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Original Article

Facilitation of AMPA receptor-mediated steady-state current by extrasynaptic NMDA receptors in supraoptic magnocellular neurosecretory cells

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ABSTRACT In addition to classical synaptic transmission, information is transmitted between cells via the activation of extrasynaptic receptors that generate persistent tonic current in the brain. While growing evidence supports the presence of tonic NMDA current (I_{NMDA}) generated by extrasynaptic NMDA receptors (eNMDARs), the functional significance of tonic I_{NMDA} in various brain regions remains poorly understood. Here, we demonstrate that activation of eNMDARs that generate INMDA facilitates the α-amino-3-hydroxy-5-methylisoxazole-4-proprionate receptor (AMPAR)-mediated steady-state current in supraoptic nucleus (SON) magnocellular neurosecretory cells (MNCs). In low-Mg^{2+} artificial cerebrospinal fluid (aCSF), glutamate induced an inward shift in $I_{holding}$ (I_{GLU}) at a holding potential ($V_{holding}$) of –70 mV which was partly blocked by an AMPAR antagonist, NBQX. NBQX-sensitive I_{GLU} was observed even in normal aCSF at $V_{holding}$ of –40 mV or –20 mV. I_{GLU} was completely abolished by pretreatment with an NMDAR blocker, AP5, under all tested conditions. AMPA induced a reproducible inward shift in $I_{holding}$ (I_{AMPA}) in SON MNCs. Pretreatment with AP5 attenuated I_{AMPA} amplitudes to ~60% of the control levels in low-Mg^{2+} aCSF, but not in normal aCSF at $V_{holding}$ of –70 mV. I_{AMPA} attenuation by AP5 was also prominent in normal aCSF at depolarized holding potentials. Memantine, an eNMDAR blocker, mimicked the AP5-induced I_{AMPA} attenuation in SON MNCs. Finally, chronic dehydration did not affect I_{AMPA} attenuation by AP5 in the neurons. These results suggest that tonic I_{NMDA}, mediated by eNMDAR, facilitates AMPAR function, changing the postsynaptic response to its agonists in normal and osmotically challenged SON MNCs.

INTRODUCTION

Accumulating evidence over the last few decades suggests that in addition to classical synaptic transmission, information is transmitted between cells via the diffusion of neurotransmitters into the extracellular space and the activation of extrasynaptic receptors in the brain [1,2]. The activation of extrasynaptic ionotropic receptors generates slow, persistent tonic currents that offer unique mechanisms of neuronal control [3,4], while synaptic receptors mediate rapid, phasic excitatory (E) or inhibitory (I) postsynaptic currents (EPSCs or IPSCs, respectively). In this sense, activation of extrasynaptic N-methyl-D-aspartate receptors (eNMDARs) by glutamate in the extracellular space can evoke a persistent tonic NMDA current (tonic I_{NMDA}) [5-8], while synaptic

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NMDA receptors are responsible for classical EPSCs. The idea that a glutamate signaling mechanism can be compartmentalized via synaptic and extrasynaptic NMDARs has been also supported bysyudies showing that synaptic and extrasynaptic NMDARs are linked to distinct, and even opposing, downstream biological actions [9-11]. However, despite growing evidence that supports the presence of tonic I_{NMDA} generated by eNMDARs, the functional significance of tonic I_{NMDA} in various brain regions remains poorly understood.

In the brain, α-amino-3-hydroxy-5-methylisoxazole-4-proprionate receptors (AMPARs) are responsible for the bulk of glutamatergic EPSCs and their dynamic regulation ensures dynamic fitting of the receptor function that underlies much of the plasticity of excitatory transmission. For example, calcium influx through open ATP-gated channels leads to reduced surface AMPARs in dendrites and at synapses in the hippocampus [12], while it facilitates AMPAR function in the hypothalamic paraventricular nucleus (PVN) neurons [13,14]. Given that activation of either synaptic or extrasynaptic NMDARs causes changes in intracellular calcium concentration [9], it is plausible that both synaptic and extrasynaptic receptors are involved in the regulation of AMPAR function. Despite the wealth of information regarding the role of synaptic NMDARs, little is known about the role of eNMDARs in regulating AMPAR function in the brain.

Magnocellular neurosecretory cells (MNCs), composed of vasopressin and oxytocin neurons in the hypothalamic PVN and supraoptic nucleus (SON), play a major role in fluid-balance homeostasis and reproductive function [15]. As in other brain regions, glutamate is a critical excitatory neurotransmitter in SON MNCs [16]. Glutamate-generating tonic I_{NMDA} under glial control efficiently influences neuronal activity in the magnocellular neurosecretory system. Enhanced activation of eNMDARs, with blunted glial GLT-1 clearance, contributes to increased MNC activity and hormone release during dehydration and heart failure conditions [4,6]. However, the functional role(s) of eNMDARs that generate tonic I_{NMDA} must be further elucidated in SON MNCs. In the present study, we demonstrated that activation of eNMDARs that generate tonic I_{NMDA} facilitates AMPAR function, resulting in enhanced AMPA-induced steady-state current in SON MNCs.

METHODS

Experimental animals

All animal experiments adhered to the Chungnam National University policies regarding the care and use of animals. Male Sprague–Dawley rats (60~80 g) were housed under a 12/12-h light/dark schedule. Rats were randomly divided into two groups: euhydrated (EU) and chronic dehydarated (DE) animals. The EU group was allowed free access to normal tap water, whereas DE rats exposed by 2% saline for 7 days. All rats had access to food water *ad libitum* except for the DE periods throughout the experiments. Plasma osmolality was measured by freezing-point depression (Fiske Associates, Norwood, MA, USA) prior to sacrifice.

Electrophysiological recordings and data analysis

Hypothalamic slices (300 μm) were obtained as previously described [17]. Brian slices containing the SON were cut using a vibroslicer (Leica VT 100s, Leica, Bensheim, Germany) and placed in a holding chamber containing standard artificial cerebrospinal fluid (aCSF) until use. Standard aCSF consists of 126 mM NaCl, 26 mM $NaHCO_3$, 5 mM KCl, 2.4 mM NaH_2PO_4, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 10 mM glucose, pH 7.3~7.4. The medium was saturated with 95% O_2 and 5% CO_2.

Patch-clamp recordings were obtained using an Axopatch200-B amplifier (Axon Instruments, Foster City, CA, USA). For voltage-clamp recordings, a low Mg^{2+} aCSF (20 μM $MgCl_2$) was used to facilitate NMDAR-mediated currents at a holding potential ($V_{holding}$) of –70 mV. Periods of 180 sec of synaptic activity were analyzed using the Minianalysis 6.0.3 program (Synaptosoft Inc., Decatur, GA). In some cases, recordings were also obtained at a $V_{holding}$ of –40 mV or –20 mV in normal standard aCSF. Currents were recorded in the presence of picrotoxin (100 μM) to inhibit ionotropic GABA receptors, if not mentioned. Current output was filtered at 2 kHz and digitized at 10 kHz (Digidata 1322A, Axon Instruments) in conjunction with pClamp 9.2 software. Patch pipettes (3~5 MΩ) were filled with a solution containing 140 mM K-gluconate, 10 mM KCl, 10 mM HEPES, 0.5 mM $CaCl_2$, 5 mM EGTA and 5 mM Mg^{2+}ATP, pH 7.3.

Persistent activation of AMPARs and NMDARs was defined as the difference of holding current ($I_{holding}$) before and after application of the receptor agonists: the glutamate AMPA/kainate receptor antagonists, NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione) or DNQX (5,7-dintroquinoxaline-2,3-dione, 10 μM), and the NMDA receptor antagonist AP5 (D,L-2-amino-5-phosphonopentanoic acid, 100 μM), respectively [17-19]. The $I_{holding}$ was measured in 50 ms epochs of traces lacking PSCs using Mini Analysis (Synaptosoft, Decatur, GA, USA).

Series resistance was monitored throughout the experiment. Neurons with series resistance changes >15% during experiments were not included in the analysis.

Statistics

Numerical data are presented as the mean±standard error of the mean (SEM). Statistical significance of the data was determined using independent or paired Student's *t*-test as needed.

RESULTS

Electrophysiological recordings were obtained from 112 SON MNCs under bright-light microscopy [20]. In a low-Mg^{2+} bath solution, AP5 induces an outward shift in the holding current ($I_{holding}$), named tonic I_{NMDA}, mediated by eNMDAR in SON MNCs [6,7]. The mean amplitude of tonic I_{NMDA} in SON MNCs was 12.1±2.5 pA (n=7) in our recording conditions (20 μM of $MgCl_2$ and $V_{holding}$ of –70 mV). In contrast, AP5 caused no significant changes in $I_{holding}$ in normal aCSF containing 1.2 mM $MgCl_2$.

Tonic activation of AMPARs by glutamate in SON MNCs

To determine whether I_{NMDA} generated by eNMDARs alters AMPARs function to change the response to its agonists, glutamate was bath applied with a known concentration of the agonist (10 μM) in low Mg^{2+} aCSF. Glutamate caused a steady-state inward shift in $I_{holding}$ (I_{GLU}) at $V_{holding}$ of –70 mV, which was blocked by the sequential application of NBQX and NBQX+AP5 in SON MNCs (Fig. 1A and 1B). Interestingly, glutamate failed to change $I_{holding}$ in the presence of AP5 in SON MNCs (Fig. 1A and 1B), suggesting that glutamate induced persistent tonic activation of AMPARs in an NMDAR-activation-dependent manner in SON MNCs. In agreement with this, glutamate induced NBQX-sensitive currents in normal aCSF when $V_{holding}$ was depolarized to –40 mV or –20 mV, activating NMDARs. While glutamate caused minimal changes in $I_{holding}$ (p>0.2, n=5) at $V_{holding}$ of –70 mV, it induced NBQX-sensitive I_{GLU} at THE depolarized $V_{holding}$ (–40 mV, 16.6±2.9 pA, n=7; –20 mV, 14.6±4.9 pA, n=7) (Fig. 1C and 1D). Furthermore, I_{GLU} was completely inhibited by pretreatment with AP5 at all tested potentials in SON MNCs (data not shown). In a subset of experiments, we further confirmed that I_{GLU} was blocked by the additional application of AP5 (Fig. 1C inset).

NMDARs blockade reduced AMPA-induced current in SON MNCs

To determine whether NMDAR activation caused an alteration in the steady state sensitivity of the AMPA receptor-activated channels to its agonists, AMPA was bath-applied in low-Mg^{2+} aCSF and normal aCSF. Application of AMPA (1 μM) induced a reproducible steady-state inward current (I_{AMPA}) in the SON MNCs, which was blocked by an AMPAR antagonist, DNQX or

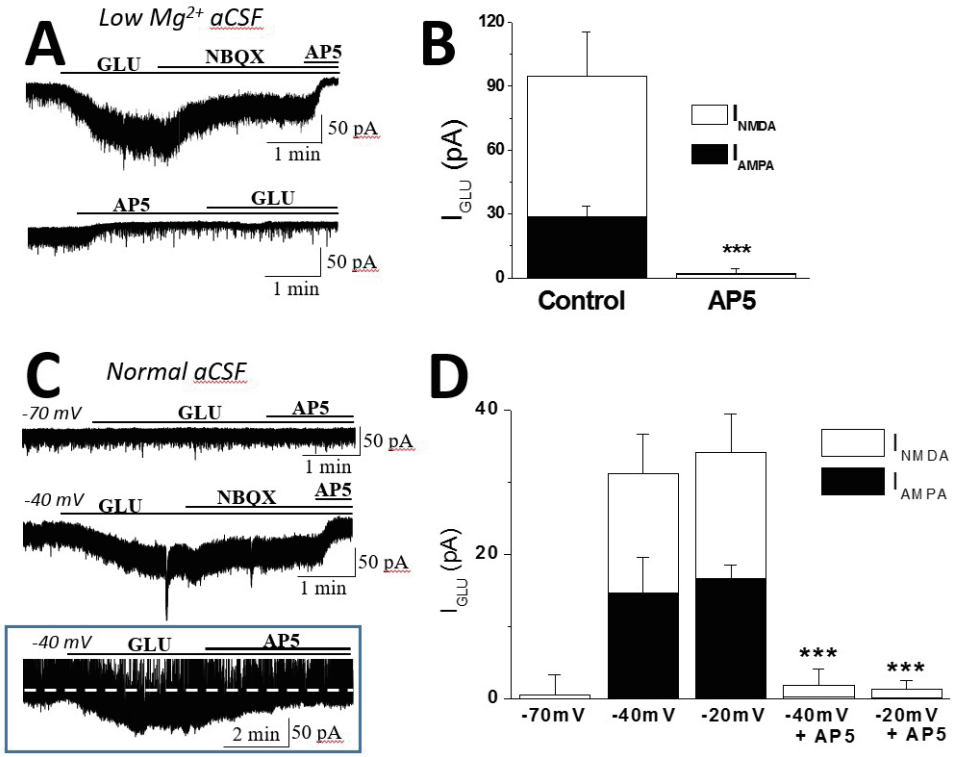

Fig. 1. Glutamate evoked NBQX-sensitive tonic currents in an NMDAR activation-dependent manner in SON MNCs. (A) Representative current traces showing that I_{GLU} was blocked by the sequential application of NBQX (I_{AMPA}) and NBQX+AP5 (I_{NMDA}) in low-Mg^{2+} aCSF. (B) The mean I_{AMPA} and I_{NMDA} in the absence (n=7) and presence (n=3) of AP5 are summarized as in A. (C) Representative current traces showing that glutamate evoked I_{AMPA} in an I_{NMDA}-dependent manner at depolarized holding potentials in normal aCSF. Note that I_{Glu} was efficiently blocked by AP5 alone, which was not affected by picrotoxin (inset, $V_{holding}$ of –40 mV in normal aCSF). (D) The mean I_{AMPA} and I_{NMDA} at different holding potentials (n=3~7) are summarized as in C. ***p<0.001 compared to each control.

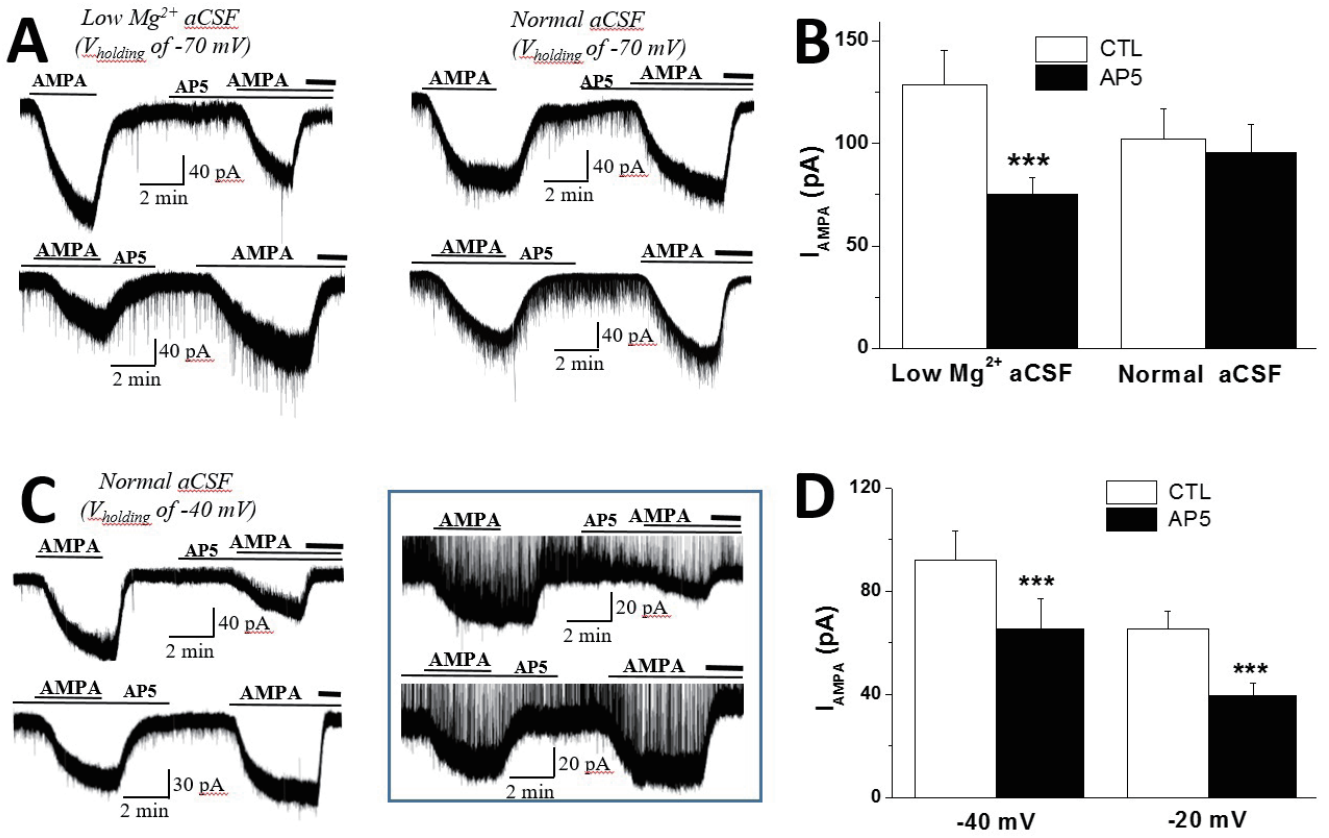

Fig. 2. NMDAR activation facilitated AMPA-induced steady-state inward currents (I_{AMPA}) in SON MNCs. (A) Representative current traces showing I_{AMPA} in low and normal Mg^{2+} aCSF ($V_{holding}$ –70 mV). Note that pretreatment with AP5 consistently reduced I_{AMPA} in low-Mg^{2+} aCSF, while repeated applications of AMPA increased I_{AMPA} in normal aCSF. Bold lines at each trace represent the application of an AMPAR antagonist, DNQX or NBQX. (B) The mean I_{AMPA} amplitudes in low-Mg^{2+} (n=12) and normal (n=10) aCSF are summarized as in A. Effects of AP5 on the first and second I_{AMPA} were tested and combined from the same number of neurons in each group. (C) Representative current traces showing that the pretreatment of AP5 consistently reduced I_{AMPA} recorded at $V_{holding}$ of –40 mV in normal aCSF. I_{AMPA} inhibition by AP5 was not affected by picrotoxin in normal aCSF ($V_{holding}$ –20 mV, inset). Bold lines at each trace represent the application of AMPAR antagonist as in A. (D) The mean I_{AMPA} amplitudes at $V_{holding}$ of –40 mV (n=12) and –20 mV (n=10) are summarized. Effects of AP5 on the first and second I_{AMPA} were pooled as in B. ***p<0.001 compared to respective controls.

NBQX (Fig. 2). We recorded and compared I_{AMPA} amplitudes in the absence and presence of the NMDAR antagonist.

Although there is a tendency for I_{AMPA} amplitude to increase with repeated AMPA applications, pretreatment with AP5 consistently attenuated I_{AMPA} amplitude in the first and second AMPA applications (Fig. 2A). The inhibitory effects of AP5 on the first and second I_{AMPA} amplitudes were tested in the same number of neurons and combined to compare with the control values (Fig. 2B). In the presence of AP5, I_{AMPA} amplitude was decreased to 60.3±4.06% of that in the absence of the antagonist in low-Mg^{2+} aCSF (control: 134.8±17.0 pA; AP5: 77.8±8.5 pA; n=12, p<0.001) (Fig. 2A and 2B), while pretreatment with AP5 failed to affect I_{AMPA} amplitudes in normal aCSF (control: 91.7±10.9 pA; AP5: 86.9±11.5 pA; n=10, p>0.15). I_{AMPA} facilitation by I_{NMDA} was also evident in normal aCSF at depolarized $V_{holding}$ activating NMDARs (Fig. 2C and 2D). In agreement with I_{AMPA} facilitation by I_{NMDA} in low-Mg^{2+} aCSF, AP5 reduced I_{AMPA} amplitudes from 92.0±11.2 pA to 65.3±11.7 pA (n= 12, p<0.001) at $V_{holding}$ of –40 mV in normal aCSF. Pretreatment with AP5 also significantly reduced I_{AMPA} at $V_{holding}$ of –20 mV (control: 65.2±7.0 pA; AP5: 39.3±5.1 pA; n=10, p<0.001) (Fig. 2D). In a subset of experiments, AP5 inhibition of I_{AMPA} was tested in the absence of picrotoxin (Fig. 2C, inset). Pretreatment with AP5 efficiently reduced I_{AMPA} in the absence of picrotoxin at $V_{holding}$ of –20 mV in normal aCSF (control: 55.8±6.7 pA; AP5: 35.3±5.3 pA; n=4, p<0.01).

These results suggested that NMDAR activation enhanced the steady-state activation of AMPARs, altering the neuronal response to the receptor agonists in SON MNCs.

Roles of eNMDAR in I_{AMPA} potentiation in SON MNCS

In the next experiments, we investigated whether eNMDARs that generate tonic I_{NMDA} contribute to I_{AMPA} facilitation in SON MNCs. To isolate eNMDAR function, we adopted an eNMDAR selective antagonist, memantine [21,22]. To investigate the role of eNMDARs in I_{AMPA} facilitation, we recorded and compared I_{AMPA} amplitude in the absence and presence of memantine in low-Mg^{2+} aCSF (Fig. 3). Memantine induced an outward shift in $I_{holding}$

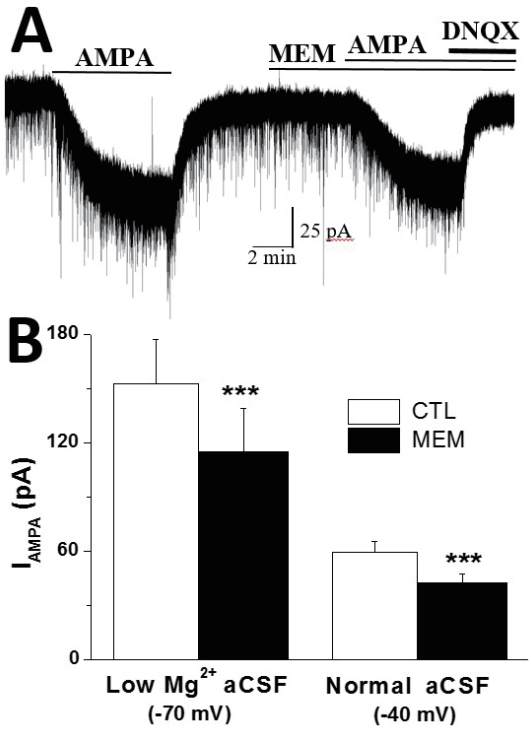

Fig. 3. Extrasynaptic NMDARs mediate I_{AMPA} facilitation in SON MNCs. (A) Representative current traces showing that the extrasynaptic NMDAR antagonist, memantine (MEM), attenuated I_{AMPA} in low-Mg^{2+} aCSF ($V_{holding}$ −70 mV). (B) Effects of memantine on I_{AMPA} amplitude in low-Mg^{2+} aCSF ($V_{holding}$ −70 mV, n = 8) and normal aCSF ($V_{holding}$ −40 mV, n = 6) are summarized. Effects of MEM on the first and second I_{AMPA} were pooled as in Fig. 2. ***p<0.001 compared to respective control.

(13.2±3.8 pA, n=6), which is comparable to tonic I_{NMDA} uncovered by AP5 (12.1±2.5 pA, n=7, p>0.7) in low-Mg^{2+} aCSF.

Pretreatment with memantine reversibly and significantly reduced I_{AMPA} amplitudes from 152.3±24.4 pA to 114.8±24.0 pA (n=8) in low-Mg^{2+} aCSF (p<0.05) (Fig. 3A and 3B), while it failed to affect I_{AMPA} in normal aCSF (control: 108.8±12.7 pA; memantine: 105.0±13.2 pA; n=6). The inhibition rate of I_{AMPA} by pretreatment with memantine (65.8±5.4% of control, n=8) was comparable to that of AP5 (60.3±4.06% of control, n=12, p>0.4) in low-Mg^{2+} aCSF. Pretreatment with memantine also significantly reduced I_{AMPA} at $V_{holding}$ of −40 mV in normal aCSF (control: 59.2±6.1 pA; memantine: 42.3±5.1 pA; n=6, p<0.01) (Fig. 3B).

These results suggested that eNMDAR activation facilitates I_{AMPA} in SON MNCs.

I_{AMPA} potentiation by NMDARs during chronic dehydration

To investigate the functional significance of I_{AMPA} facilitation by tonic I_{NMDA} in SON MNCs, we examined and compared the I_{AMPA} potentiation by I_{NMDA} in euhydrated (EU) and chronic dehydrated (DE) rats (Fig. 4). Chronic dehydration with a 7-day salt loading (2% NaCl) protocol increased plasma osmolarity from 306.7±2.3

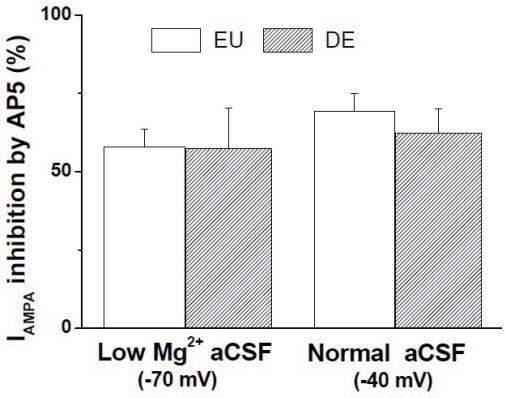

Fig. 4. I_{AMPA} facilitation by NMDAR in SON MNCs from chronically dehydrated (DE) rats. Inhibition of I_{AMPA} by AP5 was compared in euhydrated (EU) and DE SON MNCs. AP5 inhibited I_{AMPA} at similar rates in DU and DE SON MNCs.

mOsm (n=4) to 378.5±7.1 mOsm (n=5). Consistent with a previous report [23], DE significantly increased EPSC frequency in SON MNCs (EU: 1.81±0.35 Hz, n=6; DE: 4.09±0.62 Hz, n=7).

Bath application of AMPA induced reproducible I_{AMPA} in DE SON MNCs as in EU SON MNCs. Pretreatment with AP5 significantly reduced I_{AMPA} in low-Mg^{2+} aCSF at $V_{holding}$ of −70 mV (control: 68.6±15.0; AP5: 45.9±12.8 pA; n=8, p<0.05) and in normal aCSF at $V_{holding}$ of −40 mV (control: 70.1±10.4 Pa; AP5: 47.9±8.6 pA; n=8, p< 0.001) in DE SON MNCs. Although I_{AMPA} amplitudes were slightly lower in DE than in EU neurons, the difference did not reach statistical significance (p>0.2 in both cases). Furthermore, pretreatment with AP5 inhibited I_{AMPA} at similar rates in EU and DE SON MNCs (Fig 4), suggesting that I_{AMPA} potentiation by I_{NMDA} was preserved in DE SON MNCs.

DISCUSSION

The main findings of this study may be summarized as follows: 1) glutamate induced the tonic activation of AMPARs in a tonic I_{NMDA}-dependent manner; 2) tonic I_{NMDA} mediated by eNMDARs facilitated AMPA-induced steady sate inward currents (I_{AMPA}); and 3) I_{AMPA} potentiation by eNMDARs was preserved in SON MNCs during chronic dehydration. To our knowledge, these data are the first to demonstrate that eNMDAR activity modulates the steady-state sensitivity of the AMPA receptor channels to their agonists in neuroendocrine systems.

Tonic I_{NMDA} mediated by eNMDARs in SON MNCs

Although receptors are often considered extrasynaptic if they are located more than 100 nm from the postsynaptic density, the precise delineation of the synaptic-extrasynaptic receptors seems to be specific to the parameter under considerations [24]. In terms of electrophysiological activities, synaptic NMDARs

are defined as receptors recruited in response to spontaneous glutamate release, generating EPSCs, or during low-frequency afferent stimulation (less than 0.05 Hz), while eNMDARs correspond to those not activated during such conditions. In this sense, there is a general consensus that eNMDARs are responsible for the persistent tonic excitatory current in SON MNCs [6,25], while their synaptic counterparts mediate conventional EPSCs. In the present study, $I_{holding}$ shift by a NMDAR antagonist, AP5, supported the presence of a persistent inward current with basal glutamate release mediated by NMDARs in SON MNCs. Combined with the fact that memantine failed to affect the basic properties of glutamate EPSCs in SON MNCs [6], our results showed that memantine, a selective eNMDAR blocker, mimicked the AP5-induced $I_{holding}$ shift, which confirmed the notion that eNMDARs generate tonic I_{NMDA} in the neurons. The subunit composition of the eNMDAR mediating tonic I_{NMDA} in various brain regions has not been well clarified, while eNMDARs containing the NR2B subunit have been known to be partly responsible for tonic I_{NMDA} [6]. Future studies are warranted to delineate the subunit composition of the eNMDARs mediating I_{AMPA} facilitation in SON MNCs.

Modulation of AMPARs function by tonic I_{NMDA} in SON MNCs

Our results showing that I_{AMPA} was inhibited by AP5 or extracellular Mg^{2+} in low-Mg^{2+} aCSF suggested that activated NMDARs facilitated AMPARs function in SON MNCs. Furthermore, similar inhibitory effects of AP5 and memantine on I_{AMPA} facilitation suggested that eNMDARs facilitated AMPAR function in the neurons. Combined with the fact that I_{GLU} amplitudes were dependent on AP5 (Fig. 1), these results suggested that eNMDAR activity modulates the steady-state sensitivity of AMPA receptors to their agonists in SON MNCs. Given that binding glutamate/AMPA to the AMPA receptor results in Na^{+} influx, which causes depolarization of the membrane, our results showing that AP5 inhibited I_{AMPA}/I_{GLU} could indicate that AP5 inhibited eNMDAR activated by the agonists. However, our results showing that AMPARs antagonists completely abolished I_{AMPA} in both the absence and presence of NMDAR antagonists (Fig. 2) argued against this possibility. It is not likely that a bath application of AMPA recruited additional NMDARs generating I_{NMDA} in our recording conditions. This idea was further supported by the finding that I_{AMPA} was insensitive to the following application of AP5 (data not shown).

In general, there has been a consensus that extrasynaptic glutamate receptors generate slow, persistent tonic currents, while their synaptic counterparts mediate AMPAR- or NMDAR-EPSCs. In the present study, it is not clear whether extrasynaptic AMPARs (eAMPARs) generate I_{AMPA}, and, if so, what portion of the currents is mediated by eAMPARs in SON MNCs. However, it is interesting to note that eAMPARs are highly mobile and move rapidly between the plasma membrane and the intracellular compartments by exocytosis and endocytosis, and diffuse laterally to and from synaptic sites [26-29]. Such continuous AMPAR exchanges between synapses and different cellular pools ensures a dynamic fit of synaptic AMPAR numbers in synaptic plasticity, including long-term potentiation (LTP). A prevailing two-step model is that NMDAR-dependent LTP is mediated by surface insertion and synaptic delivery of AMPARs, in which AMPARs deliver to the extrasynaptic sites first, and their synaptic targeting requires synaptic NMDAR activation that likely triggers the signal transduction cascade necessary to anchor AMPARs in the synapse. Given that activated eNMDARs cause Ca^{2+} influx [9], it is reasonable to assume that eNMDARs regulated AMPARs trafficking in the present study, resulting in I_{AMPA} facilitation. As a gliotransmitter, ATP has been known to activate purinergic receptors in PVN MNCs, promoting the insertion of AMPARs at the surface and, strengthening the excitatory synapses [14]. However, it is noteworthy that NMDAR-induced CaM-KII activation alters AMPARs channel properties in both a Ca^{2+}-dependent and a Ca^{2+}-independent manner [30,31]. It is also possible that I_{NMDA} facilitated AMPAR-mediate currents via increased single channel conductance and/or open channel probability of AMPAR activated channels in SON MNCs. Future studies are warranted to delineate the cellular mechanisms of eNMDAR-mediated I_{AMPA} facilitation in SON MNCs.

Functional significance of I_{AMPA} potentiation by eNMDARs in SON MNCs

A peculiar property of the SON is that it undergoes anatomical remodeling under certain physiological conditions, such as lactation and chronic dehydration. This remodeling includes a reduction in astrocytic coverage of neurons [32] and is associated with increased extracellular levels of glutamate in the SON [33]. The increased extracellular level of glutamate in the nucleus is also in agreement with enhanced EPSC frequency [23] and increased numbers of excitatory synaptic contacts in SON MNCs. Combined with our results showing that I_{AMPA} facilitation by I_{NMDA} is conserved in DE SON MNCs, these results suggested that I_{AMPA} facilitation by I_{NMDA} could contribute to enhanced neuronal activity and hormone release from the neurons during the osmotic challenge.

Spontaneous glutamate release from astrocytes synchronizes neuronal activity via eNMDAR activation [34,35], and increases the frequency of AMPAR-mediated EPSCs via the activation of metabotropic glutamate receptors facilitating presynaptic release in CA1 pyramidal neurons [36] and kainite receptors in hippocampal interneurons [37], respectively. Combined with the fact that Ca^{2+} influx triggers the signal transduction cascade necessary for anchoring AMPARs in synapses [26,38], our results showing that eNMDAR activation potentiated AMPAR-mediated steady-state currents in SON MNCs are in line with astrocytic

glutamate release altering AMPARs, resulting in strengthening of excitatory synapses in SON MNCs.

In summary, our results support the view that tonic I_{NMDA} generated by activated eNMDARs facilitates AMPA receptor function in SON MNCs, which gives the gliotransmitter glutamate the ability to regulate postsynaptic efficacy during normal and physiological challenges, including chronic dehydration.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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