp recording from pyramidal cells and interneurons of the rat hippocampal thin slice preparation. Bath application of 10 μM TRH induced an increase in the frequency of spontaneous postsynaptic currents from 1.07 ± 0.68 to 3.16 ± 0.73 Hz in pyramidal neurons and interneurons of the stratum lacunosum-moleculare (SL-M). In tetrodotoxin solution TRH did not change miniature postsynaptic currents. Application of TRH to minislices containing only the CA1 region still produced an increase in the spontaneous postsynaptic current frequency, indicative of an action by TRH upon a local GABAergic circuitry. Paired recordings from one pyramidal cell and one stratum lacunosum moleculare interneuron displayed synchronous events whose frequency rose after TRH application, suggestive of a common, TRH-sensitive input. In a small subset of cells TRH induced the appearance of highly rhythmic large postsynaptic currents at a frequency of ~2 Hz, as confirmed by autocorrelation analysis. Postsynaptic currents electrically evoked by focal stimulation of stratum lacunosum-moleculare were depressed from 90 ± 27 to 44 ± 15 pA after application of TRH. This phenomenon was solely due to an increase in the number of synaptic failures. It is proposed that the effect of TRH on the GABAergic system was primarily exerted on a subset of interneurons controlling the activity of pyramidal cells as well as stratum lacunosum-moleculare interneurons.

Introduction

Thyrotropin-releasing hormone (TRH) is a naturally occurring neuropeptide which is found in high concentration in the hippocampus (Sharif, 1989). The origin of the TRH-containing fibres is currently unclear, although a major component seems to course through the fornix (Low *et al.*, 1989). TRH-containing fibres appear to make a diffuse mesh through various areas of the hippocampus, with particularly high concentrations of the peptide and its receptor binding sites in the dendritic layers of the CA1 pyramidal neurons (Manaker *et al.*, 1985; Low *et al.*, 1989; Eymin *et al.*, 1993). Nevertheless, the function of TRH remains unknown. Since TRH has been shown to exert slow modulatory effects on brainstem (Reckling, 1990) and spinal neurons (Bayliss *et al.*, 1992; Fisher and Nistri, 1993), attention has recently been paid to the possibility that TRH might exert a modulatory role in the hippocampus as well. Within this framework it has been shown that TRH mimics the action of serotonin in depressing a high-threshold Ca^{2+}-dependent K^+ conductance (Ballerini *et al.*, 1994) and that it selectively enhances glutamatergic transmission mediated by NMDA receptors (Stocca and Nistri, 1994, 1995) in analogy to the phenomenon reported in the cerebral cortex (Kasparov *et al.*, 1994). The mechanism through which TRH produces these effects is unclear, but in accordance with the well documented effect of this peptide on the phosphoinositol metabolism of cultured pituitary cells (Gersherngorn, 1986) it has been suggested that a similar phenomenon may take place in hippocampal neurons (Ebihara and Akaike, 1993). Should this be the case one might expect that other neurotransmitter pathways might be susceptible to a modulatory action by TRH. In particular, inhibitory neurotransmission mediated by GABA has not, to the best of our knowledge, been investigated for its sensitivity to TRH. The present investigation using patch-clamp electrophysiological techniques examined the action of exogenously applied TRH on $GABA_A$ receptor-mediated synaptic transmission at the level of the CA1 region of the rat hippocampus.

Materials and methods

Slices

Thin hippocampal slices (250–300 μm) were cut from the brain of 2- to 3-week-old Wistar rats at 2–4°C with a vibroslicer and subsequently stored at 32°C for 30–60 min in a solution containing (mM): NaCl 132, KCl 3.5, $NaH_2PO_4.H_2O$ 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 10 and glucose 10. The solution was oxygenated with a

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mixture of 95% O_2 and 5% CO_2. A single slice was subsequently transferred to a recording chamber continuously supplied with the same physiological solution at room temperature (20–22°C). The chamber was mounted on the stage of an upright immersion microscope (Axioscope, Zeiss) with Nomarski optics (400×). In some cases minislices were cut from the whole hippocampal slice after removing the dentate gyrus or the CA3 area (or both) with a scalpel blade.

Patch-clamp recording

Whole-cell voltage clamp (3–5 MΩ resistance electrodes) was performed at a holding potential of −70 mV from visually identified pyramidal neurons or interneurons of the CA1 area. The identification of the cell type was also confirmed by their distinctive firing pattern in response to depolarizing current pulses lasting ~1 s, delivered in current-clamp mode (Lacaille and Schwartzkroin, 1988a). The pipette solution contained (mM) KCl 135, HEPES 10, EGTA 1.0, NaATP 1.5. In a few experiments the principal salt contained in the pipette solution was CsCl (135 mM) instead of KCl; no difference was observed from results obtained with intracellular KCl, and the data were thus pooled. All experiments were performed in the presence of the glutamate ionotropic receptor antagonist kynurenic acid (1 mM). Since application of 20 μM bicuculline reversibly abolished all postsynaptic currents (PSCs), whether spontaneous, miniature or evoked, these responses were identified as $GABA_A$ receptor-mediated currents (Pearce, 1993). TRH was bath-applied at the concentration of 10 μM (unless otherwise stated). The average series resistance was 20 ± 5 MΩ. Cells with series resistance >33 MΩ were discarded. In some experiments pipette KCl was replaced by CsCl to decrease cell leak conductance. Pulses of 2–5 mV were given every 10 s to monitor access and input resistance. The signals were amplified with two LIST EPC-7 amplifiers, stored on video cassettes, and digitized off-line at 5 kHz with the pCLAMP 6.0.2 software (Axon Instruments). Focal stimulation (by a patch electrode filled with extracellular solution) was applied to the cell body of visually identified interneurons in the stratum lacunosum-moleculare (SL-M) region. In each cell stimulation was adjusted to elicit responses characterized by a consistent number of failures ('minimal stimulation'; Voronin, 1993), which are reputed to be generated by activation of one (or very few) synapses. The number of failures observed in different cells was variable (range 5–50%) presumably because of differing excitability of tested interneurons. The stimulus frequency was 0.1 Hz to avoid fatigue. Measurements of evoked PSC amplitudes were done by averaging a series of 20–50 responses. Analysis of PSCs in terms of their frequency, amplitude, rise time and decay time was performed with the program N05 generously supplied by Dr S. Traynelis. The peaks of high-frequency background noise were in the range 2.5–5 pA, over which the threshold for event acceptance was fixed. Only non-overlapping events were considered for the analysis. To detect any significant variations in spontaneous PSC (sPSC) frequency the number of events during short (30 s) intervals was first calculated and subsequently evaluated over longer (150–300 s) intervals. Autocorrelation analysis was performed on event lists generated by the N05 program and processed further with the help of a home-made program written in Quick Basic. Data are reported as mean ± SEM. Statistical analysis was performed with Student's *t*-test, taking $P < 0.05$ as a statistically significant difference.

Results

Spontaneous activity

Pyramidal cells

Spontaneous PSCs were present in all recorded pyramidal cells, identified by visual inspection. These cells displayed a typically accommodating firing pattern (Fig. 1a). Applications of 10 μM TRH elicited (after 30–60 s) an increase in the frequency of sPSCs in 21/24 CA1 pyramidal cells, as shown by the sample tracings in Figure 1b. The phenomenon of increased frequency of sPSCs usually outlasted the 3 min application of TRH, with a gradual return to baseline values after ~20 min. When the application of TRH was repeated at this time, no response was observed. However, TRH application after a further 30 min wash elicited a response virtually identical to the first one. Therefore, an interval of at least 30 min was assumed as a minimum recovery time between successive applications. Because of such a long-lasting desensitization to the action of TRH it was difficult to make a dose–response frequency curve. Using individual responses from single cells was impracticable because it was not possible to normalize them to take into account intercell variability. Preliminary tests showed that, when TRH was continuously superfused for 20 min, the rise in frequency peaked at ~60 s and lasted for up to 15 min ($n = 3$), followed by a gradual decline in frequency and amplitude of the sPSCs. Washout for 30 min was sufficient for substantial recovery. Hence, increasing concentrations (0.1–5 μM) of the peptide were applied to the same cells at 3 min intervals. Within the constraint of tachyphylaxis, this approach allowed us to establish that the apparent maximal effect of TRH was present at 0.5 μM concentration and that the approximate ED_{50} value was 0.1 μM, as shown in the graph of Figure 2b and the accompanying original traces (Fig. 2a). These data, while unable to provide a strict quantification of the pharmacological potency of TRH, did justify the use of the 10 μM concentration as capable of inducing maximal responses.

On average, after application of TRH the frequency of sPSCs increased from 1.07 ± 0.68 to 3.16 ± 0.73 Hz ($P < 0.01$, $n = 21$), as evaluated from records starting 1–2 min after the drug application. In the majority of cells (16/21) TRH did not significantly change the amplitude of sPSCs (29.4 ± 7.6 pA in control versus 33.7 ± 12.1 pA in TRH); only in 5/21 cells was the amplitude significantly increased (108 ± 13%) after 3 min of application of TRH. On a sample of 24 neurons the cell input resistance did not vary in the presence of TRH (133 ± 34 MΩ in control versus 125 ± 45 MΩ in TRH). Even when the input resistance was examined for the subset of five neurons upon which TRH elicited an increased amplitude of sPSCs, no significant change was found (−10 ± 14%), which ruled out any increase in postsynaptic current as due to increased membrane resistance. TRH application did not significantly change the mean decay time of the sPSC (39.7 ± 12.8 ms in control versus 35.2 ± 4.4 ms in TRH, $n = 9$) or rise time (3.2 ± 0.5 ms in control versus 2.9 ± 0.5 ms in TRH, $n = 11$). In order to check for a possible direct regulation by TRH of GABA release mechanisms, miniature PSCs (mPSCs) were recorded after block of action potentials by tetrodotoxin (1 μM). Application of TRH in tetrodotoxin solution did not significantly affect miniature PSC frequency (0.25 ± 0.05 Hz in control versus 0.23 ± 0.05 Hz in TRH, $n = 6$).

Localization of the TRH effect

To identify the regional location of TRH-sensitive GABAergic neurons, minislices were cut from transverse hippocampal slices by removing the dentate gyrus, the CA3 area or both. Seven of eight cells displayed sPSCs in control or after the application of 10 μM TRH, and in all cases TRH increased sPSC frequency. Assigning the numbers I, II and III to the groups lacking respectively CA3, dentate gyrus and both, the increase in frequency was from 3.49 to 5.98 Hz for group I ($n = 3$), from 1.12 to 2.65 Hz for group II ($n = 2$) and from 0.57 to 3.36 Hz for group III ($n = 3$). The variation of the frequency for the whole group was from 1.56 ± 0.95 in control to

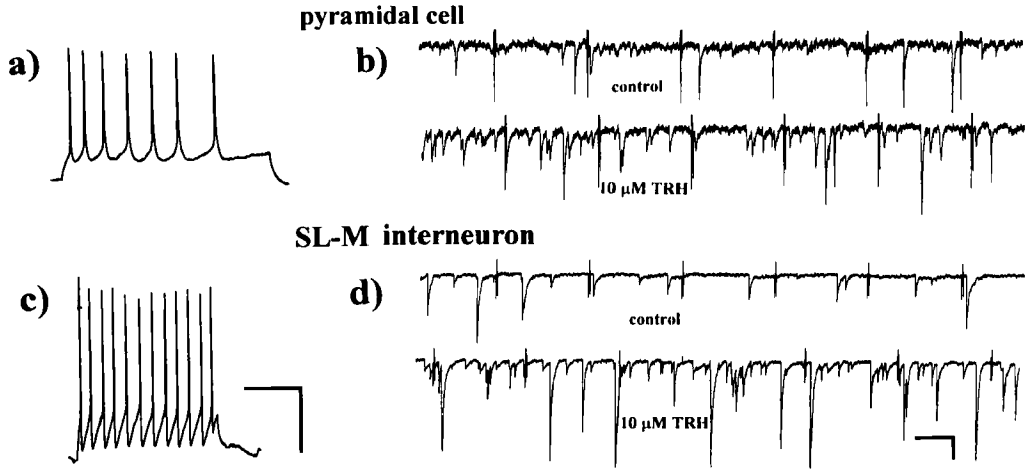

FIG. 1. Effect of a maximal dose (10 μM) of TRH on sPSCs. Patch-clamp recording was performed from visually identified pyramidal neurons or from interneurons of SL-M layer. Panels (a) and (c) show current-clamp responses to a 40 pA current pulse (0.8–1 s). The pyramidal cell displays a typical accommodating firing pattern while the interneuron supports regular high-frequency spiking with virtually no accommodation. Calibration bars: 400 ms, 50 mV. Spontaneous PSCs recorded under voltage clamp from pyramidal cell (b) as well as from SL-M interneuron (d) are shown as downward deflections (inward currents) and are sensitive to TRH application. Note that traces also contain outward (upwards) current responses (plus transients) elicited by 5 mV step pulses (40 ms) used to regularly monitor cell input resistance. In each pair of traces (b and d) upper records are controls while lower records are after 2 min application of TRH (10 μM). Kynurenic acid (1 mM) was present throughout to eliminate the glutamatergic drive. The increase in frequency was from 1.9 Hz to 6.1 for the pyramidal cell and from 1.8 to 8.0 for the SL-M interneuron. Mean sPSC amplitude is not significantly varied by TRH. Calibration bars: 1 s, 20 pA.

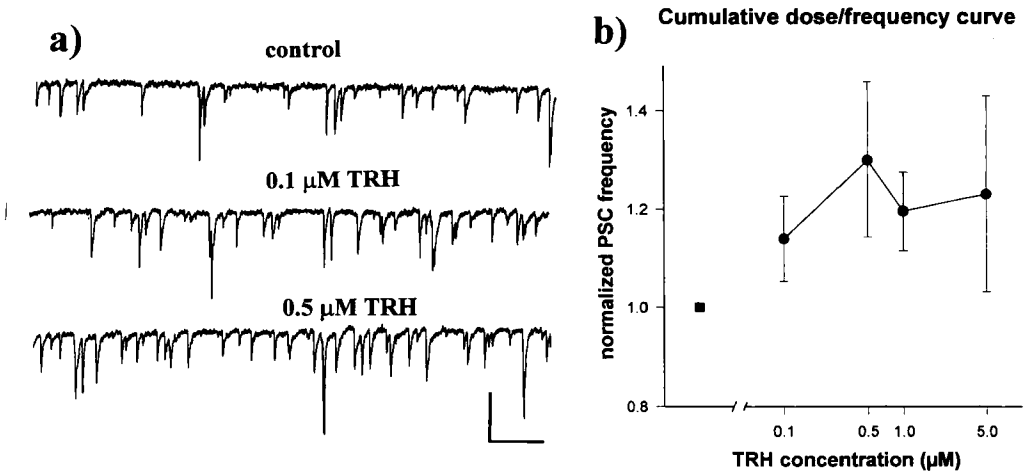

FIG. 2. Variation of sPSC frequency in response to varying doses of TRH. Doses of TRH were consecutively applied to obtain a cumulative dose–frequency curve normalized to sPSC frequency in the presence of 1 mM kynurenic acid (control). (a) A rise in frequency is already present at 0.1 μM TRH (from 2.5 Hz in control to 3.2 Hz in 0.1 μM TRH) and is maximal at 0.5 μM (4.0 Hz). (b) Dose–frequency curve (n = 3). Calibrations: 1 s, 100 pA.

3.76 ± 1.22 Hz after TRH. The variations in frequency for the whole set and for group III were statistically significant ($P < 0.05$).

Interneurons of stratum lacunosum-moleculare

Non-pyramidal interneurons were identified in SL-M and patch-clamped using the same conditions as for pyramidal cells. The presence (or absence) of accommodation in the firing pattern (in current-clamp mode) provided a functional identification of the cell type (Fig. 1c). Mean amplitude (23.8 ± 5.7 pA), rise time (3.2 ± 0.5 ms) and decay time (42.7 ± 4.8 ms) of sPSCs were not significantly different from those recorded from pyramidal cells (n = 6). Application of 10 μM TRH elicited also in this case an increase in sPSC frequency from 1.63 ± 0.61 to 3.28 ± 0.63 Hz (n = 13), as shown in the example in Figure 1d. Cell input resistance in control solution (436 ± 159 MΩ, n = 6) was significantly different ($P <$ 0.02) from that of pyramidal cells and was not affected by TRH application (+8 ± 27%).

Double recording from pyramidal cell–stratum lacunosum-moleculare interneuron pairs

Simultaneous double recording from a visually identified pyramidal neuron and a neighbour interneuron was performed in six cases. Two spontaneous events were considered simultaneous if they occurred at 3 ms interval or less. The frequency of simultaneous events was

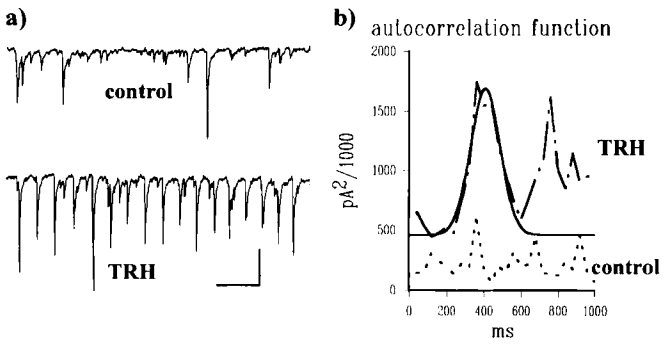

FIG. 3. TRH induces rhythmic GABAergic PSCs recorded from an SL-M interneuron in the presence of kynurenic acid (1 mM). (a) Spontaneous activity in control solution (top) and after ~2 min TRH application (bottom) when large PSCs appear in a rhythmic fashion. Calibration bars: 1 s; 5 pA. (B) Plot of the autocorrelation function in control (dotted line) and after application of TRH (broken line). The first harmonic of the autocorrelation function in the presence of TRH is fitted by a Gaussian (continuous line) centred at 408 ± 6 ms with 80 ± 9 ms variance. The ratio between the amplitude of the Gaussian and the baseline level is 2.66 ± 0.18.

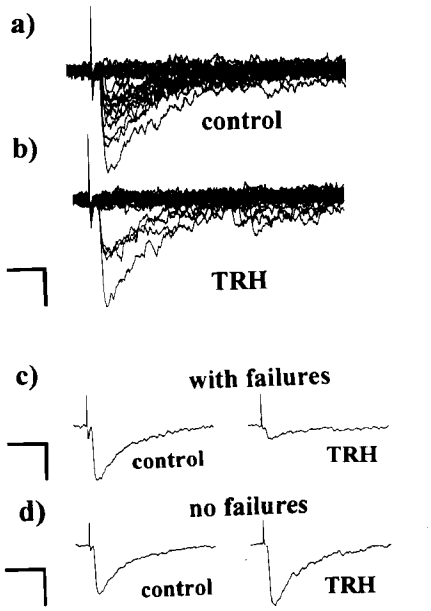

FIG. 4. Effect of TRH on evoked PSCs. PSCs are recorded from a pyramidal cell following stimulation of the SL-M layer with a patch electrode filled with extracellular solution. Panels (a) and (b) show the first 20 superimposed raw traces before and after bath application of 10 μM TRH (in the presence of 1 mM kynurenic acid). For these responses the latency from the stimulus artefact is 4.5 ms; the rise and decay times are ~1.5 and ~24 ms respectively. Calibration bars: 20 ms, 150 pA. (c) Averaging over 50 responses (including failures) yields a mean amplitude of 88 ± 106 pA in control versus 20 ± 87 pA in 10 μM TRH. Calibration bars: 40 ms; 60 pA. (d) After excluding failures from averages it is clear that TRH does not depress evoked responses. The number of failures rises from 28/50 in control solution to 42/50 in TRH solution. Calibration bars: 40 ms; 160 pA.

compared with the frequency of randomly expected concurrences, given by:

$$\nu = \nu_1 \cdot \nu_2 \cdot \Delta t$$

where ν_1 and ν_2 are the event frequencies of the two cells and Δt is the width of the window. The calculated frequency of random concurrences was 0.022 ± 0.012 Hz, while the experimentally observed frequency was an order of magnitude larger, i.e. 0.103 ± 0.089 (n = 6). In all six recordings it was possible to test the action of TRH on the concurrences. In this case sPSC frequency for both cell types was largely increased by TRH (1.87 ± 0.35 Hz in control versus 4.31 ± 0.72 Hz in TRH for pyramidal cells and 1.67 ± 0.32 Hz in control versus 3.21 ± 0.39 Hz in TRH for interneurons), as also found with individual cell recording. The ratio between the frequency of measured and random concurrences was 4.32 ± 1.36 in control solution, a value virtually equal to that observed in TRH solution (4.49 ± 0.95). This finding indicates that in the presence of TRH the degree of coupling between GABAergic events of the two neurons in the pair was preserved.

TRH-induced rhythmic postsynaptic currents

In contrast to the effects of TRH described above, a small number of neurons (3/25 interneurons and 1/42 pyramidal cells) displayed an unusual response to TRH characterized by large-amplitude PSCs regularly time-spaced at ~2 Hz, as in the example in Figure 3a. Cells presenting this phenomenon were not different from the others possessing the more commonly observed response, in terms of their input resistance and baseline holding current. Even if the occurrence of this particular response was low, it seemed interesting to examine it in some detail because it indicated that the responsiveness of hippocampal neurons to TRH was not homogeneous. Such PSCs displayed the typical kinetic and pharmacological profile of GABAergic events since they were blocked by 10 μM bicuculline and their rise and decay times were not significantly different from control values. Autocorrelation analysis was performed on series of rhythmic sPSCs, considering each event as a point-like Dirac delta function $A \cdot \delta(t - t_0)$, where t_0 is its starting point, having an area equal to the event peak amplitude A. The intervals between each PSC and its next ten adjacent events were calculated and histogrammed in each trace. A peak at ~500 ms in the autocorrelation function corresponding to the first harmonic was fitted by a Gaussian curve plus a constant describing the randomly occurring intervals, as exemplified in Figure 3b. The centre of the Gaussian was at 526 ± 96 ms with a variance of 65 ± 8 ms (n = 4), corresponding to a frequency of 1.90 ± 0.21 Hz. The ratio between the amplitude of the Gaussian and the constant was 4.39 ± 1.10, significantly different from 0 ($P < 0.03$). In one case rhythmic PSCs were detected in an SL-M interneuron during a simultaneous recording from a pyramidal cell. In the pyramidal cell no large-amplitude events were readily recognizable synchronous with those in the interneuron.

TRH effects on evoked synaptic activity

In order to detect any influence of TRH on the phasic release of GABA PSCs were evoked by direct electrical activation of the local GABAergic circuitry. The stimulating electrode was placed onto the cell body of a visually identified neuron of SL-M while recording from a CA1 pyramidal cell. Focal stimulation (5–50 V; 50–200 μs) of SL-M with a patch electrode evoked PSCs at a fixed latency in the range 3–6 ms (mean latency was 4.9 ± 0.7 ms), suggestive of a monosynaptic event, as shown in the example of Figure 4. In 11 cells the application of TRH elicited a statistically significant decrease in the mean PSC amplitude from 90 ± 27 to 44 ± 15 pA ($P < 0.05$, n = 11) as shown in the example of Figure 4a, b. After elimination of the failures from the calculation, the difference in PSC amplitude was, however, no longer statistically significant (122 ± 47 pA in control versus 82 ± 26 pA in TRH, n = 11). Rise time (3.3 ± 0.7

ms in control versus 3.8 ± 1.0 in TRH, $n = 6$) and decay time (35.0 ± 4.7 ms in control versus 33.1 ± 3.1 ms in TRH, $n = 6$) were also unchanged. TRH increased the probability of failure from 27 ± 8 to 41 ± 10% ($P < 0.05$, $n = 11$). An example of this effect is shown in Figure 4c, d, in which the mean of all responses including failures (displayed in Fig. 4c) is clearly larger in control (left) than in TRH solution (right). Conversely, after eliminating failures from the calculation, the mean amplitude of response did not decrease after TRH application (Fig. 4d).

Discussion

The principal finding of the present study is the novel demonstration of the modulation by the endogenously occurring neuropeptide TRH of pharmacologically isolated $GABA_A$ receptor-mediated transmission on rat hippocampal neurons. GABAergic transmission was not uniformly affected by TRH since sPSCs were increased in frequency while electrically evoked PSCs were depressed. This result may appear paradoxical because the same neurotransmitter, namely GABA, was differentially modulated depending on whether it was released spontaneously or not. In order to resolve this apparent discrepancy, it seems advantageous to consider first the site of action of TRH and secondly to attempt to fit the available data within a unitary hypothesis which regards the dissimilar action of TRH on GABAergic transmission as due to local network properties. A large body of electrophysiological evidence supports the presence of distinct subsets of GABAergic interneurons regulating the activity of CA1 pyramidal cells of the hippocampus (for a review see Bernard and Wheal, 1994); further confirmation has recently been obtained with combined electrophysiological and histochemical methods (Buhl *et al.*, 1994). GABAergic inhibition of pyramidal neurons is typically induced by electrical stimulation of the stratum radiatum/lacunosum-moleculare layers (e.g. Alger and Nicoll, 1982), but it should be noted that GABAergic interneurons of this region usually display little spontaneous firing (Lacaille *et al.*, 1987), thus providing minimal contribution to the sPSCs recorded from CA1 neurons. Similarly, most GABAergic interneurons within the pyramidal layer itself have a rather low spontaneous firing rate (Lacaille *et al.*, 1987). Conversely, GABAergic interneurons of the oriens–alveus layer, which establish inhibitory connections with a number of hippocampal cells including pyramidal neurons and LM interneurons (Bernard and Wheal, 1994), exhibit prominent and persistent spontaneous firing (Lacaille, 1991), although their sparse distribution makes it difficult to stimulate them with electrical pulses. These observations are relevant to the interpretation of the present results since the differential action by TRH on evoked or spontaneous PSCs may simply reflect distinct cellular targets for the action of the peptide rather than dissimilar sensitivity of transmitter release mechanisms within the same cell type. Thus, TRH might regulate the excitability of pyramidal cells via different GABAergic cells to depress or augment specific inhibitory inputs.

Effects of TRH on spontaneous postsynaptic currents

In the present study visually identified pyramidal cells or interneurons of SL-M displayed spontaneous GABAergic synaptic activity which was similar in terms of frequency, mean amplitude, rise time and decay time. TRH elicited a persistent, large increase in the frequency of sPSCs of pyramidal cells as well as of SL-M interneurons. Since the frequency of mPSCs in tetrodotoxin solution was not increased by TRH, direct involvement of TRH in the mechanisms of GABA release is unlikely. In most cells the increased sPSC frequency was not accompanied by changes in the amplitude of the postsynaptic currents. Furthermore, there was no effect of TRH on baseline current and input conductance. All these observations suggest that the action of TRH was predominantly presynaptic at the level of the action potential-dependent release of transmitter by GABAergic interneurons. In a small subset of neurons TRH increased the amplitude of sPSCs; according to the classical interpretation of quantal analysis of synaptic transmission (Voronin, 1993), an increase in the amplitude of a synaptic current may originate from either enhanced responsiveness of the postsynaptic cell or presynaptic facilitation of transmitter release. Since no change in postsynaptic cell properties was detected, the augmented amplitude of sPSCs occasionally observed seems to be consistent with a presynaptic action of TRH which might have changed the composition of quanta, particularly if the peptide raised intracellular free Ca^{2+} (Ebihara and Akaike, 1993). The frequency of sPSCs in minislices lacking the dentate gyrus or the CA3 region (or both) demonstrated that TRH-sensitive neurons projecting to CA1 pyramidal cells and SL-M interneurons were localized within the CA1 area itself. Simultaneous measurements of synaptic activity from pyramidal neurons and SL-M interneurons directly indicated a high degree of functional coupling between them, as shown by the high ratio between randomly expected and measured concurrences of synaptic events. This ratio remained unchanged after application of TRH, indicating virtually the same action of the peptide on these two cell types.

Several possibilities could account for the observed synchronicity of synaptic currents. The first one is that the two cells directly influenced each other, but this interpretation would require that the activity of one cell would follow regularly the activity of that of the other at constant latency, a fact not borne out by experimental observations. Moreover, as both cells were clamped at –70 mV their ability to fire action potentials was blocked, thus preventing cross-communication. A more likely possibility is that the high degree of synchronicity derived from the fact that synaptic responses from each neuron were driven by the activity of a third, distinct cell type directly sensitive to TRH. This proposal accords with the similarity of sPSCs from pyramidal neurons and SL-M interneurons. Although direct histological validation of this view was lacking, it seems possible to suggest that the common source of inhibitory inputs might have been the subset of the oriens–alveus GABAergic interneurons according to the scheme of Bernard and Wheal (1994). The notion of selectivity of a particular class of GABAergic interneurons to TRH does not exclude effects of this peptide on other neurotransmitters. Recent studies have observed a large potentiation of NMDA receptor-mediated excitatory synaptic transmission in the hippocampus (Stocca and Nistri, 1995) and in the cerebral cortex (Kasparov *et al.*, 1994), while the synaptic responses mediated by AMPA receptors remained unaffected. These findings indicate that TRH affected more than one neurotransmitter system in a discrete fashion because it did not involve an indiscriminate modulation of neurotransmission via non-specific changes in cell properties. A comment may be added on the tachyphylaxis of the effects of TRH which, once developed, required a long recovery (~30 min) to be replicated. It seems probable that tachyphylaxis was not the result of whole cell dialysis by the patch pipette in view of the response recovery, but it might have been due to changes in the signal transduction mechanisms mediating the action of TRH (Gershengorn, 1986), as previously discussed for other central neurons recorded with sharp microelectrodes (Fisher and Nistri, 1993).

TRH-induced rhythmic postsynaptic currents

Even though large-amplitude GABAergic rhythmic events were detected in a small minority of cells, it seemed of interest to observe that they were elicited by TRH. The rhythm of such oscillatory PSCs

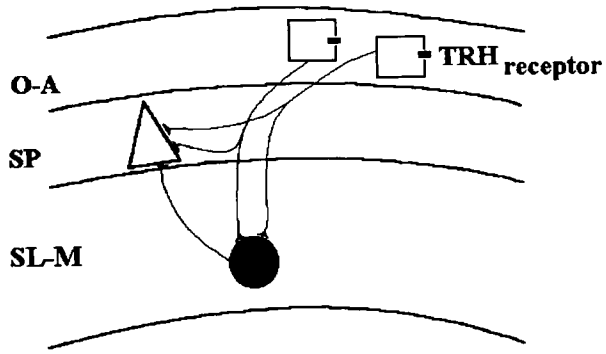

FIG. 5. Idealized representation of minimal hippocampal circuitry to account for the modulating action of TRH on GABAergic transmission. GABAergic interneurons (open squares) present in the oriens–alveus (O–A) layer possess TRH receptors as indicated and are therefore the primary target for the effect of such a neuropeptide. By stimulating GABA release from these cells TRH will enhance the inhibition of SL-M interneurons (filled circle) and of CA1 pyramidal cells (triangle). This will be observed as increased frequency of sPSCs recorded from pyramidal cells and SL-M interneurons while evoked PSCs, electrically evoked by stimulation of SL-M interneurons, are depressed.

(~2 Hz at room temperature) was near the lower values (4 Hz) of the hippocampal theta rhythm of *in vivo* animals (Ylinen *et al.*, 1995), especially when considering the large disparity in temperature. Although the theta rhythm is traditionally supposed to be dependent on the cholinergic projection from the septal area which is modulated by TRH (Lamour *et al.*, 1985), it seems that GABAergic interneurons of the hippocampus are important in shaping it (Stewart and Fox, 1990; Ylinen *et al.*, 1995). The complex circuitry underlying the generation of the theta rhythm prevents the use of the hippocampal slice as a suitable model for theta oscillations. Nevertheless, at least in the few cases found in the present study TRH could trigger GABAergic rhythmic oscillations even after severance of septal afferents in the process of preparing the slices.

Effects of TRH on the evoked postsynaptic currents

In the presence of kynurenic acid, focal stimulation applied to the SL-M area elicited (after constant delay) bicuculline-sensitive (probably monosynaptic) PSCs recorded from pyramidal cells. The stimulus intensity was adjusted to activate only a very small number of fibres (minimal stimulation), as judged by the presence of failures. When averaging the amplitude of a large number (~50) of PSCs, their mean peak was depressed after application of TRH. The changes in PSC amplitude were merely due to the increased number of failures of GABAergic currents as all the other parameters pertaining to these responses were essentially unaltered by TRH. A straightforward explanation might be that TRH directly inhibited the membrane excitability of SL-M interneurons. Against this hypothesis stand the findings obtained with direct recording from SL-M interneurons, in which no inhibitory action by TRH could be demonstrated in terms of variations in baseline current or input conductance. In TRH solution the increased frequency of sPSCs indicated that the GABAergic input received by these cells was actually increased. This suggests an indirect action by TRH, namely that the higher number of failures of evoked PSCs observed in pyramidal cells was probably due to the increase in background inhibition impinging upon GABAergic SL-M interneurons which reduced their firing probability and thus GABA release onto pyramidal cells. Figure 5 presents a highly simplified schematic diagram to show the minimal wiring required by a network comprising SL-M interneurons, CA1 pyramidal cells and oriens–alveus interneurons. While it is emphasized that the present scheme is only a working model, it nevertheless helps to interpret apparently paradoxical data with a unitary hypothesis. In the present study the action of TRH on firing patterns recorded under current-clamp conditions was not investigated; however, in a previous report it was noted that TRH reduced the afterhyperpolarization (following a train of action potentials) consequent to a block of a high-threshold Ca^{2+}-activated K^+ current (Ballerini *et al.*, 1994). In the present study it is unlikely that quiescent cells like pyramidal neurons and SL-M interneurons could have reached the activation range of this current because their excitation was routinely prevented by pharmacological block of glutamate receptors. This condition might not have applied to oriens–alveus interneurons, which are known to be spontaneously active (Lacaille and Williams, 1990; Lacaille, 1991) and are thought to be the origin of the increased GABAergic drive to pyramidal neurons and SL-M interneurons.

Cellular mode of action of TRH

The effects of TRH on a large variety of cells ranging from pituitary cells (Geshengorn, 1986) to brain neurons (Toledo-Aral *et al.*, 1993) appear to be due to activation of intracellular second messenger pathways leading to the formation of inositol triphosphate and the activation of protein kinase C. These events produce a transient rise in intracellular free Ca^{2+}. It is difficult to translate these effects into the observed increase in spontaneous GABAergic activity particularly because the identity of the TRH-sensitive neurons remains elusive. On pyramidal cells (Ballerini *et al.*, 1994), septal neurons (Toledo-Aral *et al.*, 1993) and pituitary cells (Bauer *et al.*, 1990; Kramer *et al.*, 1991) TRH can depress various K^+ conductances which contribute to inhibition of spike generation. One might suspect that, if a similar suppression of intrinsic K^+ conductances by TRH took place in a subset of GABAergic interneurons, the excitability of such cells and consequently their spike-dependent release of GABA would be increased. This phenomenon might have functional relevance to the operation of the network comprising CA1 pyramidal cells, particularly since it may help to complement the increase in NMDA receptor-mediated synaptic transmission (Stocca and Nistri, 1995).

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Abbreviations

CA1/CA3	cornu Ammonis 1/3
EGTA	ethylene glycol tetraacetic acid
GABA	γ-amino-butyric-acid
HEPES	*N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid)
NMDA	*N*-methyl-D-aspartate
SL-M	stratum lacunosum-moleculare
sPSC	spontaneous postsynaptic current
TRH	thyrotropin-releasing hormone

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