Modulation of dendritic AMPA receptors by different forms of synaptic plasticity

Thesis of Ph.D.

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Contents

CA1 pyramidal neurons have tens of thousands of excitatory and inhibitory synaptic connection with other neurons and the integration of these thousands of inputs determinate the electrical activity of the certain neuron. Through the synaptic connections the information transfer are mainly mediated by two neurotransmitters: glutamate and γ-amino-butyric acid (GABA). The excitatory (glutamatergic) synaptic inputs are widely spread out on several hundreds of micrometers on the dendritic arbor, meanwhile the action potential output localized at the proximal site of the axon. These excitatory synaptic transmissions are conveyed by glutamate activated ionotropic channels (iGluR) α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA), *N*-methyl-D-aspartate (NMDA) and kainite (KA), which are named for the agonists that selectively stimulate them, located at the postsynaptic site and activated by the presynaptically released glutamate. AMPA receptor mediated glutamate currents are responsible for the rapid information transfer between neurons, whereas the NMDA receptors have a detector function for specific patterns of activity which can induce long-term changes in the synaptic strength by modulating the AMPA responses.

AMPA receptors are tetrameric or pentameric ionotropic channels, built from subunits GluR1-4. These subunits can form certain type of heteromeric or homomeric channels. Recently, several details of transport and insertion of different types of heteromeric AMPA receptor have become evident including the different physiological roles of different subunits determined mostly by their C-termini, where different phosphorylation and

transport protein binding sites are located. Recent studies together strongly suggest that different subunit combinations of AMPA receptors are differentially involved in the three types of plasticity (I. Homeostatic, II. Hebbian-type and III. Distance-Dependent-Synaptic-Scaling) and offer a great opportunity to investigate them by manipulation of the receptor subunits.

I. Homeostatic plasticity provides network and cellular stability, globally optimizes synaptic connections and regulates excitability, adjusting gain of the neurons in dynamically changing environment

II. Hebbian type plasticity /LTP, LTD, etc/ produces associative changes of individual synaptic strength, progressively modifies network properties increasing synaptic weight differences between synapse (depending on synaptic event history). It is believed that it is crucial in computational tasks, learning and memory.

III. Distance dependent synaptic scaling (DDSS) adjusts the weight of every synapse to make them equally capable to have same impact on output regardless of their distance. Distance-dependent synaptic scaling (DDSS) shares many similar features with the previously distinguished, two major forms of synaptic plasticity: homeostatic and Hebbian type. Like homeostatic plasticity, DDSS globally optimizes synaptic connections. The major target of all three forms of plasticity is the alteration of the level of synaptic transmission by changing the number and/or properties of postsynaptic glutamate receptors Homeostatic plasticity globally alters the number of

synaptic AMPA receptors by changing their half-life over minutes or hours/days. Hebbian plasticity can change AMPA receptor number within minute. The DDSS also works through increasing the number of AMPA receptors but proportional to the distance from the soma.

Previous works suggested a strong relationship between different forms of synaptic plasticity and the density/properties of synaptically active glutamatergic, GABAergic receptors.

The last few years, we have focused on the DDSS and the Hebbian-type plasticity, to determine the underlying mechanism of these two plasticity forms. We have directly examined single synaptic transmission before and after different electrical/molecular manipulations in control and genetically modified mice avoiding the spatial and temporal influence of other synapses and/or anatomically uneven dendritic conductance differences that distort conventional somatic recordings. To characterize biophysical properties such as single-channel conductance, open probability, kinetic features and agonist affinity of glutamate-activated channels we used outside-out patches excised from dendrites. This provided insight into specific alterations of excitation in chemically and genetically manipulated rats and mice.

Hippocampal slices (350-400 μm) were prepared from adult rats and 42-54 day-old GluR1 -/- mice and wild type littermates using previously described standard procedures, perfusing and holding the slices with physiological ACSF bubbled with 95% O_2 and 5% CO_2 at ~33 °C (pH 7.4). Patch pipettes (5-8 MΩ) were pulled from borosilicate glass and filled with an internal solution contained KCH_3SO_4 or Cs-gluconate (depended on experiments). All neurons had resting potentials between -60 and –75 mV. Series resistances from dendritic whole-cell recordings were between 10 and 30 MΩ. Unitary synaptic events were evoked by pressure ejection of a hyperosmotic external solution, and AMPA currents were isolated by the presence of external NMDA and GABA blockers. Currents were recorded at –70 mV using an Axopatch 200B amplifier, filtered at 5 kHz and digitized at 50 kHz.

Miniature EPSCs crossing an approximate 4 pA threshold level were selected for further examination using a template fit algorithm written in Igor Pro. Events were fit with a sum of two exponential functions to obtain peak amplitude, rise and decay time constants. Events that had rise-time constants greater than 400 μs were eliminated from analysis since these events were unlikely to be from local synapses. Local dendritic recordings were done in different distance from soma, proximally (60-80 μm, at mice) and distally (200-220 μm) to investigate the distance dependence of them.

Outside-out patches were pulled from dendritic locations by patch pipettes to describe kinetic and biophysical properties of iGluR receptors, using fast application of glutamate to evoke AMPA or NMDA currents.

Currents were recorded at –80 mV using an Axopatch 200B amplifier, filtered at 2 kHz and digitized at 20 kHz.

Single-channel properties of dendritic AMPA receptor, namely the single-channel conductance (γ), maximum open probability ($P_{o,max}$) and channel number was estimated using non-stationary fluctuation analysis of current evoked by 1 ms pulses of saturated concentration of glutamate. Between 20 and 100 traces per patches were obtained for analysis. The mean variance determined from all responses, was plotted against the mean current for all responses.

To exclude the possible presynaptical changes we used MNI-glutamate (MNI-glu) on isolated, visualized spines. Two-photon microscope was used to un-caging MNI-glu by focused laser beam on single synapse. The evoked synaptic currents were recorded by local dendritic pacth pipette.

Distance-dependent increase in AMPA receptor number in the dendrites of adult hippocampal CA1 pyramidal neurons

The main finding is that the mean amplitude (I) of the AMPA current at least doubles (I at 100 µm 276 ± 42 pA; distal 250 µm 566 ± 74 pA; $p<0.005$) with distance from the soma. This distance-dependent increase in AMPA receptor current could be the result of an increased receptor number, receptor density or some modification of receptor properties. Modified subunit composition or different phosphorylation states can change the kinetic properties, ionic permeability, agonist affinity (EC_{50} proximal 436µM, n=6; distal 479µM, n=7) current-voltage relationship (E_{rev} proximal 4.2 ± 1.82 mV, n=4; and distal 4.4 ± 1.45 mV, n=6), single-channel conductance (γ at 125 µm 9.83 ± 0.69 pS, n=5 and at 250 µm 9.42 ± 0.95 pS, n=5) and maximum open-probability ($P_{o,max}$ in both locations 0.83 ± 0.01 and 0.84 ± 0.02, respectively) of AMPA channels. We examined all of these receptor properties and compared them with distance from the soma, and found no significant differences in any of them, while on the other hand, channel number increased approximately two-fold (N=467 ± 42, proximal 125 µm and N=1041 ± 206, distal 250 µm from soma, $p<0.05$). These data then strongly suggest that the distance dependent increase in AMPA current is due to a progressive increase in number of AMPAR in the patches and not to alterations in the basic properties of the receptor/channels.

Impaired regulation of synaptic strength in hippocampal neurons from GluR1-deficient mice

We have characterized the properties of dendritic AMPA receptors, spontaneous synaptic currents and postsynaptic AMPA receptor responsiveness in hippocampal CA1 pyramidal neurons from both wild type and GluR1 -/- mice. The AMPA receptor currents from outside-out patches pulled from the apical dendrites of GluR1 -/- mice are severely reduced (~97%) in amplitude (~20 μm from the soma, WT: 541 ± 108 pA, n = 9; KO: 11 ± 2 pA, n = 9; $p<0.001$). Similar magnitude of reduction was also detected at a more distal region (170-200 μm) where spine and synaptic density is high (WT: 874 ± 136 pA, n = 8; KO: 25 ± 8 pA, n = 12; $p<0.001$). These currents from the KO mice also appear to decay faster and have a lower probability of opening than WT currents. Spontaneous synaptic currents are also smaller in amplitude in the KO mice and the degree of this reduction is dependent on the dendritic location of the synapse (at 100 μm WT: 11.7 ± 0.9 pA, n=5; GluR1-/-: 8.2 ± 0.8 pA, n=6), with distal synapses showing the greatest reduction (at 200 μm WT: 24.2 ± 2.3 pA, n=6; GluR1-/-: 10.1 ± 0.9, n=8). In WT mice, the mEPSCs increased 107 % with distance from soma, meanwhile in KO mice this increase was only 23%. Statistical analyses of the synaptic currents indicate that the distal SC synapses of KO mice lack a normal increase in postsynaptic responsiveness, and focal application of glutamate onto postsynaptic spines confirms this scenario. We interpret these data to indicate that the extra-synaptic pool of AMPA receptors is almost entirely composed of GluR1-containing AMPA receptors (probably GluR1/2 heteromeres) and that distance-dependent scaling of SC synaptic weight is

the result of an increased delivery of these receptors to distal synapses. Furthermore, this regulated delivery probably involves receptor cycling between the synaptic and extra-synaptic pools of AMPA receptors.

Changes in AMPA Receptor Currents Following LTP Induction on CA1 Pyramidal Neurons

In the third part of our investigation we have characterized the changes in dendritic AMPA receptors in hippocampal CA1 pyramidal neurons from adult rats that are produced by, or at least coincide with, LTP induction. The main observations is that tetanus induced potentiation significantly increased (~75%) the amplitude of AMPA receptor mediated glutamate currents in outside-out patches pulled from the apical dendrites of adult rats (control: I=1019 ± 47 pA, n=12; LTP: I=1742 ± 173 pA, n=11). This increase is due to an increase in the number of AMPA receptors in the patches (control: N=1642 ± 126, LTP: N=2771 ± 249). These currents also appear to decay more slowly (control: τ=3.2 ± 0.3 ms, n=7; LTP: τ=4.4 ± 0.3 ms, n=11, $p<0.01$) and have a slightly higher probability of opening ($P_{o,max}$ 0.87 ± 0.03 vs 0.92 ± 0.01, respectively) than control or unpotentiated neurons. Other properties of AMPA receptors like single-channel conductance, channel rectification and glutamate affinity do not show any alterations following potentiation.

Increases in intracellular CaMKII activity mimic the changes in AMPA receptor properties that were observed following tetanus-induced potentiation. The dendritic region affected by the synaptic stimulation is not much greater than the area receiving input and requires the presence of

excitatory synapses. We interpret our data to indicate that the stimuli used here produce an increased delivery of AMPA receptors (probably GluR1/2 heteromeres) to synaptically-active regions of the apical dendrite without inducing any significant changes in their basic biophysical properties. We suspect that the primarily extra-synaptic receptors sampled by our patches will ultimately arrive at activated PSDs via the regulated process of receptor cycling between the synaptic and extra-synaptic pools of AMPA receptors.

All of our results are compressed into a simplified model, indicating that the changes in number and/or properties of AMPA receptors are the crucial underlying mechanism of different forms of synaptic plasticity, and that certain types of subunit composed channels are differentially involved. The GluR1 containing AMPA receptors are a basic component of the synaptic pool of glutamate receptors at Schaffer-collateral synapses, particularly at distant synapses. Also GluR1 containing AMPA receptors are the main component of the extra-synaptic AMPA receptor pool. In addition Location-dependent insertion of GluR1 containing AMPA receptors mediates distance-dependent scaling in hippocampal CA1 pyramidal neurons. Together these data suggest that the highly regulated cycling of AMPA receptors between synaptic and extra-synaptic receptor pools is dependent upon the presence of GluR-1 containing receptors, and furthermore that two functionally diverse forms of synaptic plasticity, LTP and distance-dependent scaling, may both use this cycling system to regulate synaptic strength. Also, the lack of GluR1 subunits in KO animals suggests that every synapse is formed by roughly the same number of GluR2/3 subunits regardless of distance from soma. This observation indirectly

suggests, that GluR1 containing receptors are added to ″top-up″ synapses depending on their distance from the soma. On the top of these distance scaled synapses, more GluR1 containing receptor can be added during long-term potentiation. It is certain that, these plasticity forms have an affect on each other and do not work separately to maintain the homeostasis of the neuronal network and retain the ability to react properly in a dynamically changing environment.

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