# ISAS - INTERNATIONAL SCHOOL FOR ADVANCED STUDIES

## Modulatory effects of thyrotropin-releasing hormone (TRH) on hippocampal CA1 neurons

Thesis submitted for the degree of

*Magister Philosophiae*

CANDIDATE      Gabriella Stocca

SUPERVISOR      Prof. Andrea Nistri

Academic Year 1992/1993

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# 1. INTRODUCTION

## 1.1 TRH AND ITS LOCALIZATION

Thyrotropin releasing hormone, TRH, is a tripeptide (pGlu-His-Pro-NH$_2$) produced by enzymic cleavage from a high molecular weight precursor containing five copies of the peptide itself (Segerson et al.,1987). It is principally produced in the hypothalamic cell bodies, transported to the end of their axons and then secreted into the portal vessel system via which TRH reaches its target cells in the anterior pituitary gland, where it induces the production of TSH. Nevertheless, the endocrine action of TRH appeared to be not only restricted to its influence on TSH release but it did also affect release of other hormones (Griffiths,1987).

Recently, more interest was focused on the extrahypothalamic functions of TRH rather than its endocrine role. More than two thirds of the brain content of TRH are localized outside the hypothalamus. Quantitative autoradiographic techniques (Sharif,1989) have established that TRH receptor binding sites were widely distributed in the CNS, both in the spinal cord and in many regions of the brain with particular respect to amygdaloid complex and hippocampal formation (Sharif,1989). Further studies using molecular biology techniques obtained very similar results concerning the regional distribution of TRH receptor messenger ribonucleic acid (mRNA) in the brain (Kaji et al.,1993).

TRH administration showed a wide range of clinical effects, including a general

excitatory action which can reverse general depression phenomena of CNS (Stanton et al.,1980). Furthermore, in motoneurons of the spinal cord TRH increases the activity level and it acts as a trophic factor during the development (Engel et al.,1983) and after injuries (Faden et al.,1983).

## 1.2. TRH AS A NEUROPEPTIDE

It was generally accepted for many years that neurons were communicating to each other through the release of relatively small weight molecules acting as neurotransmitters. During the last decades the existence in the central nervous system (CNS) of peptides previously considered only for their endocrine function, brought about the idea of their possible role in affecting neurotransmitters action. Immunohistological techniques proved the coexistence of various peptides and classical neurotransmitters in the same neuron and sometimes also in the same vesicle (Kow and Pfaff,1988). There is evidence that these peptides can act synergistically with the neurotransmitters at the postsynaptic site (Agnati et al.,1981).

The modulatory action exerted by these peptides may be different on a single neurotransmitter and depending on the region of the CNS on which it acts. That does mean that the neuropeptide receptors may be coupled to different metabolic pathways. TRH has been found to have a neuromodulatory action on both motoneurons and cortical neurons. In the latter TRH is capable of potentiating the excitatory action of acetylcholine (Braitman et al.,1980) while in rat spinal motoneurons in vivo TRH

enhances glutamate and aspartate evoked activity (White,1985). Furthermore, in hypoglossal motoneurons the peptide can potentiate NMDA evoked responses in a voltage dependent manner but not the responses to glutamate, aspartate or quisqualic acid (Rekling,1992). Other studies showed a facilitatory effect of TRH on LTP in the hippocampus, a phenomenon that seem to be mediated by glutamate receptors. In spinal cord motoneurons TRH was blocking an apparently novel $K^+$conductance (Nistri et al.,1990).

## 1.3. PRESENT EXPERIMENTAL STUDY

In the present project it has been investigated the effect of TRH on the CA1 hippocampal neurons from adult rat. In order to clarify at which level, either presynaptic or postsynaptic, TRH was exerting its influence we used current clamp techniques in order to examine its effect on membrane passive and active properties. On the other hand, voltage clamp techniques were used in order to investigate possible variations in voltage activated conductances.

# 2. MATERIALS and METHODS
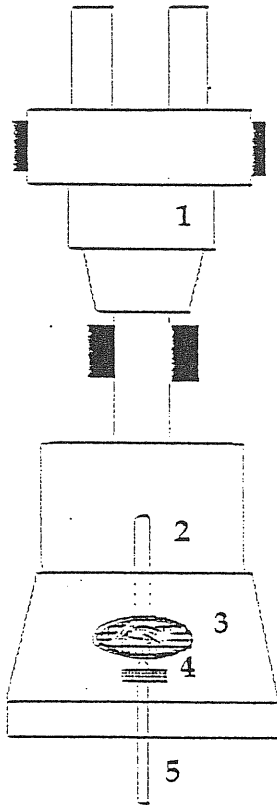
## 2.1. SLICE PREPARATION

Adult (16-38 days) Wistar rats were housed at constant temperature (20°C) and humidity with unrestricted access to standard chow and water. Animals previously anaesthetized were decapitated, their brain removed from the skull and rapidly submerged in artificial cerebrospinal fluid (ACSF) of the following composition (mM):

NaCl 126, KCl 3.5, CaCl$_2$ 2, NaH$_2$PO$_4$ 1.2, MgCl$_2 \cdot$ 6H$_2$O 1.3, NaHCO$_3$ 25, glucose 11.

The ACSF was equilibrated with 95% O$_2$ and 5% CO$_2$ to reach pH of 7.3-7.4 at room temperature. The two emispheres of the brain were separated with a sharp blade and the two hippocampi carefully isolated. Each one of them was laid on an Agar bed (4 gr Agar in 100 ml of a physiological saline obtained diluting 9 gr of NaCl in 1000 ml distilled water) and transversally cut into slices with a Mc Ilwain tissue chopper (The Mickle Laboratory Engeeniring Co.Ltd.). The slices (500 µm thick) were allowed to recover in oxygenated ACSF at room temperature for about 1 hour before use. A single slice was transferred to a recording chamber (about 1 ml volume) which contained a plastic ring with a nylon mesh on which the slice was laid (Fig.1A) and continuously superfused at room temperature with ACSF saturated with oxygen at a rate of 2.5-4 ml per minute. The fluid was gravity fed in order to reduce possible electrical noise due to a power-operated pump. The bath was transilluminated by optic fibers and the layer of CA1 pyramidal cells unambiguously identified (see Fig.1B).
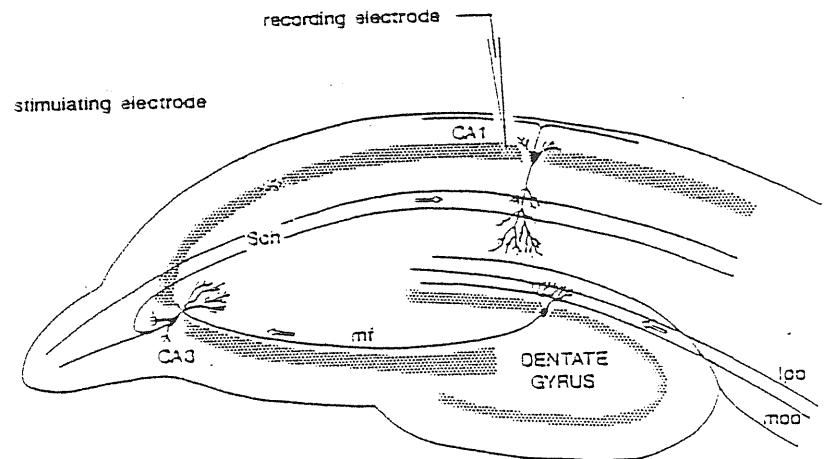
**Figure 1.** A: diagram of the recording chamber arrangement: 1 microscope, 2 inlet pipe, 3 nylon mesh with hippocampus slice, 4 paper wick, 5 outlet pipe.
B: Schematic diagram of the rat hippocampal slice preparation: CA1 and CA3 regions, lateral (lpp) and medial (mpp) perforant paths, mossy fibers (mf) and Schaffer collateral-commissural (Sch) pathway(Collingridge and Davies, 1989) .

## 2.2. INTRACELLULAR TECHNIQUES

Intracellular recordings, current injection, discontinuous current clamp (DCC) and single electrode voltage clamp (SEVC) were conducted using an Axoclamp 2A amplifier (Axon Inst. Burlingame, CA). Microelectrodes with a resistance of 50-110 M$\Omega$ were pulled by a Brown-Flaming Micropipette Puller (Sutter Inst. San Francisco, CA) using borosilicate glass tubing, 1 and 1.5 mm O.D., (Clark Electromedical Instruments) and filled with 3 M KCl, 3 M CsCl or 4 M K-acetate (pH adjusted to 7.2 with glacial acetic acid). It were then inserted in a holder filled with 3 M KCl connected through a pin to the headstage

of the Axoclamp. The headstage is a preamplifier that allows electronic control of the signal before it leaves the Faraday cage.

## BRIDGE MODE

Cells were impaled in Bridge mode (socalled because it refers to the original Wheatstone bridge circuit once used to balance the voltage drop of the electrode and now replaced by an operational amplifier) after having properly compensated the capacitance of the pipette, balanced the bridge and offset the voltage. Searching for cells was done after positioning of the electrode in the CA1 region and applying short (10-15 ms) hyperpolarizing pulses of 0.3-0.4 nA amplitude at high frequency (10Hz) while advancing through the slice in small steps with the aid of a Narashige micromanipulator. When the surface of a cell was reached, a sudden increase in resistance was detected on the oscilloscope and the impalement was obtained using the "clear" switch of the Axoclamp through which a large, transient current can be sent down the pipette making its tip to oscillate and enter the cell. Just after impalement a negative steady current (about -0.3 nA) was injected into the cell, to stabilize recording.

During all the current clamp experiments two pulses of current, one second apart, an hyperpolarizing and a depolarizing one (about 0.1 and 0.3 nA amplitude, 400 ms and 300 ms duration respectively) at a frequency of 0.1 Hz were routinely applied to the cell for continuous monitoring of the membrane input resistance ($R_m$).

DCC

The DCC mode was switched on after optimizing capacitance neutralization as well as sampling rate in order to obtain a full decay of voltage transients. This technique allowed more reliable measurement of the membrane potential (Vm) even in the presence of changes in microelectrode resistance since sampling (3-4 KHz) of the membrane voltage is performed after transient modifications of potential, due to electrode resistance, have dissipated. If requested by the protocol the DCC mode was then followed by discontinuous SEVC.

dSEVC

This procedure was started by ensuring that the clamp level corresponded to resting membrane potential. The gain control and the anti-alias filter were set to a minimum and a repetitive -10 mV step was applied. At this point the gain was gradually increased to $\geq 1$ nA/mV with fine tuning of the phase shift for current/voltage switching until a satisfactory voltage control of the onset/offset of the step was achieved coincident with a smooth current response. In the recorded currents the leak subtraction was performed on the basis of the IV curve obtained at potentials at which all the membrane voltage activated conductances were supposed to be shut.

Only cells in good condition were studied, with initial Vm of -70 mV and spike amplitude exceeding 85 mV.

Current and voltage responses were recorded both on a GOULD chart recorder and on a video cassette recorder (PHILIPS) using a VR-10A CRC digital data recorder

(Instrutech-Corporation).

## 2.3 CHEMICALS

All chemicals used were purchased from the drug companies listed below:

TOCRIS-NEURAMIN:

N-methyl-D-aspartate (NMDA),

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)

6-cyano-7-notroquinoxaline 2,3-dione (CNQX)

(-)-bicuculline methochloride

3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP)

7-chlorokynurenic acid

SIGMA Chemical Co.:

Tetrodotoxin (TTX)

atropine sulphate salt

tetraethylammonium-chloride (TEA)

4-aminopyridine (4AP)

CYANAMID-TAKEDA:

thyrotropine-releasing hormone tartrate salt (TRH)

## 2.4 DATA ANALYSIS

Data presented were averaged and standard error of the mean for the population was calculated. In order to assess the statistical significance of result the Student's $t$ test for paired samples was used.

# 3. RESULTS

## 3.1 REPEATED APPLICATIONS OF GLUTAMATE AGONISTS

Results were obtained from 32 hippocampal neurons with a membrane potential of -61±1 mV and 69±6 MΩ input resistance at rest. In Fig.2 a record from one of these cells can be observed: spontaneous spiking activity on top of synaptic events was superimposed with regular upward and downward deflections of membrane potential elicited by depolarizing and hyperpolarizing steps, respectively. Repeated bath applications of NMDA (5-15 µM) (usually <1 min duration) (Fig.2) were delivered every 10-15 minutes.
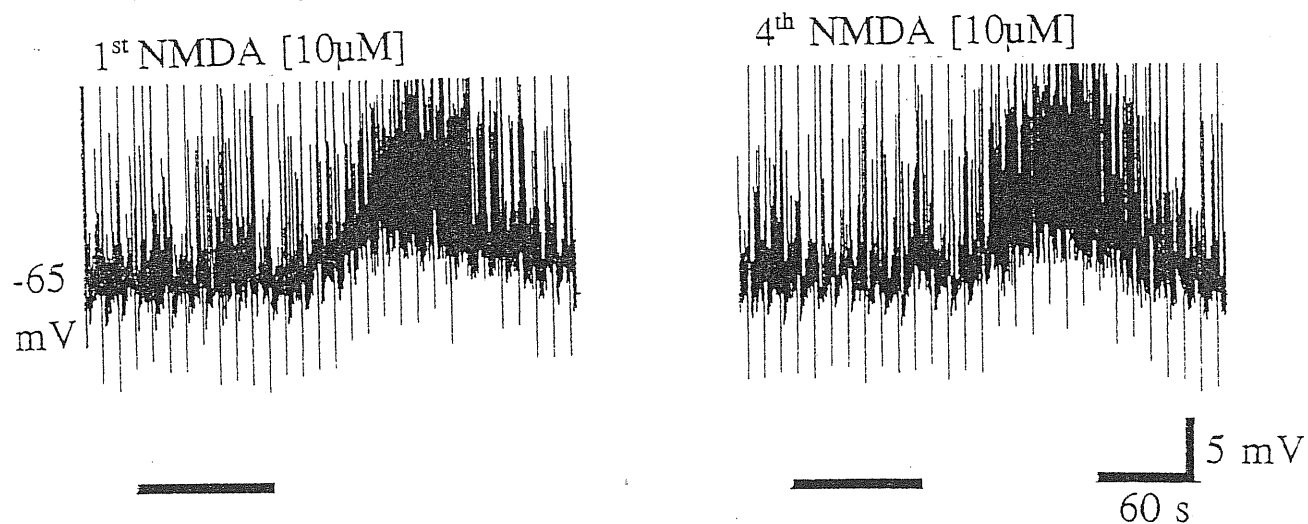


**Figure 2.** Effect of repeated applications of NMDA on CA1 hippocampal neurons. Chart record of membrane potential recording in control solution. NMDA was applied for the duration of the bar. Hyperpolarizing (400 ms) and depolarizing (350 ms) current pulses (0.15 and 0.3 nA, respectively) were regularly injected via the microelectrode at 0.1Hz. Time interval between applications was 10min. Responses were not significantly changed. Spikes truncated by frequency response of recorder.

The duration of application and the agonist concentration were adjusted in each cell to elicit a small depolarization (11±3 mV). NMDA responses were developing within a few seconds after the end of application and were characterized by an augmentation of synaptic activity leading to a depolarization and a further increase in spiking activity. Repolarization of the membrane potential was attained after about 4 minutes, and complete recovery to resting level activity in about 10 minute time.

There was an apparent increase (about 20%) in the third and forth response with respect to the first one, but this phenomenon was not statistically significant (Fig.4, open triangles).

Another set of experiments was performed on a different group of cells (n=5) using AMPA, a non-NMDA glutamate agonist (Fig.3).
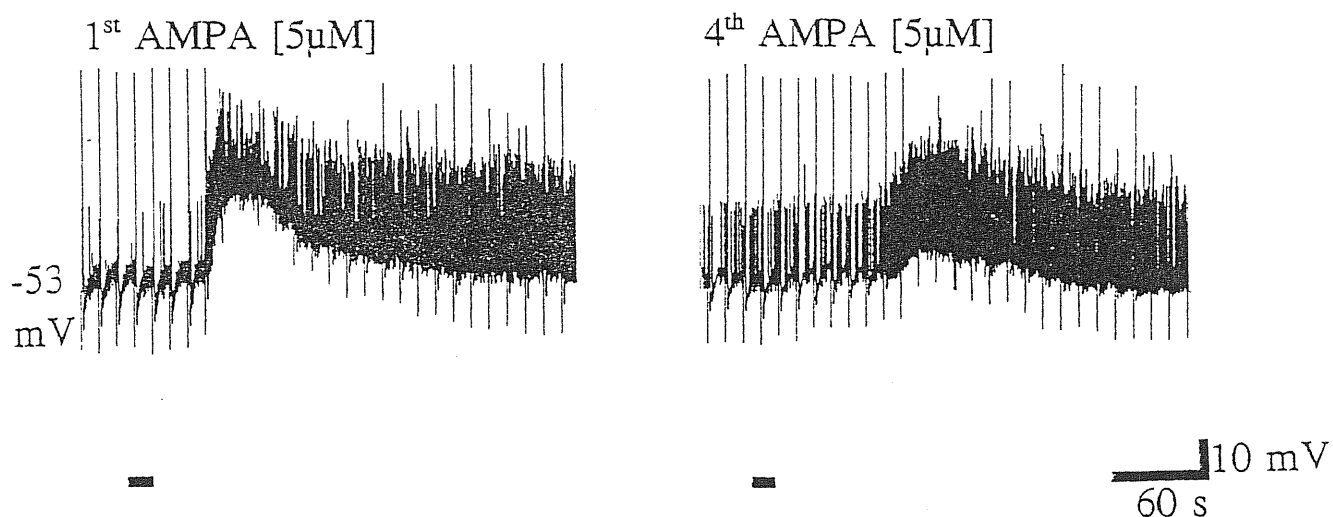


Figure 3. Effect of repeated applications of AMPA. Chart record of membrane potential recording in control solution. AMPA applications (see bar) elicited desensitized responses. AMPA was slower to wash, time interval between applications was 15 min. (for current pulses see Fig.2)

AMPA (5-10 μM) was repeatedly applied following the same protocol as with NMDA. To elicit similar depolarizations (11.2±2.8 mV) the duration of applications was shorter (10-30 s) compared with the NMDA ones, and the depolarizations produced were faster to develop and slower to come back to the same resting membrane potential ($V_m$) preceding the application. Usually, even after recovery of membrane potential, spiking activity persisted (indicating lowering of spike threshold) so further applications of AMPA were delivered after a longer wash (20-25 minutes in ACSF). The amplitude of AMPA responses was decreasing with further administrations (Fig.4, open circles), a phenomenon due to desensitization (Trussel et al., 1993).
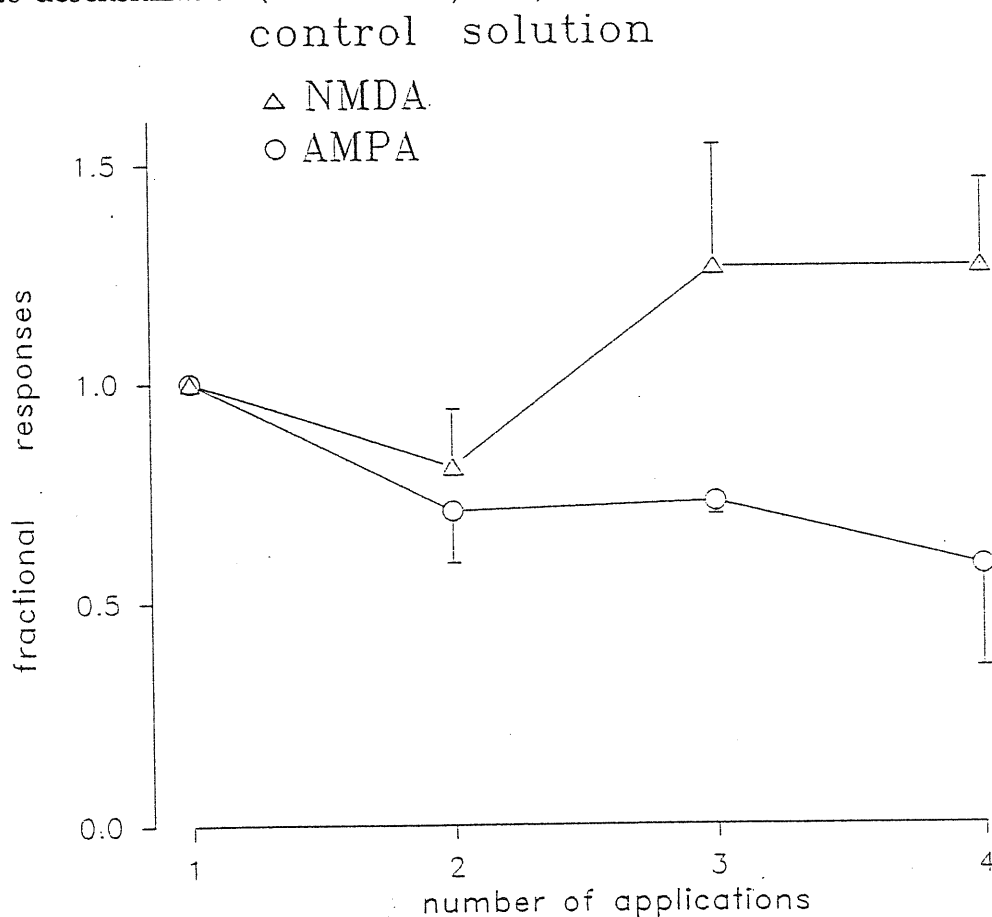


Figure 4. Comparison between NMDA and AMPA responses amplitudes in control solution. NMDA (open triangles) and AMPA (open circles) responses amplitudes (ordinate) vs number of agonist applications (abscissa). Responses were normalized with respect to the first response. Note that AMPA desensitization reached the steady state already at the second application.

## 3.2 EFFECT OF TRH ON HIPPOCAMPAL NEURONS

Records obtained with electrodes filled with KCl or K-acetate filled showed that applications of TRH (5-10 µM) did not significantly change either the $V_m$ (depolarization elicited: $1\pm0.44$ mV) or membrane input resistance ($68\pm6$ vs $69\pm6$ M$\Omega$), unlike the results obtained from spinal cord motoneurons (Nistri et al.,1990). Nevertheless, TRH elicited (after 5-10 minutes from the application onset) a clear and sustained increase in spontaneous electrical activity (see, for example, Fig.5: tract of the record before NMDA application), probably due to a combination of presynaptic phenomena (e.g. modulation of transmitter release) and postsynaptic effects (e.g. increase in the excitability of the postsynaptic membrane, even if undetected by input resistance measurements).

## 3.3 INHIBITORS OF TRH EFFECT

Preliminary tests were made to identify via which transmitter(s) TRH was upregulating spontaneous neuronal activity. CNQX (10 µM) and atropine (1 µM), antagonists of non-NMDA and muscarinic acetilcholine receptors respectively, did not block this effect of TRH. On the other hand 20-50 µM bicuculline (an antagonist of $GABA_A$), 1 µM kynurenic acid (a broad spectrum excitatory aminoacids antagonist) and 10 µM CPP (an NMDA receptors antagonist) blocked the increase in synaptic activity induced by TRH (data not shown).

## 3.4 EFFECT OF TRH ON GLUTAMATE AGONISTS

Since it has been observed that TRH facilitated LTP in CA3 hippocampal neurons (Ishihara et al.,1991), and since glutamate receptor activation is presumably involved in this process (Bliss and Collingridge,1993), the effect of TRH on responses to glutamate agonists was investigated. During continuous application of TRH was repeated doses of NMDA or AMPA were administered with the same duration and concentration used in control ACSF. TRH showed a modulatory effect leading to a potentiation of NMDA induced responses as indicated in Fig.5.
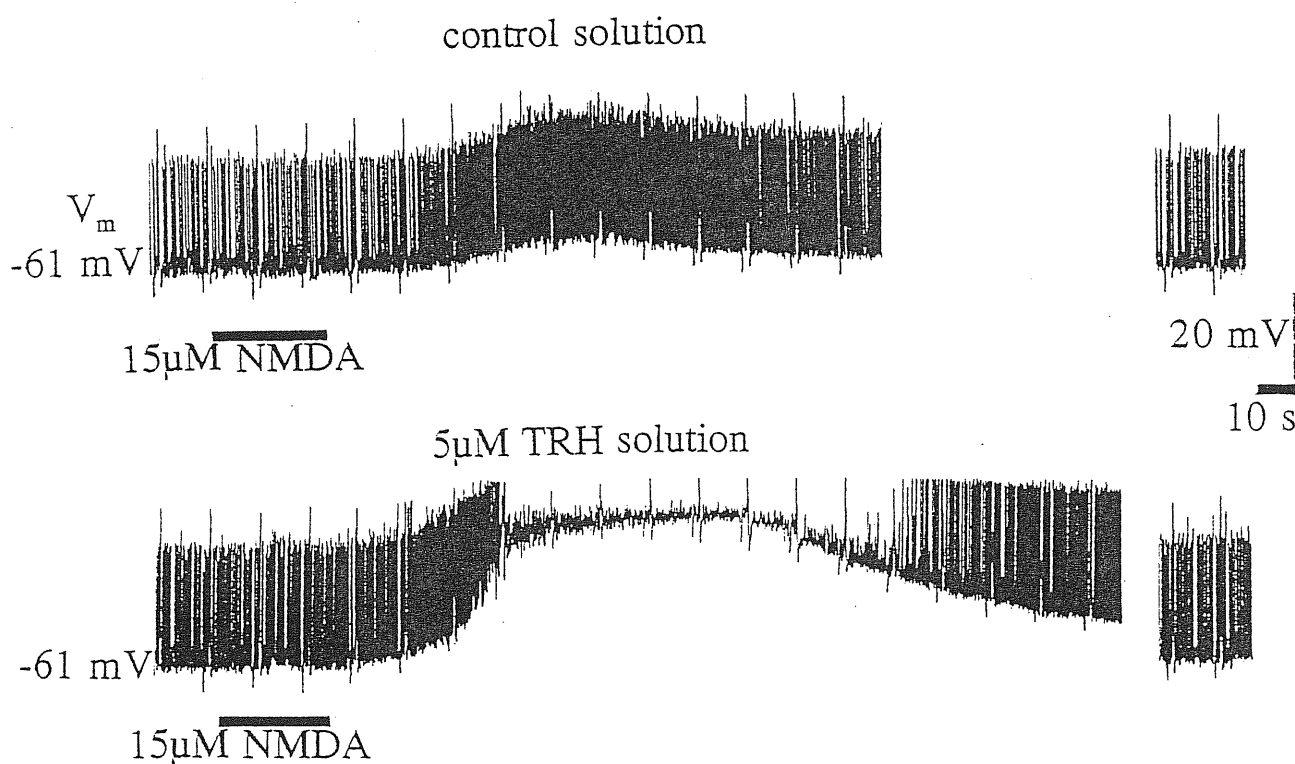


**Figure 5.** Effect of TRH on NMDA responses. Chart records of NMDA response in control solution (top trace) or in the continuous presence of TRH (bottom). The top trace shows the response produced by the fourth application of NMDA at resting membrane potential of -61 mV. The gap in the trace corresponds to about 10 min wash after which recovery was attained. The bottom trace shows that on the same cell at the same membrane potential, after 5 min exposure to TRH, the same application of NMDA elicited a much larger response. Full recovery was again obtained after 10 min (see trace after gap). Note increase of synaptic activity in TRH before NMDA application. (for current pulses see Fig.2)

Note that the depolarization onset elicited by NMDA was not significantly changed. On

the other hand, the response was increased both in terms of amplitude and duration: in

this example the depolarization itself was intense enough to block spiking activity.

Between applications the cells were washed for at least 10-15 minutes in order to reattain

to the same baseline $V_m$ as before the NMDA application (Fig.5).

This potentiation can be readily observed when responses to NMDA in control solution

(open triangles of the plot in Fig.6) are compared to NMDA responses in TRH solution

(filled circles) which were normalized with respect to the last response before TRH
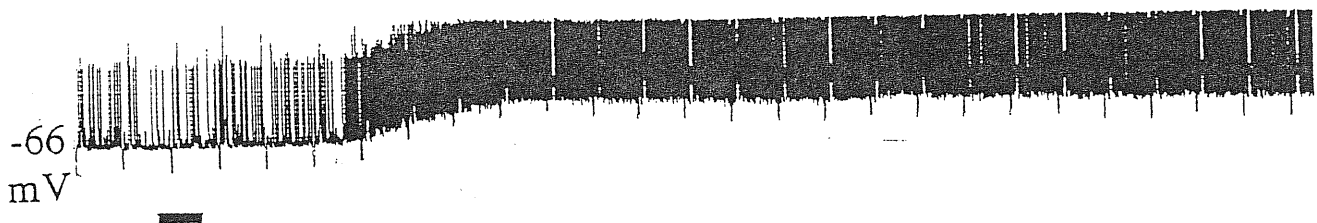
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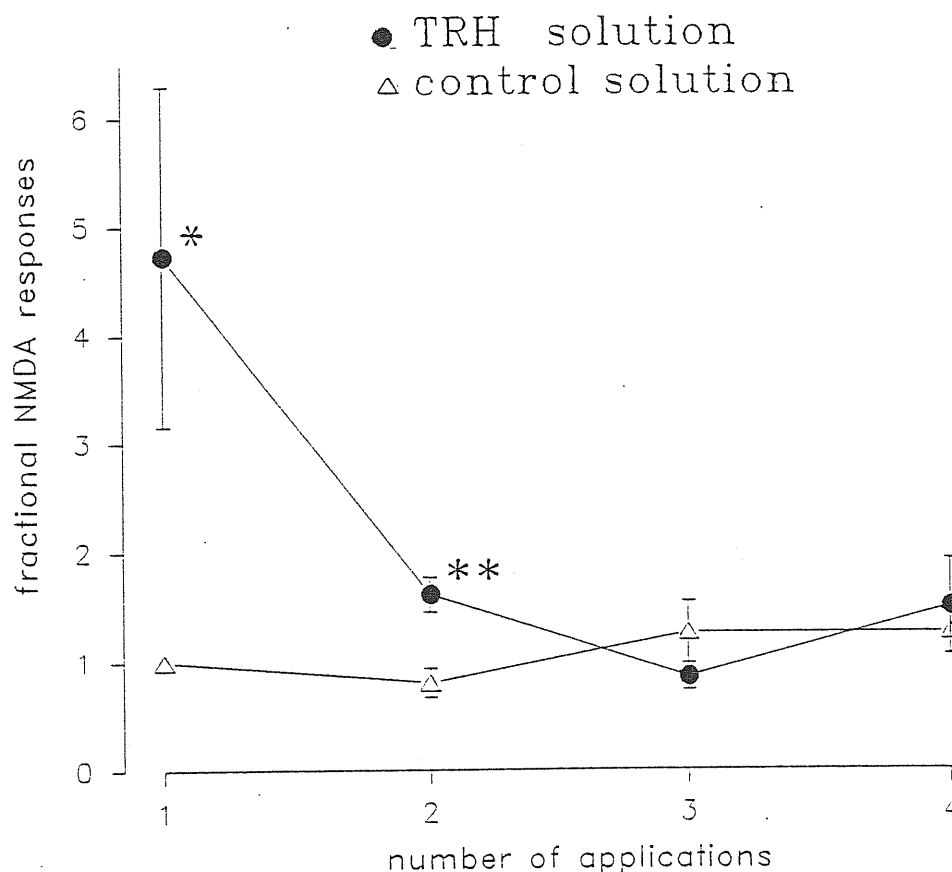


Figure 6. Effect of TRH on repetitive NMDA applications. Comparison between NMDA responses elicited in control solution (open triangles) and after 5 min in 5 µM TRH (filled circles). Control responses were normalized as in Fig.4 legend, while TRH responses were normalized with respect to the last response in control solution. Asterisks indicate that the difference between responses in the presence of TRH and their respective controls was statistically significant (*=P<0.02 and **=P<0.05, respectively when tested with the paired t-test on original data).

In 8/10 neurons the first response (Fig.6) in TRH solution was 4.7±1.5 times larger than its preceding control (P<0.02 for actual depolarization) although such a potentiation declined with the second responses being only 1.6±0.1 times larger (P<0.05). Further NMDA applications did not show a significant potentiation.

AMPA was applied to another set of neurons (n=5) on which TRH had the same enhancing effect on spontaneous activity. In this case there was a small increase in amplitude of AMPA responses but it was not statistically significant (see Fig.7 and 8).

4$^{th}$ AMPA [5µM] in control solution

-66
mV

1$^{th}$ AMPA [5µM] in TRH [5µM] solution

-66
mV

10
mV

10 s
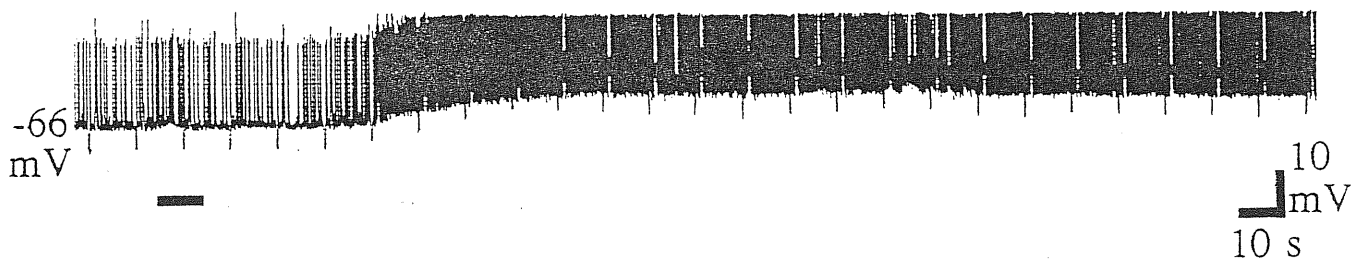
Figure 7. Effect of TRH on AMPA responses. Recording of AMPA response in control solution (top) and in the continuous presence of TRH (bottom). The two responses were not significantly different (for current pulses see Fig.2).

In the plot of Fig. 8 it is can be seen more clearly that even though there was a certain

enhancement of the response, and it was not significant, the desensitization did seem to be still there.
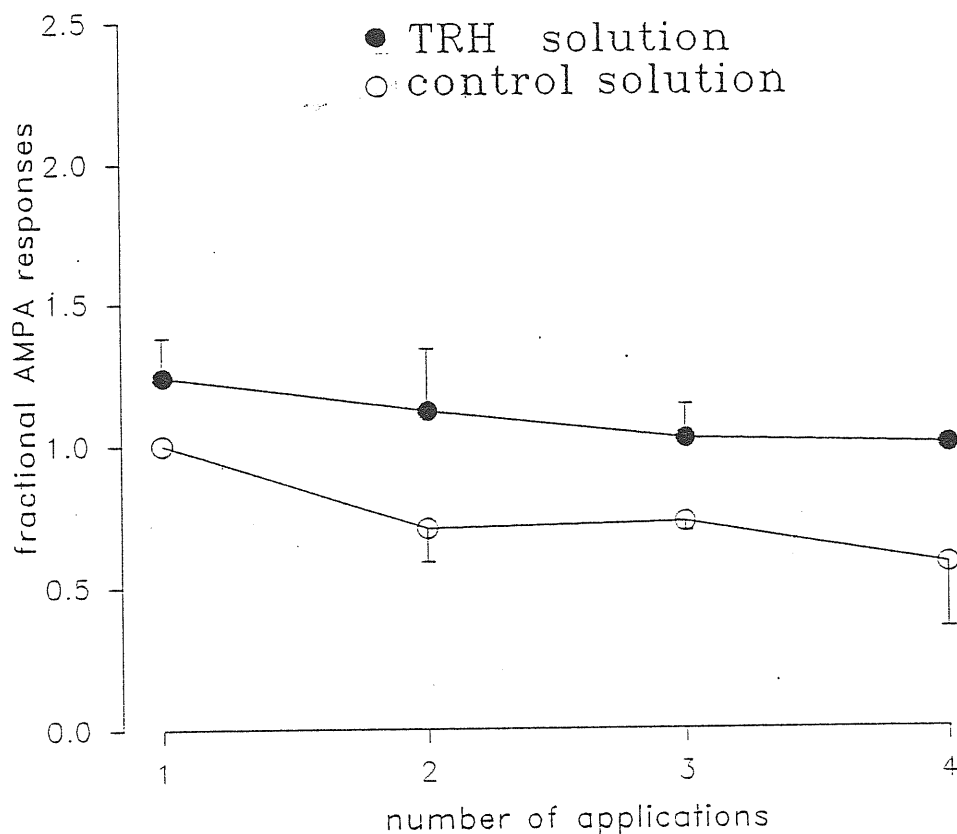


**Figure 8.** Effect of TRH on repetitive AMPA applications. Comparison between AMPA responses elicited in control solution (open circles) and after TRH application (filled circles). Normalization as in Fig.6.

## 3.5 EFFECT OF TRH IN TETRODOTOXIN (TTX) SOLUTION

In order to rule out TRH-induced presynaptic effects due to impulse dependent activity, experiments were conducted in solutions containing TTX (0.5-1 μM), a toxin extracted from the puffer fish, which blocks voltage activated $Na^+$ channels. In this solution larger doses of NMDA (30 μM) were required to elicit responses (9.1±1.4 mV) matching the

amplitude of those observed in control solution but, as shown in Fig.9 (open squares)

these responses remained stable after repeated application.
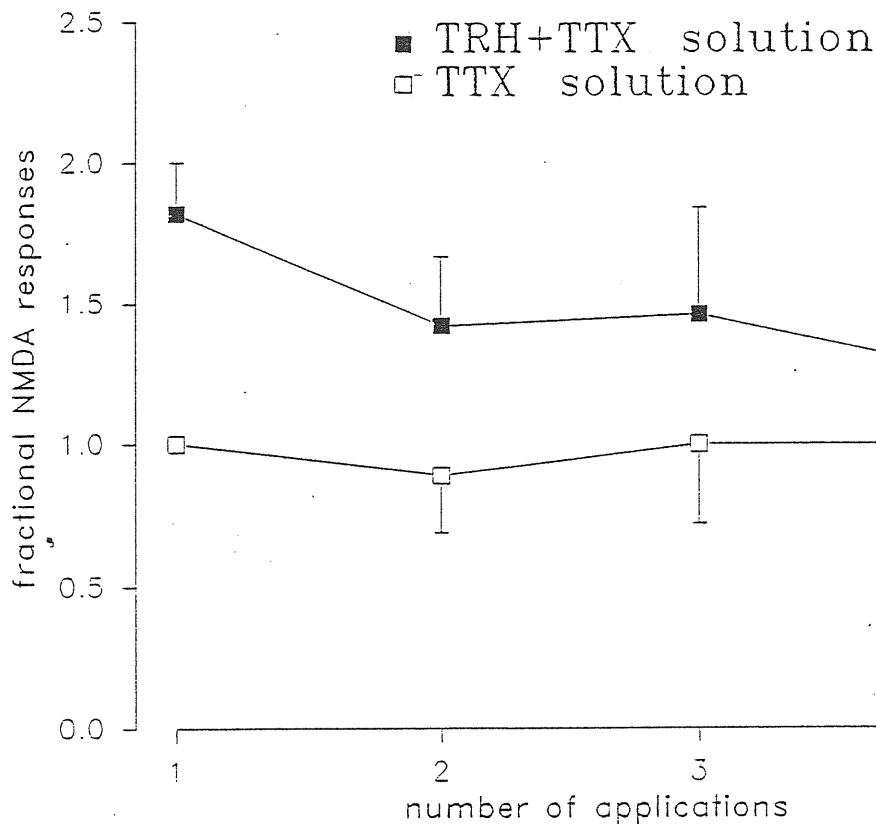


**Figure 9.** Effect of TRH on NMDA responses in TTX solution. Comparison between NMDA responses in TTX solution (open squares) and in TRH added TTX solution. Responses were normalized as in Fig.6.

In 5/11 cells bathed in TRH (5 μM) plus TTX solution the action of NMDA was initially

potentiated (1.8±0.1 times larger than its control in TTX medium; $P<0.05$) (Fig.9, filled

squares) even though the subsequent responses were not significantly enhanced. In

Fig.10 (upper traces) some aspects of the potentiation of the first response to NMDA can

be observed. In this example upward deflections were prevalently $Ca^{++}$ spikes induced
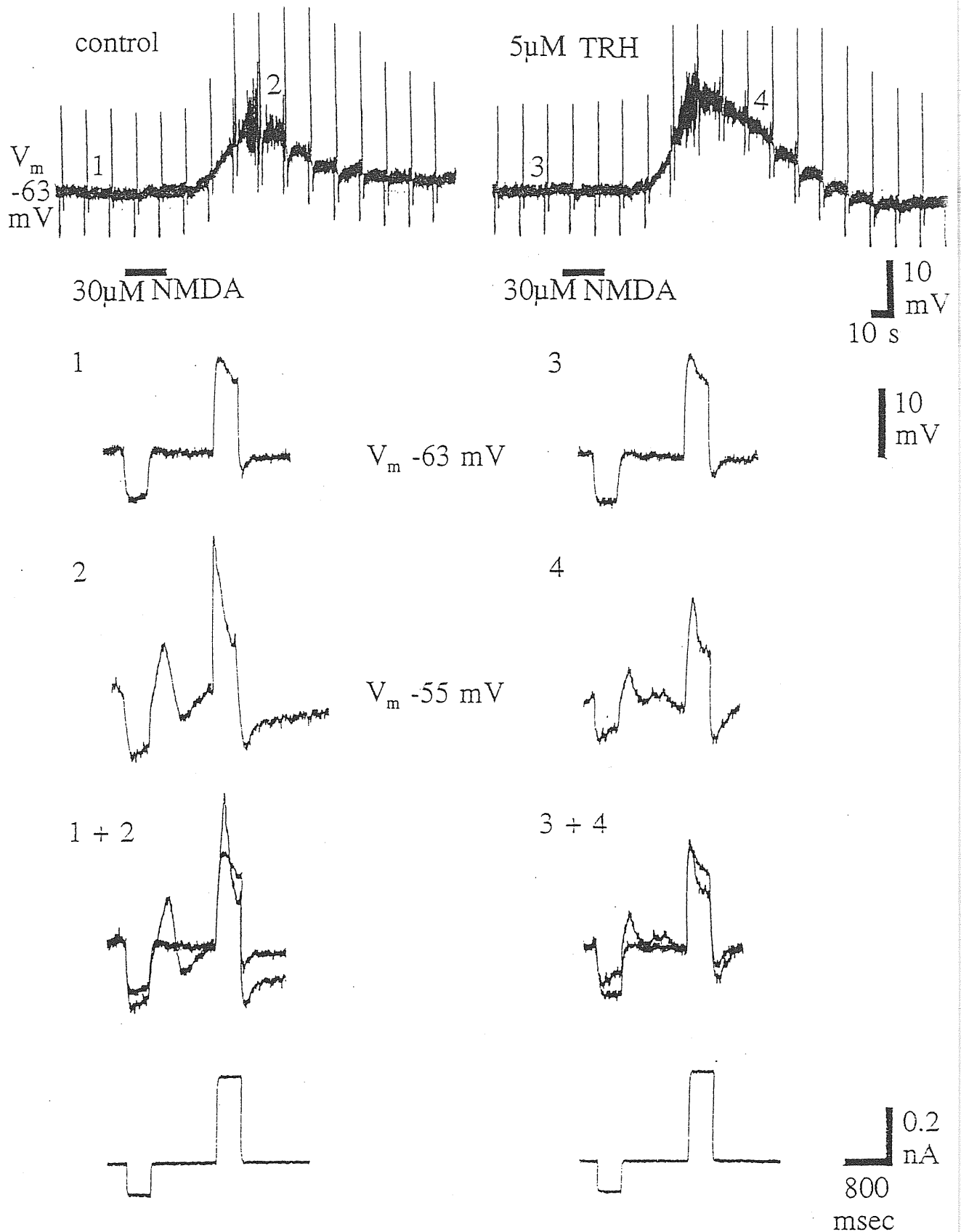
**Figure 10.** Effect of TRH on NMDA responses in TTX solution. Representative tracings from the same cell with -63 mV membrane at rest. TTX (0.5 µM) was present throughout. Top records show (on a slow time course) the potentiation of the response to NMDA (see bar for the time of application) after 5 min continuous exposure to TRH (5 µM). Hyperpolarizing and depolarizing current pulses (shown on a faster timebase in the bottom row) were regularly injected through the pipette. Middle rows present expanded, faster records of hyperpolarizing and depolarizing electrotonic potentials corresponding to those numbered from 1 to 4 in the top slow tracings. To aid comparison fast timebase responses before and during application of NMDA are superimposed (see record labelled 1+2 and 3+4). In trace 1+2 an apparent increase in input membrane potential that is reversed in 3+4 after TRH application (for more details see text).

by depolarizing current steps; downward deflections were either hyperpolarizing current steps (the larger ones) or afterhyperpolarizing potentials induced by $Ca^{++}$-dependent $K^{+}$currents following the $Ca^{++}$spikes. On top of the depolarization evoked by NMDA some membrane oscillations can also be observed. Fig.10 also depicts other interesting features of the TRH effect that may be more easily detectable in TTX solution. The slow membrane depolarization produced by the control application of NMDA was associated with an *apparent* increase in input resistance due to a characteristic of the channels involved (compare traces 1 and 2). The NMDA channel is both a ligand-gated and voltage dependent channel in the presence of extracellular $Mg^{++}$(Novak et al.,1984): when the agonist activates it, $Mg^{++}$ starts blocking it as long as the membrane potential is more negative than about -55 mV. The NMDA-induced depolarization is thus accompained by an apparent resistance increase simply because the electrotonic hyperpolarizating potentials reach $V_m$ values at which the $Mg^{++}$ block becomes operative. In the presence of TRH, at the same membrane potential, the amplitude of the hyperpolarizing potential was actually smaller, not larger, than before NMDA (Fig.10 traces 3 and 4), a result indicative of an input resistance decrease. Consistent with this phenomenon was the attenuation of the depolarizing electrotonic potential and the partial spike.

## 3.6 EFFECT OF TRH ON AFTER-HYPERPOLARIZING POTENTIAL (AHP)

In hippocampal pyramidal cells action potentials are usually followed by an after

hyperpolarization in which at least two components can be distinguished (Storm,1990):

a fast one, lasting few milliseconds, and a slow one which lasts for more than one

second. The first can follow each spike and takes part in the repolarization phase of the

action potential, whilst the latter is more easily detectable after a burst of spikes. This

after-hyperpolarization is principally due to various $Ca^{++}$-dependent $K^+$ conductances.
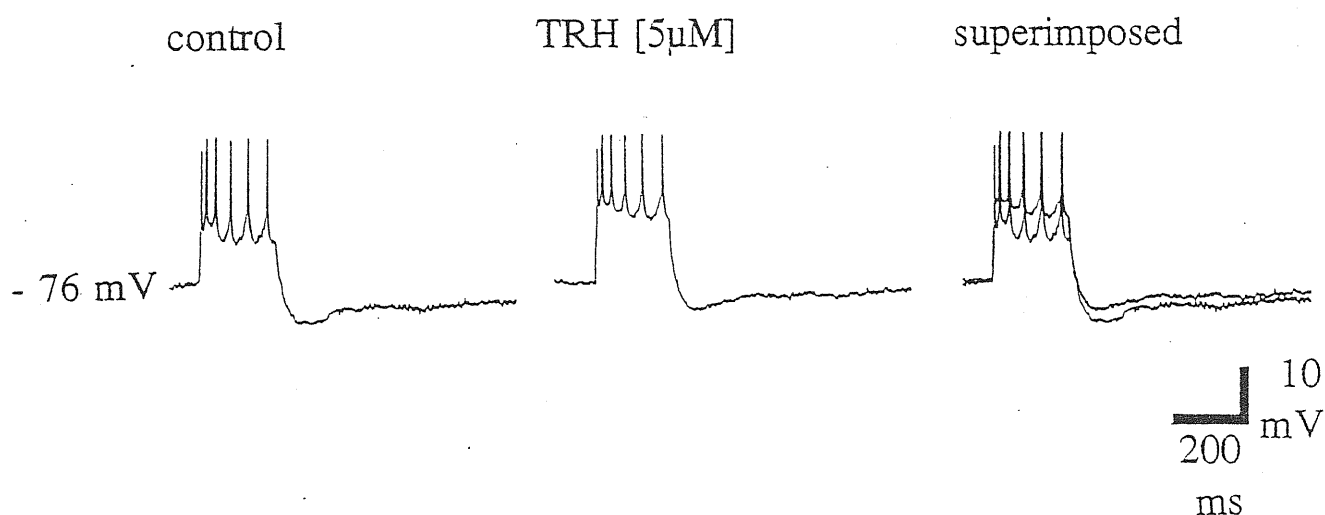


Figure 11. Effect of TRH on after-hyperpolarization. Voltage traces showing the membrane potential response to depolarizing steps of current (0.18 nA) of the duration of 250 ms. Depolarizations elicited a train of spikes followed by a long after-hyperpolarization. 5 µM TRH was decreasing the amplitude of that hyperpolarization (see superimposed traces).Stimuli delivered at 0.071Hz with interpulse delay of 2s.

Data were obtained, in DCC mode, using K-acetate (4 M) filled electrodes (70-110 MΩ)

in neurons (n=4) with a membrane potential of 74±1.55 mV and receiving

hyperpolarizing (amplitude of about 0.1 nA, duration of 400 ms) and depolarizing steps

(0.1-0.2 nA, 250 ms). The stimuli were delivered at 0.071 Hz with an interpulse delay of

about 2 seconds.

Application of TRH (5µM) reduced the AHP, even if the $V_m$ before the spike train and

the number of spikes induced by the depolarizing step were kept constant (Fig.11). Of the two components the slow one, measured at 500 ms after the end of the pulse, was more affected with a fractional amplitude of 0.67±0.14 when compared to the control one in ACSF. The peak fractional amplitude of the fast component was also diminished (0.77±0.02). The interspike interval was not changed; note also that, although TRH did not alter input resistance at rest as previously mentioned, this peptide increased the amplitude of the depolarizing envelope elicited by positive current pulses (see Fig.11). This observation suggests that a yet unidentified voltage sensitive conductance operating at relatively depolarized membrane potential might have been modulated by TRH.

## 3.7 TRH EFFECT ON $Ca^{++}$CURRENT

In order to investigate further the nature of TRH effect on AHP, $Ca^{++}$currents were studied in SEVC. To eliminate voltage-activated $Na^+$currents and the majority of $K^+$conductances, cells were superfused with a solution containing: 1 µM TTX, 20 mM TEA, 3 mM $Cs^+$, 3 mM 4AP and the pipette was filled with 3 M CsCl. From a holding potential of -40 mV, hyperpolarizing and depolarizing steps of 10 mV increments in amplitude and 1 second duration were delivered at a frequency of 0.05 Hz. Inward currents were measured at steady state (at the end of the pulse) and started to develop at -30 mV reaching its maximum around 0 mV, as it can be seen in the I/V curve of Fig.12A obtained after leak subtraction. TRH application did not significantly affect slow $Ca^{++}$currents as it can be seen in the plot of Fig.12 A and in the traces of Fig.12B (leak

not subtracted).
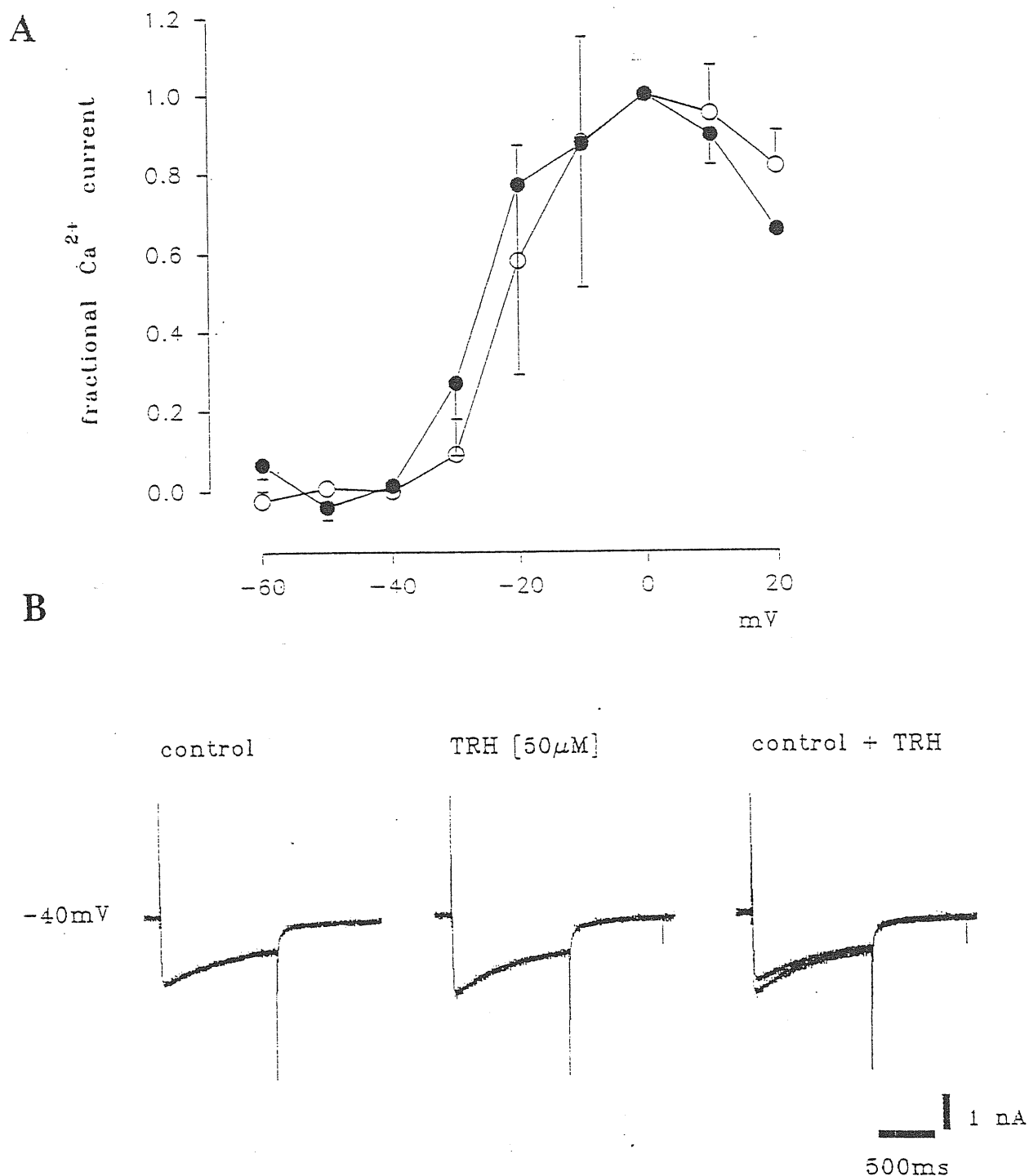


**Figure 12.** Effect of TRH on Ca$^{++}$currents. Voltage clamp Ca$^{++}$currents recorded from a neuron at a holding potential of -40 mV in the presence of 1 µM TTX, 20 mM TEA, 3 mM Cs$^+$, 3mM 4AP in the solution and 3 M CsCl in the pipette. Steps of 10 mV increments and 1 s duration were applied at 0.05 Hz. A: I/V curve obtained after leak subtraction: a comparison between values measured in control solution (open circles) and in TRH solution (filled circles). Data were normalized *vs* the current value obtained when the membrane was clamped to 0 mV. B: traces of inward current elicited clamping the cell to -10 mV (leak not subtracted).

# 4. DISCUSSION

## 4.1 GENERAL EFFECTS OF TRH ON HIPPOCAMPAL CA1 NEURONS

Several previous studies have shown that TRH can alter spontaneous and evoked activity in different kinds of neurons of the central nervous system (CNS) in lower and higher vertebrates (Lacey and Nistri,1989; Nicoll,1977; Lacey et al.,1989). In spinal cord motoneurons (Nicoll,1977 and 1978)TRH is known to induce a slowly developing, long lasting depolarization associated with an increase in membrane resistance leading to repetitive firing of action potentials. Since the presence of TRH receptors in many areas of the brain has been shown, particularly in the hippocampus (Sharif,1989), the present report investigated the TRH effect on hippocampal neurons.

In order to study TRH effects on CA1 hippocampal neurons, current clamp experiments were performed to investigate possible electrophysiological changes in active and passive membrane properties occurring during the tripeptide application. To this end, hyperpolarizing and depolarizing pulses, were delivered to the neuron at regular intervals. No changes in resting membrane potential and in input resistance were observed; on the other hand, an increase in synaptic activity slowly developed following TRH application. The duration and the amplitude of the depolarizing pulses were such as to elicit a train of spikes. The number of spikes and their interspike interval remained constant even after TRH administration. The train of action potentials was usually

followed by an AHP that is principally caused by a combination of $Ca^{++}$dependent $K^+$conductances (Storm,1989). TRH application affected the amplitude of the AHP, mainly inhibiting the slow component rather than the fast one. Furthermore, in TRH solution a larger amplitude of the response to the depolarizing current steps was clearly detectable.

All these results suggest that this effect of TRH was voltage dependent and therefore not evident at resting membrane potential (Nicoll,1977).

The action exerted by the neuropeptide on $Ca^{++}$dependent $K^+$conductances brought about the possibility of a direct effect on $Ca^{++}$currents. The kind of currents that were likely responsible for sustained entry of $Ca^{++}$necessary to elicit the AHP were deemed to be the high voltage activated (HVA) ones with a very slow inactivation rate (L-type)(Miller and Fox,1990). A voltage clamp technique was used for recording such currents measured at steady state after blocking voltage activated $Na^+$currents and various $K^+$conductances. This procedure allowed testing $Ca^{++}$currents in relative isolation. TRH applications did not significantly affect these $Ca^{++}$currents, a result indicating that TRH modulation of the AHP occurred downstream the process of $Ca^{++}$entry through the neuronal membrane. The tripeptide was found to increase clearly the baseline synaptic activity and firing rate of hippocampal cells without significantly affecting either their membrane potential or their input membrane resistance. These results suggest that the TRH modulation of the activity of these hippocampal neurons is more subtle than that one found in other CNS regions and probably it is a composite phenomenon which could include a modulation of the presynaptic transmitter release and/or an upregulation of postsynaptic responses,

especially if their remote location might have prevented direct measurements of synaptic conductance changes.

Even though the identity of the transmitter(s) involved remains to be fully characterized, preliminary tests with pharmacological receptor antagonists have suggested the presence of a combined GABAergic and glutamatergic contribution to the phenomenon. Further electrophysiological studies of quantal analysis in the presence of selective blockers of either GABA or glutamate receptors will be needed to elucidate the locus of action of TRH in facilitating synaptic transmission.

## 4.2. POTENTIATION OF RESPONSES TO GLUTAMATE AGONISTS

Many neuropeptides have already been found to be involved in neurotransmitter modulation. Interactions between TRH and excitatory aminoacids (EAA) or acetylcholine have been observed both in motoneurons (Rekling,1992) and in cortical neurons (Stone,1983; Renaud and Martin,1975; Kasparov et al.,1992) usually resulting in potentiation of responses in exogenous neurotransmitter. Clinical effects of the tripeptide on loss of memory (Barret,1983) and recent studies on its modulation of LTP in hippocampal neurons (Ishihara,1991) have suggested a possible action via glutamate receptors mechanisms.

In order to check for action of TRH on glutamate receptor mediated responses, glutamate agonists were tested before and after application of TRH. Glutamate itself was not applied in view of its wide range of excitatory effects and its strong reuptake process

which could compound observed responses. The selective agonists which act via different population of glutamate receptor, were first applied several times at regular intervals in order to investigate whether their effect was use dependent. Responses to AMPA showed clear desensitization, as previously observed (Trussel et al.,1993). On the other hand, NMDA responses did not show a similar phenomenon. In the large majority of tested neurons TRH strongly potentiated NMDA responses, as observed by Kasparov et al.,1993. This potentiation consisted in an increase in the amplitude and the duration of the response itself. During the second application the response enhancement was still present although reduced while further responses did not display any potentiation. AMPA elicited depolarizations that were not significantly changed during TRH application, revealing the existence of a specific modulatory phenomenon. These data outline a disparity in the ability to enhance responses to different glutamate receptor agonists. Of course responses elicited by glutamate agonists in control ACSF are likely to comprise a combination of presynaptic and postsynaptic components since local neurons impinging upon pyramidal cells presumed to possess glutamate receptors also sensitive to the same substances.

In order to restict the action of TRH and NMDA to postsynaptic level, some experiments were performed in TTX solution. Under these experimental conditions only half of the recorded neurons showed potentiation by TRH of NMDA responses. This time the enhancement was smaller suggesting that the site of action of TRH was predominant at the presynaptic site. In TTX solution other important features of the effect of TRH on NMDA responses could be detected. In fact although NMDA receptors are ligand gated,

they display a particular voltage dependent block of their intrinsic ionic channel by $Mg^{++}$(Nowak et al.,1984).

When NMDA binds to its receptor, the channel opens allowing $Mg^{++}$to block the pore as long as the membrane does not depolarize above about -50 mV, at which level the block is relieved. When the membrane during the depolarization is suddenly repolarized by intracellular, current injection as indeed is the case when electrotonic potential are generated to monitor input resistance, a prompt reappearance of the $Mg^{++}$blocking action takes place. Such a phenomenon is observed as an apparent increase in input resistance. In the present experiment this situation was actually found during the response to NMDA in TTX solution but it was abolished in the presence of TRH. Hence the peptide might have somehow weakened the interaction of $Mg^{++}$with the NMDA receptor.


In conclusion TRH effect on spontaneous activity was shown to be principally presynaptic and probably involving both glutamatergic and gabaergic synapses as can be suggested by preliminary tests with antagonists. Moreover the fact that TRH effects postsynaptically the input membrane resistance when the cell is depolarized by NMDA applications and without changing the resting membrane potential, gives the idea of a fine tuning role on respect to the one in motoneurons. Other experiments will be performed in order to better characterize both the effect on presynaptic release and the neurotransmitters involved.

# 5. ACKNOWLEDGEMENTS

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