THE CONSTITUTIVE ACTIVITY OF THE HISTAMINE H1 RECEPTOR INTERACTION WITH THE NMDA RECEPTOR : CONSEQUENCES IN EPILEPSY

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Summary

Objectives To explore the epileptogenic effects of several drugs specific to the antihistaminic H1 receptor on NMDA and GABA responses.

Methods With patch-clamp whole-cell recordings of hippocampal neurons of rats, we observe the effects of several histaminergic agonists and antagonists on the NMDA and GABA currents. During the NMDA rundown and GABA rundown, we applied the drugs and observe the effect on the responses, and compare them to control conditions.

Results Mepyramine with nanomolar concentrations increases significantly the NMDA responses about 35 %, this effect is mimicked by another Anti H1 drug Triprolidine. Histamine alone has no effect on NMDA rundown but 100 µM Histamine can reduce partially the effect of Mepyramine. An H1 receptor agonist 2,3 Bromophenyl Histamine also shows the same properties as Histamine. The H1 receptor of Histamine seems to be crucial during these experiments, blockade of its constitutive activity by Mepyramine induces a significant increase of the NMDA responses. On the GABA response, the same nanomolar dose of Mepyramine has no effect on the rundown and we also observed no effect of Histamine on the GABA rundown.

Significance

An interaction between Histamine H1 receptor and the NMDA is revelated by using Mepyramine in nanomolar concentration, Histamine has no effect on these responses also in these experiments Mepyramine acts as an inverse agonist blocking the constitutive activity of the H1 receptor. The constitutive activity of the H1 receptor seems to be crucial in the regulation of NMDA receptor activities.

Keywords : Histamine H1 Receptor, Epilepsy, NMDA Receptor, Constitutive Activity

Keys Points

° The use of antihistaminic H1 drugs can facilitate the apparition of epileptic seizures,

° During rundown experiments, Mepyramine, a high potent H1 antagonist increased the NMDA responses, while Histamine as no effect.

° On GABA rundown both Mepyramine and Histamine show no effect.

° Constitutive activity of the Histamine H1 receptor seems to be crucial for the NMDA receptor activity.

INTRODUCTION

In this paper we want to try to answer an old question; why are H1 antagonist drugs proconvulsant?

The effects of Histamine are mediated by four Histamine receptor subtypes (H1, H2, H3, and H4), which are all G protein-coupled receptors. Blocking H1 receptor (H1R) in the brain induces many "side effects " such as slowing reaction time and somnolence (1) and the first generation of H1R antagonist causes sedative and slowing effects on EEG (2) but it is also known that H1R antagonists, including classical anti-allergy drugs, occasionally induces convulsions in healthy children and patients with epilepsy (3-5). In animals, H1R antagonists showed proconvulsive effects in mice and in rats (6,7). More recently in rats with genetically generalized epilepsies, it has been proved that H1R densities are increased in the brain (8). All those facts indicate that H1R may play a crucial role in the brain in epileptogenesis and the control of epilepsy.

Epilepsy is a complex pathology and finding its mechanism remains complicated. One putative mechanism triggering epileptic activity is the over-excitation of glutamatergic neurons via the over-activation of the N-methyl-D-aspartate receptor (NMDAR) (9). The second mechanism implicates the GABAergic inhibition that prevents in the neuronal networks the generation and spread of paroxysmal activities (10).

Electrophysiological approaches are classical for epilepsy exploration and the rundown studies in the past from several teams have proved that it was an interesting alternative to explore the mechanism of the disease, in temporal lobe epilepsy (11) or in human partial epilepsy (12).

The rundown of the currents is a time-dependent decrease of the response. In fact, the repetitive activation receptors produce a use-dependent decrease (run-down) of the membrane currents and are observed with NMDA and GABAA. The mechanism of the rundown was studied and the decrease is due to dephosphorylation of the receptor in the case of the GABAA (13) and to a calcium and ATP process in the case of NMDA (14). Finally, it shows the time course evolution of the current which can mimic the long-term action of drugs on brain neurons.

METHODS

Electrophysiological recording in neurons; Patch-clamp

Primary neuronal cultures from rat hippocampus and whole-cell patch-clamp were performed as described (15). Ionic currents were recorded within large pyramidal neurons. A rapid perfusion system was monitored for drug application. For the NMDA experiments, the external solution for recording whole-cell currents contained 140 mM NaCl, 5 mM KCl, 1.2 mM CaCl2, 12 mM HEPES acid, 12 mM HEPES sodium, and 33 mM D-glucose. Tetrodotoxin (1 µM) was added to eliminate the voltage-gated sodium channel currents. Glycine 5 µM was added to the external solution. The pH was 7.3 and the osmolarity was adjusted to 300 mOsm with D-glucose. The internal pipette solution contained 100 mM CsF, 40 mM CsCl, 1 mM CaCl2, 20 mM HEPES acid and 10 mM EGTA. The pH was adjusted to 7.3 with CsOH. A whole-cell amplifier (AXOPATCH 1D) was used to measure the current responses, the membrane potential was clamped at -60 mV. During the recording, NMDA (50 µM) was applied for 2 seconds every 30 seconds for 30 minutes. GABA experiments, the external solution for recording whole-cell currents contained 135 mM NaCl, 3 mM KCl, 2 mM CaCl2, 10 mM HEPES acid, 1 mM MgCl2, 7 mM Triéthylamine chloride and 10 mM D-glucose. Tetrodotoxin (1 µM) was added to eliminate the voltage-gated sodium channel currents. The pH was 7.3 and the osmolarity was adjusted to 300 mOsm with D-glucose. The internal pipette solution contained 130 mM CsF, 10 mM CsCl, 4 mM NaCl, 0,5 mM CaCl2, 10 mM HEPES acid, 5 mM EGTA and 7 mM Mg-ATP. The pH was adjusted to 7.3 with CsOH. A whole-cell amplifier (AXOPATCH 1D) was used to measure the current responses. The membrane potential was clamped at -60 mV. During the recording, GABA (100 µM) was applied for 1 second every 3 minutes for 30 minutes.

All the drugs; 2,3 Bromophenylhistamine dimaleate, Mepyramine, Triprolidine, Histamine were added in all solutions bath and perfusion.

The Rundown was measured by normalizing the currents evoked after the beginning of the recording. The data are expressed as a percentage of control +- standard deviations.

Immunohistochemistry ***Immunofluorescence of primary cultures.***

Primary cultures of hippocampal neurons were fixed with 2 % paraformaldehyde in phosphate buffer (0,1M pH 7,4) at room temperature for 10 min, rinsed for 48 h in 0.1M PBS phosphate buffer, primary cultures were double-immunolabeled for GABAAR/H1R ; NMDAR/H1R ; GFAP/H1R ; MBP/H1R ; HUC/D/H1R.

List of antibodies (dilution):

Immunofluorescent labelling

Anti-alpha 1 for GABAA receptor (1:1,000) (NeuroMab)

anti-NMDA (1:1000) Ionotropic glutamate receptor subunit GluN1 (Synaptic Systems - SYSY)

anti-H1 (1:100) ((Alomone Labs - AL)

anti-GFAP (1:750) (GFAP, Glial fibrillary acidic protein of astrocyte, (Chemicon, Temecula, CA)

anti-BMP, bone morphogenetic protein of oligodendrocyte, (1:250) (Chemicon, Temecula, CA)

anti-neuronal (protéine HUC/D.somatostatin (1:30) (Invitrogen)

Statistical analyses

After normalization of the current among the time the variation of the current was reported on the time curved and compared to control with a Two-way ANOVA with Bonferroni adjustment. Significance was tested using a two-way ANOVA analysis for repeated measures followed by Dunnett's post hoc analysis (one-tailed distribution).

The area under the curve of the different conditions was compared to control conditions, thus a Dunnett's test was used as the samples were of the normal distribution.

RESULTS

Mepyramine decreases the NMDA rundown

In whole cells recordings from cultured hippocampal neurons, we observe a rundown of the NMDA R (fig 1 A) the amplitude of the NMDA R -mediated responses decrease with the successive application of NMDA to a similar degree to that observed by Rosenmund et al, (14). When we add Mepyramine 10 nM (fig 1 A) we first observe an increase of the current after 4 minutes remaining for 10 min then the second phase of decrease of the responses, we can notice that the slope of this decrease is the same slope observed during the run down in control condition (fig 1 B). We have measured the area under the curve, this can give an insight on the current during the 30 minutes of recordings and of the activity of the NMDA receptor during the experiments, we have a significant increase of 39% of the area (fig1 C). With 50 nM we reach the same saturated effect on rundown increased by 37 % of the area, 2 nM shows a weaker effect (14%) (fig1 C).

Mepyramine has no “direct” effect on the NMDA current

Parallelly to the rundown experiments we have also tested the direct effect of Mepyramine 10 nM on the NMDA current, in order to exclude a direct effect of Mepyramine on the NMDA responses. In this case, we first applied NMDA alone three times every 3 minutes, the 3 minutes time is used to counteract any rundown apparition, and then the same with NMDA + Mepyramine every three minutes. When we compared the responses with and without Mepyramine we do not observe any difference in the current between the two conditions (n=10) data not shown.

Triprolidine decreases the NMDA rundown

We tested another H1 antagonist Triprolidine 10 nM (fig 2), it also shows the same significant effect, an increase of the responses (20% increase of the area under the curve) and a decrease of the rundown that's lower than the one with mepyramine.

Histamine decreases the effect of Mepyramine but alone has no effect on the NMDA rundown

In order to characterize this effect, we tried to antagonize the Mepyramine effect, with Histamine. Histamine 100 µM can significantly reduce about 50 % the effect of the Mepyramine 10 nM (fig 3 A). We also observe that Histamine 100 µM alone has no effect on the NMDA rundown.

2,3 Bromophenyl Histamine decreases the effect of Mepyramine but alone has no effect on the NMDA rundown

A high potent H1 agonist 2,3 Bromophenylhistamine dimaleate was tested (fig 3 B), with 10 µM. It can reduce significantly about 50 % the effect of Mepyramine 10nM and Bromophenylhistaminewas also tested alone has no effect on the NMDA rundown.

Mepyramine has no effect on GABA rundown

In whole cells recordings from cultured hippocampal neurons, we observe a rundown of the GABAAR (fig 4) the amplitude of the GABAA -mediated responses decrease with the successive application of NMDA to a similar degree to the one observed by Laschet et al.(12). When we add Mepyramine 10 nM, we do not observe any change in the rundown, the same observation with 50 nM (fig 4).

Histamine has no effect on GABA rundown

Histamine (n=6) alone has an effect on the GABA rundown.

H1 is not colocalized with NMDA nor GABA receptor

We studied the localization of the H1 receptor in the hippocampal cells culture, on neurons. The H1 receptor immunoreactivity was observed (fig S1 A) but not on oligodendrocyte and astrocytes (fig S1 B, C). The co-expression of the NMDA receptor and the H1 receptor was analyzed and there is no colocalization of the receptors in the neurons (fig S2 A).

The co-expression of the GABAA receptor and the H1 receptor was also analyzed and there is no colocalization of the receptors neurons (fig S2 B).

DISCUSSION

Our results demonstrate that the Histamine receptor H1 is important in the functioning of the NMDA receptor, the blockage of this receptor induced a strong reduction of the rundown of the NMDA receptor holding the receptor in a more active statement. It can explain the proconvulsant effect of the pharmacological class of the H1 receptors antagonist.

H1 antagonist reduces the NMDA rundown

The H1 antagonist effect is dose-dependent and is maximum with 10 nM Mepyramine about 40 % of increase of the current compared to control conditions, Mepyramine is a high potent antagonist (16) of the receptor and to confirm this observation we tested the effect of Triprolidine, another formerly designated H1 antagonist. When we used 10 nm of Triprolidine the effect on rundown was the same as with Mepyramine but weaker certainly due to a lower affinity on the receptor compared to Mepyramine (16). During the experiments we observed with 10 nM Mepyramine a small increase of the NMDA current after 4 minutes application of NMDA, during about 10 minutes with a 20 % maximal effect, this increase of the current is difficult to explain with a simple patch-clamp analysis of the whole-cell current, we observed more current passing through the NMDA receptor but the aspect of the recording line do not give any other special indication. After this 10 minutes under Mepyramine we have a decrease of the NMDA current and the slope of this decrease among the times is mimicking the slope of rundown in control conditions (fig 1 B), it seems that we have the same effect but with a decay of 10 minutes. Mepyramine holds up the beginning of the rundown! It is very important to notice that the same concentration of Mepyramine in no rundown (spacing out the applications) condition has no effect on the NMDA current. Finally, something happens during the rundown conditions that increase the NMDA currents. In order to characterize pharmacologically the effect of the Mepyramine we have tested the effect on the rundown of Histamine (100 µM), alone, Histamine has no effect on NMDA rundown and when combining Histamine and Mepyramine to counteract Mepyramine, Histamine can reduce partially the effect of Mepyramine 10 nM about 50 % (fig 3A). The traditional maximal dose use of 100 µM Histamine can just partially reduce the effect of the Mepyramine which has a

very high affinity with the H1 receptor (16). Then we tested a high potent agonist of the H1 receptor 2,3 Bromophenylhistamine dimaleate at 10 µM (17,18), it has also no effect alone and reduce in the same range as Histamine the effect of Mepyramine on NMDA rundown. These two experiments confirm and characterize a pure H1 effect of Mepyramine on the NMDA rundown.

H1 receptor constitutive activity regulates the NMDA receptor activity.

H1 receptor exhibits a constitutive activity (19). It is a crucial point in our experiments because when we add Histamine 100 µM, we have no effect on NMDA rundown and when we block the H1 receptor we diminish the rundown. This effect is due to the particular pharmacological properties of both Mepyramine and Triprolidine which are inverse agonists of the H1R (16). These two drugs can oppose the constitutive activity of the receptor. Finally, the constitutive activity of the H1 receptor seems to be important for the NMDAR. The blockade of the constitutive activity by the H1R antagonist induces an increase of the NMDA current leading to an increase of the excitability which is a theoretically proepileptogenic effect.

Is the H1 receptor pathway able to interact with NMDA receptor trafficking?

We have first observed the aspect of the NMDA responses during the rundown in normal conditions and under Mepyramine more particularly the slopes of the current's traces during the 30 min of experiments and they were not significantly changed.

In order to increase the NMDA responses, the recruitment of a larger quantity of NMDA receptors at the membrane is a hypothesis, it could be due to a change in the receptor trafficking slowing the disappearance of the receptor at the membrane for example.

H1 receptor is coupled to Gαq and its activation increases phosphotidyl inositol turnover (20) and induced [Ca2+]i increase (21), to abrogate this calcium rise could be enough to modulate the NMDA receptor. H1R also interacts with actin filament (22). Histamine reduces actin filament in endothelial cells and in T cells (23) could explain a part of our effect but we must notice that this is not observed for the moment in neurons. The calcium and ATP dependence of the rundown is well described (14) and this influences NMDA receptor activity by altering the state of polymerization of the actin filament (24). The rundown is attenuated if actin filaments are stabilized with a high concentration of intracellular ATP. The high intracellular

concentration of calcium disturbs the connection between NMDA receptor and actin filament proteins kinase A and C phosphorylate NMDA and increase his activity (25,26) In this case, the H1R does not interact with these pathways, H1 has just an activating Phospholipase C effect which seems to have no consequence with MNDA rundown (27).

Another possibility is a direct interaction between the receptor and it is documented that both Histamine H1 and H2 receptors have been shown to co-localized with dopamine D1 and D2 in neurons (28) but in our experiments we cannot observe any significant colocalization of the receptor NMDA with the receptor H1, it means that the effect that we observed is not linked to possible direct contact of the receptors.

The Complex role of Histamine and H1 receptor pathway in the brain role in epilepsy

The results of our experiments on the interaction of Mepyramine or Histamine on GABA rundown give us no evidence of any effect of these drugs, and we conclude that the GABAergic system is surely not involved in the proconvulsive effect of the H1 antagonist,

In the brain, Histamine also acts as a positive allosteric modulator of the N-methyl-D-aspartate receptor (NMDAR) (29). It potentiates NMDA currents in isolated (30), and cultured (31) hippocampal neurons, and this effect results from direct interaction with NMDARs containing NR1 but this direct effect never was related to epileptiform activities. The results of Kamei clearly suggest that the epileptogenic activity of first-generation H1-antagonists is dependent upon a centrally acting Histaminergic mechanism through the Histaminergic receptors (7). Histamine has anticonvulsant effects, H3 receptor antagonists, which enhance endogenous Histamine release in the brain, have been demonstrated to have a potent anticonvulsant action. (32). Histamine attenuated amygdaloid-kindled seizures in rats effect (33), Histamine as also an anticonvulsant effect on pentetrazole (PTZ)-induced and electroconvulsive seizure threshold in mice (34). Both authors conclude that this effect is mediated with the H1 receptor because H1 antagonist can counteract the Histamine effect but we know that H 1 antagonist have proconvulsant effects and we propose that the Histamine effect on epilepsy is probably not due to direct interaction with the H1 receptor but through another pathway. In our experiments, Histamine as well as a pure H1 agonist have alone no effect on the NMDA rundown they can just reduce the effect of the antagonist H1 drugs by competition interacting on the binding site of the dugs on the H1R. We can hypothesize that

they are two different mechanisms one for the anticonvulsant effect of Histamine and one for the proconvulsant effect of H1 antagonist this indicates the crucial role of the H1 in the central nervous system.

Midzyanovskaya et al(8) describe an increase of the densities of the H1 receptor in the brain of rats with generalized epilepsies and Kukko-lukjanov et al.(35) note that H1 R knock out mice the status epilepticus is more severe than in control mice, they conclude that the Histamine receptors H1 play a role in the control of epilepsy.

To conclude, the intracellular microenvironment with calcium (24) but also extracellular (36) regulates the signaling of NMDAR and the role of actin filament seems to be crucial for the presence of new NMDA receptors at the membrane but we will need another approach than whole-cell recording to explore the intracellular movement of the NMDAR such as for example those from Ferreira et al, ref (36)

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

FIGURES

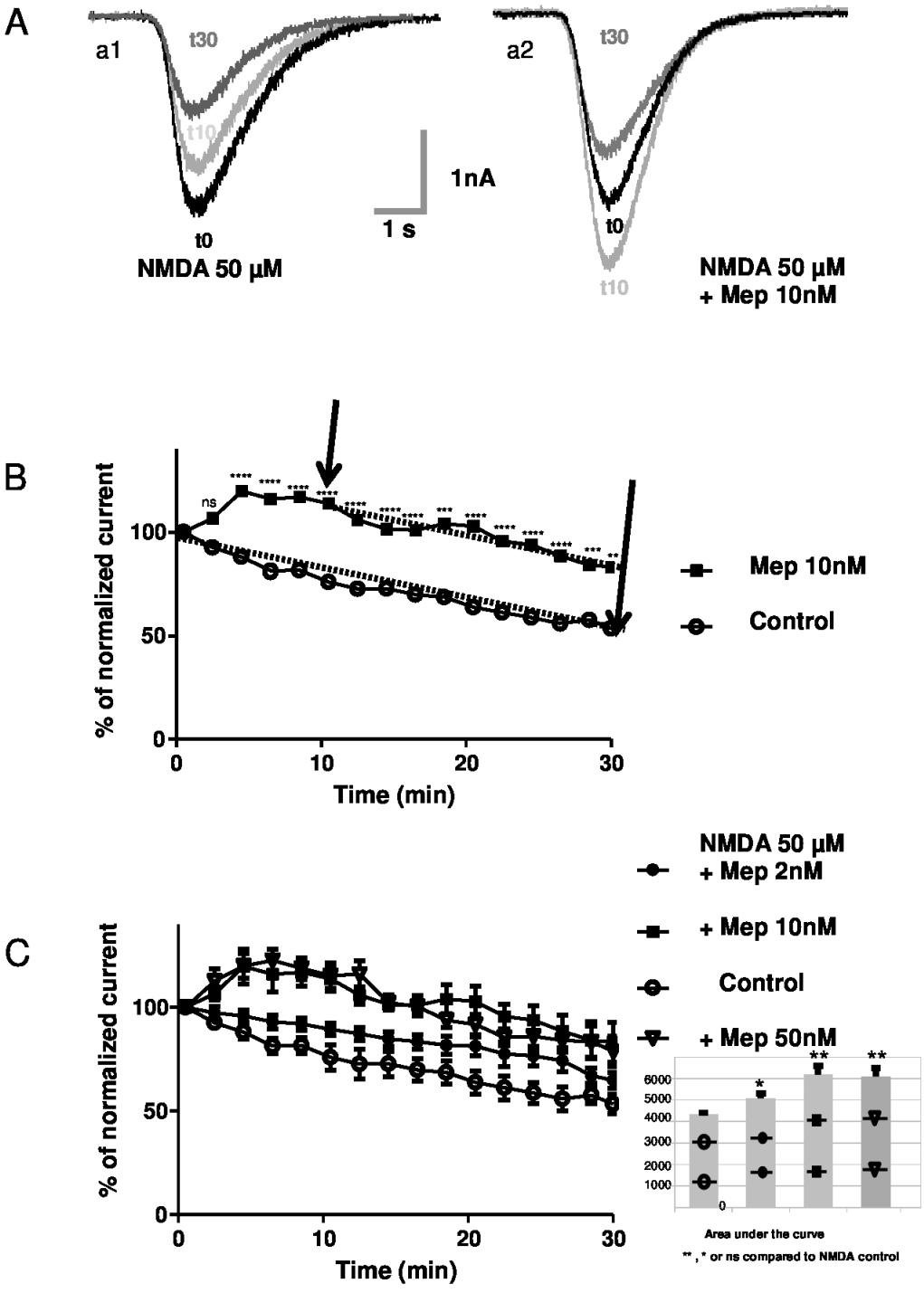

Figure 1.

Rundown of NMDA current ; effect of Mepyramine **(Mep)**

Rundown of NMDA current during whole-cell recording, inward currents evoked by 2 s application of 50 µM NMDA at 30 s intervals in control and with Mepyramine.

A Currents evoked after the first application **t0** , 10 min **t10** and 30 min **t30** of recording are superposed in NMDA control condition (a1) or in presence of 10 n M of Mepyramine (a2).

B The graphs show the normalized currents in control (n= 10) and in presence of Mepyramine 10 (n=10) Currents were normalized to the first application. Note that the slope in both conditions is the same (dotted line) indicated with an arrow.

C The graphs show the normalized currents in control (n= 10) and in presence of Mepyramine 2 , 10 and 50 nM (n=10 for each condition) Currents were normalized to the first application. The area under the curve for all the conditions tested with statistical comparison *, ** or ns to NMDA control.

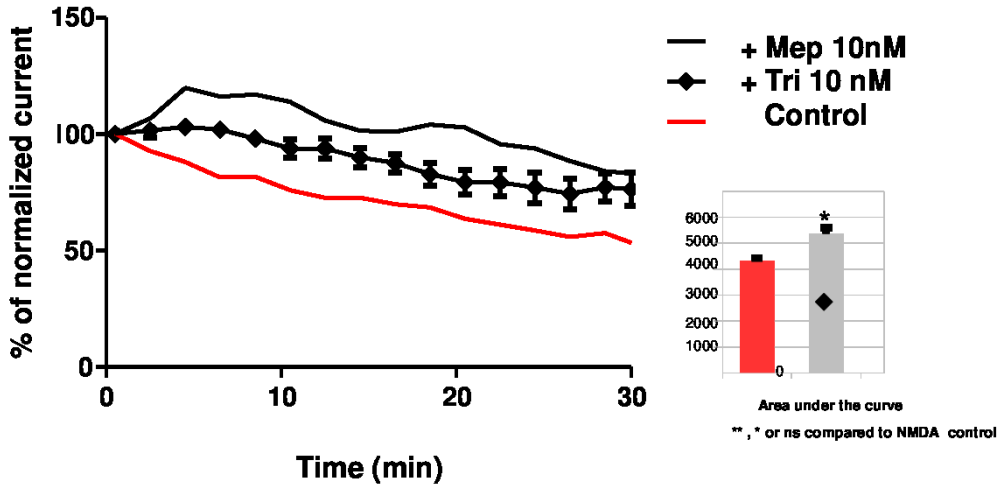

Fig 2

Figure 2. Rundown of NMDA current ; effect of Triprolidine (**Tri)**.

The graphs show the normalized currents in control red and in presence of Mepyramine 10 nM in black and with Triprolidine 10 nM (n=10) Currents were normalized to the first application. The area under the curve for all the conditions tested with statistical comparison *, ** or ns to NMDA control.

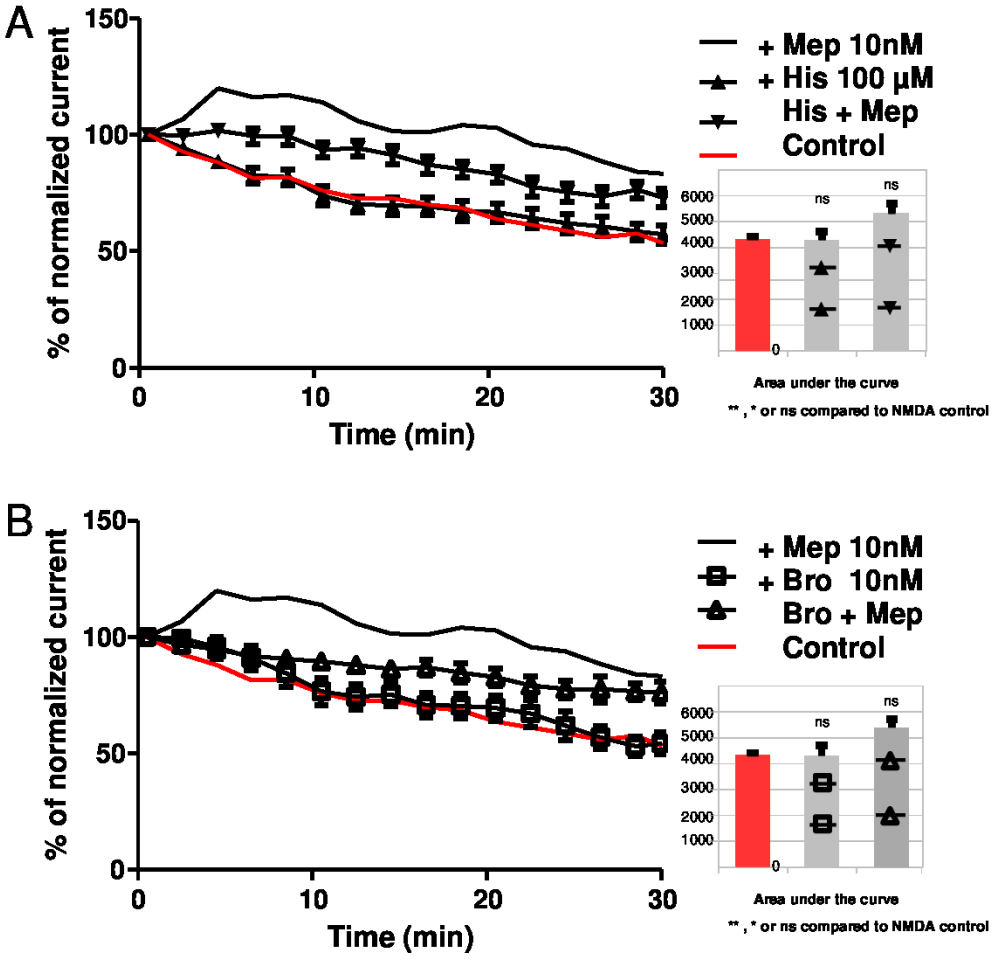

Fig 3

Figure 3. Rundown of NMDA current effect of Histamine (**His)** and 2,3 Bromophenylhistamine dimaleate (**Bro)**,

A The graphs show the normalized currents in control red and in presence of Mepyramine 10 nM in black, with Histamine 100 µM (n=10) and Histamine against Mepyramine 10 nM (n=10). Currents were normalized to the first application. Tha area under the curve for all the conditions tested with statistical comparison *, ** or ns to NMDA control.

B The graphs show the normalized currents in control red and in presence of Mepyramine 10 nM in black, with 2,3 Bromophenylhistamine dimaleate 10 µM (n=10) and 2,3 Bromophenylhistamine dimaleate against Mepyramine 10 nM (n=10). Currents were normalized to the first application. The area under the curve for all the conditions tested with statistical comparison *, ** or ns to NMDA control.

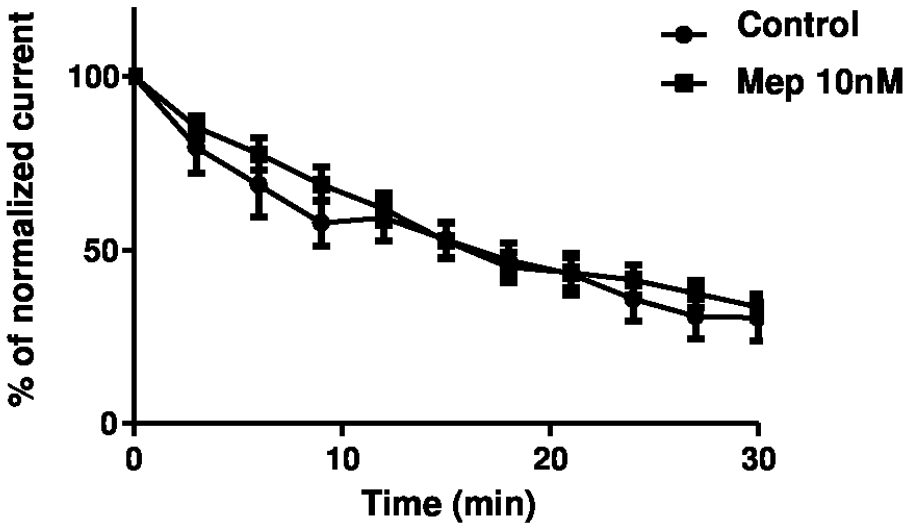

Fig 4

Figure 4. Rundown of GABA current ; effect of Mepyramine **(Mep)**

Rundown of GABA current during whole-cell recording, inward currents evoked by 1 s application of 100 µM GABA at 3 min intervals in control (n=12) and Mepyramine 10 nM presence (n=10).

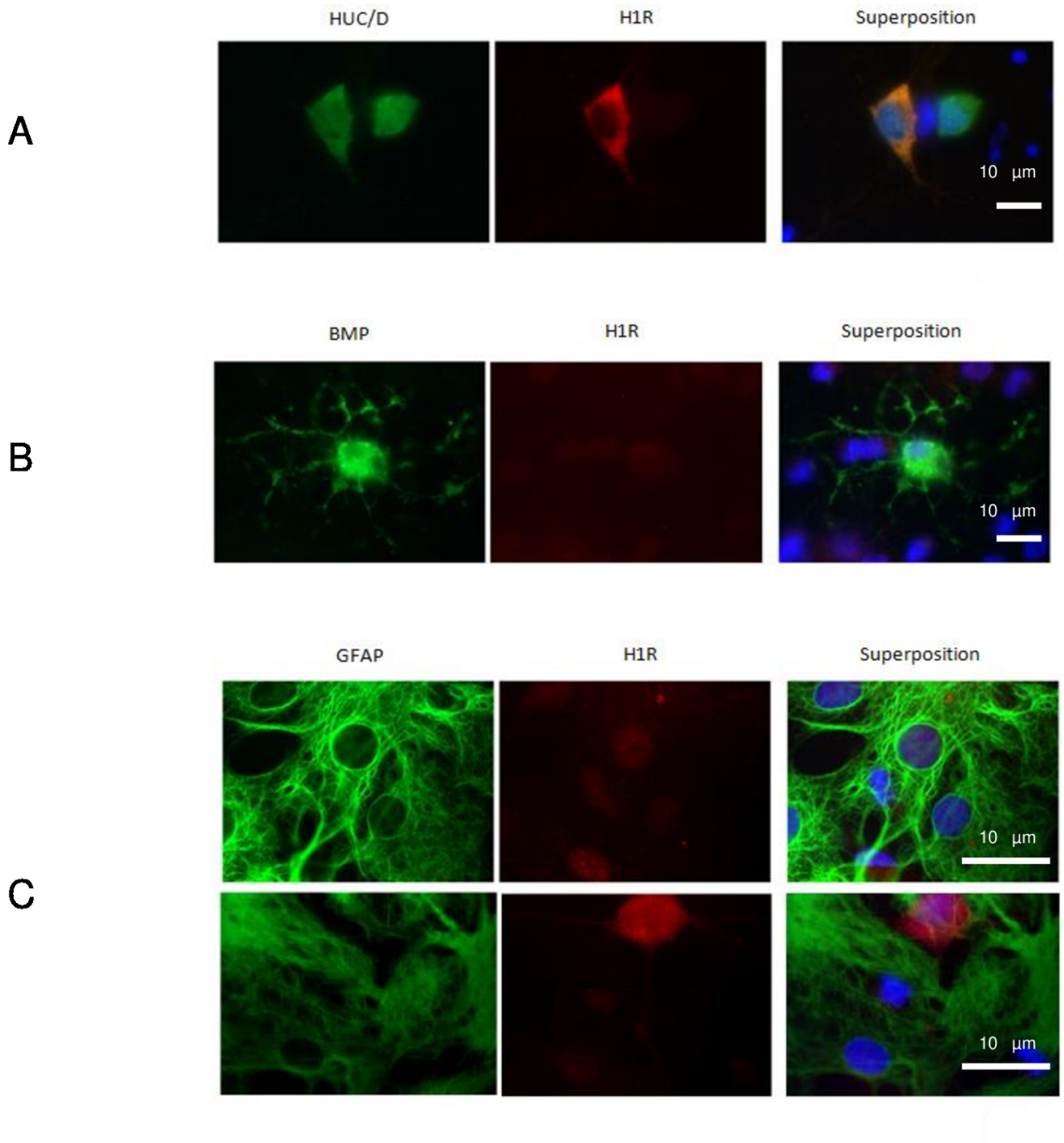

Fig S1

Figure S1. Immunostaining for HUC/ D (A), BMP (B) and GFAP (5C) co-expression with the H1 receptor in cultured hippocampal cells. Scale bar, 10 µm

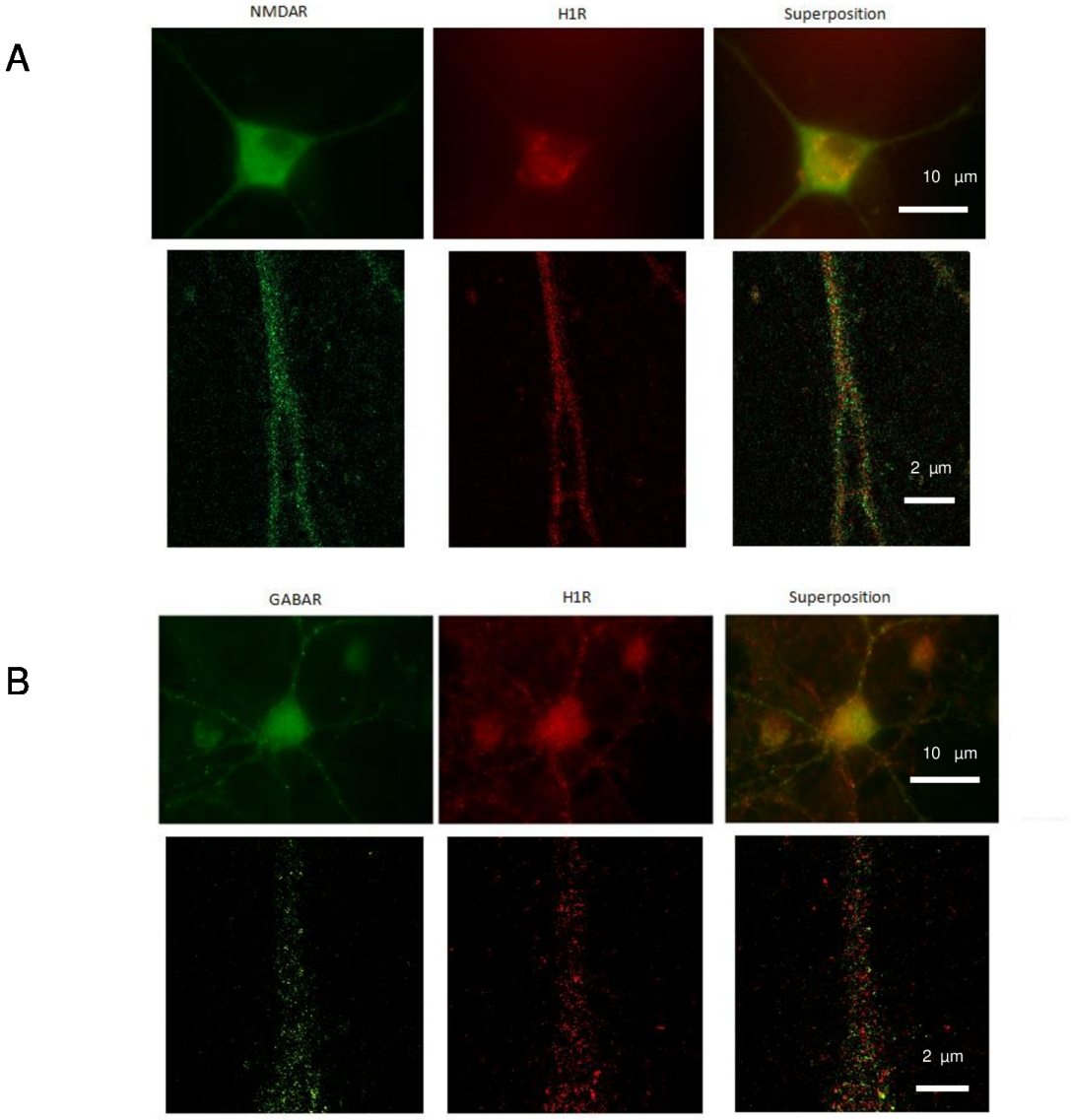

Fig S2

Figure S2. Immunostaining fo**r** NMDA receptor (A) and receptor $GABA_A$ receptor (B) co-expression with the H1 receptor in cultured hippocampal cells. Scale bar 10 µm and 2 µm as indicated