Theta-Frequency Facilitation of AMPA Receptor-Mediated Synaptic Currents in the Principal Cells of the Medial Septum

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Armstrong, John N. and Brian A. MacVicar. Theta-frequency facilitation of AMPA receptor-mediated synaptic currents in the principal cells of the medial septum. J Neurophysiol 85: 1709–1718, 2001. Recent evidence suggests that Ca2+-permeable AMPA receptors display rapid, short-lasting current facilitation. In this study, we investigated the properties of AMPA receptor-mediated synaptic currents in medial septal neurons of the rat in an in vitro slice preparation. Immunocytochemistry with a selective antibody to the GluR2 subunit revealed that both choline acetyltransferase-containing and parvalbumin-containing neurons of the medial septum express no detectable GluR2 subunit immunoreactivity. We used whole cell voltage-clamp recordings to measure synaptically evoked AMPA receptor-mediated currents from medial septal neurons following stimulation of midline afferents. The responses were sensitive to external application of philanthotoxin-343 (PhTx-343, 50 μM), a potent, high-affinity antagonist of Ca2+-permeable, GluR2-lacking AMPA receptors. Rectifying AMPA receptor-mediated currents also displayed a rapid increase in amplitude when evoked at low frequencies. In contrast to currents observed in large medial septal neurons, AMPA receptor-mediated currents evoked in the remaining small (8–11 μm) neurons were nonrectifying and displayed rapid synaptic depression when stimulated five times at 6 Hz. The currents evoked in these cells were unaffected by external application of PhTx-343 and were therefore GluR2-containing AMPA receptors. The results of the present study demonstrate that the principal projection neurons of the medial septum contain PhTx-343-sensitive, GluR2-lacking AMPA receptors that display rapid current facilitation when stimulated at low frequencies.

INTRODUCTION

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors mediate fast, excitatory glutamatergic synaptic transmission throughout the CNS. A single AMPA receptor is a heteromeric tetramer containing any of the four AMPA receptor subunits, GluR1–4 (Rosenmund et al. 1998). Of the four subunits the GluR2 subunit appears to be most abundant (Geiger et al. 1995). When the GluR2 subunit is present, the channel properties of the GluR2 subunit dominate the entire heteromeric receptor complex (Boulter et al. 1990; Nakanishi et al. 1990). Therefore several unusual features of AMPA receptor mediated synaptic transmission are observed when GluR2 subunits are not present. These unusual features include Ca2+-permeability (Burnashev et al. 1992; Hollmann et al. 1991; Hume et al. 1991), rectification (Hollmann et al. 1991; Rozov et al. 1998; Washburn et al. 1997) and sensitivity to block by external polyamines (Brackley et al. 1993; Herlitze et al. 1993; Washburn et al. 1997). Ca2+ impermeability is generated in the AMPA receptor through RNA editing of the GluR2 subunit (Hume et al. 1991; Seeburg 1996). Virtually all GluR2 subunits appear to be edited in the rat brain (Jonas and Burnashev 1995; Seeburg 1996). Therefore AMPA receptors lacking GluR2 subunits are the only native AMPA receptors that are Ca2+-permeable (Hollmann and Heinemann 1994; Washburn et al. 1997).

Rozov et al. (1998) recently showed that recombinant, calcium permeable AMPA receptors, display rapid, short lasting (<5 s) activity-dependent facilitation when stimulated at low frequencies (<50 Hz). Both the inward rectification and facilitation were the result of block of open AMPA receptor channels by intracellular polyamines (Rozov et al. 1998). The following study was designed to determine whether similar low-frequency facilitation could be synthetically activated at calcium permeable AMPA receptors in the medial septum.

Previous studies have suggested that some medial septal neurons possess GluR2-lacking, Ca2+-permeable AMPA receptors. First, AMPA receptor activation caused Ca2+ influx (Schneeggenburger et al. 1993a,b; Waters and Allen 1998). Second, as few as 15% of these neurons contain detectable levels of GluR2/3 immunoreactivity (Page and Everitt 1995), whereas all medial septal neurons have been reported to contain GluR4 AMPA receptor subunits. Unfortunately the determination of the distribution of GluR2-lacking AMPA receptors has been hindered by the lack of a GluR2 antibody that did not show some high degree of cross-reactivity with GluR3.

In the following study, we present evidence from immunocytochemistry using a GluR2-selective antibody that the principal projection neurons of the medial septum possess no...
detectable GluR2 immunoreactivity. AMPA receptor-mediated synaptic currents evoked in these neurons rectify are sensitive to blockade by phosphatase-343 (PhTx-343) and undergo rapid current facilitation when stimulated at 6 Hz.

METHODS

Protein isolation, SDS-PAGE, and Western blots

Total protein was isolated from rat brain tissue that was rapidly dissected and frozen on dry ice. Each 100 mg of tissue was then sonicated in 200 μl of 0.32 M sucrose and refrozen in 10-μl aliquots. Approximately 25 μg of protein was then diluted in sample buffer, electrophoretically separated on a 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) (Laemmli 1970), transferred to a Immobilon-P PVDF membrane (Millipore, Bedford, MA), and blotted with a rabbit anti-GluR2 polyclonal antibody (Chemicon, Temecula, CA) and an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Piscataway, NJ).

Immunocytochemistry

The immunocytochemistry protocol used in the present study was based on the protocol described by Sloviter and Nilaver (1987) as reported previously (Armstrong et al. 1996). Briefly, 10- to 30-day-old rats were anesthetized with pentobarbital sodium (100 mg/kg) and transcardially perfused with phosphate-buffered saline (PBS, pH 7.4) containing 0.1% sodium nitrite followed by 4% paraformaldehyde/0.4% glutaraldehyde. After 3 h additional glutaraldehyde-free fixation, the tissue was sectioned in the coronal or sagittal plane at 25 or 50 μm on a vibrating microtome (VT100, Leica Microsystems, Willowdale, ON). Collected sections were then washed in PBS and incubated in 1% hydrogen peroxide for 30 min. After rinsing several times in PBS, sections were incubated for 1 h in PBS containing 5% normal donkey serum. The sections were then transferred to a fresh solution of PBS containing 0.005% BSA and rabbit anti-GluR2 (1:3,000; Chemicon) and incubated overnight at room temperature. The next day, sections were rinsed several times in PBS and incubated for 1 h in PBS containing biotinylated donkey anti-rabbit IgG (1:1000, Jackson Immunoresearch Laboratories, West Grove, PA); Sections were then washed several times in PBS and incubated in streptavidin-peroxidase complex (1:1000; ABC Elite; Vector Laboratories) for 1 h. Sections were washed again several times in PBS and reacted for 15 min in PBS containing 0.003 mg/ml glucose oxidase, 0.4 mg/mL-1 ammonium chloride, 2 mg/mL-1 β-D-glucose, and 0.01% dianidobenzidine-tetrachloride (DAB, Sigma, St. Louis, MO). Occasionally cobalt chloride was added to this solution to produce a black reaction product that was easier to visualize than the standard brown reaction product. Sequential black/brown DAB was carried out by first completing an entire reaction using the cobalt chloride enhanced DAB followed by an entire reaction using standard (brown) DAB. Finally, following 15 min in DAB solution, the sections were washed several times in PBS and mounted on gelatin-, chrom-alum-coated slides, air-dried overnight, dehydrated in a series of ethanol, cleared in xylene, and coverslipped with Permount (Sigma).

For co-localization studies, immunocytochemistry was first carried out for GluR2 as described above using a Cy5-conjugated donkey anti-rabbit secondary antibody (1:1,000, Jackson Immunoresearch Laboratories). The GluR2 labeled sections were then incubated overnight in PBS containing 0.005% BSA, 0.1% TritonX-100, mouse anti-parvalbumin (PARV; 1:20,000; Sigma) and goat anti-choline acetyltransferase (ChAT; 1:3,000; Chemicon). The following day the sections were rinsed several times in PBS and incubated overnight at 4°C in buffer containing Cy2-conjugated donkey anti-mouse IgG and Cy3-conjugated donkey anti-goat IgG (Jackson Immunoresearch Laboratories) at a dilution of 1:600 PBS containing 0.1% TritonX-100 and 0.005% BSA. Sections were then washed several times in PBS and mounted on gelatin-, chrom-alum coated slides, air-dried overnight, coverslipped with FluorSave (Calbiochem, La Jolla, CA) and imaged on a LSM510 Laser Scanning Axioplan 2 Microscope (Carl Zeiss Mikroskopie, Jena, Germany).

Slice preparation

Slices containing rat basal forebrain were obtained from male and female neonatal Sprague-Dawley rat pups. All rat pups were housed with their dam and littermates according to the guidelines set by the Canadian Council on Animal Care. Successful whole cell recordings were made in slices obtained from 5- to 21-day-old rat pups; however, the majority of recordings were made in slices prepared from rat pups that were 9–10 days of age.

To obtain slices, rats were decapitated, their brains rapidly removed, and the basal forebrain containing the medial septum/diagonal band of Broca and surrounding structures blocked. This tissue block was rapidly fixed to a mounting tray and immersed in ice-cold (4°C) modified oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF) that contained (in mM) 205 sucrose, 2.0 KCl, 7.0 MgCl2, 0.5 CaCl2, 1.25 NaH2PO4, 26 NaHCO3, and 11 t-glucose, pH 7.35. Coronal (300–400 μm) slices were then cut through the tissue block with a vibrating microtome (VT100, Leica Microsystems, Willowdale, ON) equipped with a sapphire blade (Delaware Diamond Knives, Wilmington, DE). Once cut, the slices were transferred to a storage chamber containing ACSF consisting of (in mM) 120 NaCl, 3.0 KCl, 1.4 MgSO4, 2 CaCl2, 1.5 KH2PO4, 26 NaHCO3, and 10 t-glucose, pH 7.35 (20°C).

Slices were then individually transferred from the storage chamber to a recording chamber on an upright microscope where they were submerged and anchored in rapidly flowing (1 ml/min), oxygenated ACSF (20°C). A bipolar stimulating electrode was placed in the ventral segment of the vertical limb of the diagonal band of Broca and afferent fibers were activated by a single, 30-V, 200-μs pulse every 15–30 s, or every 5 s for PhTx-343 experiments.

Whole cell recordings

Whole cell voltage-clamp recordings (Hamill et al. 1981) were obtained in the medial septum of the basal forebrain slice using the “blind-patch” technique (Blanton et al. 1989) with either an Axoclamp 2A or Axopatch 1D amplifier (Axon Instruments, Foster City, CA). All recordings were digitized at 5–10 kHz and filtered at 2 kHz. Patch electrodes were pulled from borosilicate thin-walled glass (1.5 mm OD; 150F-4, World Precision Instruments, Sarasota, FL) in three stages on a Flaming-Brown micropipette puller (model P-87; Sutter Instrument, Novato, CA). Patch electrodes had a resistance of 2–6 MΩ when filled with (in mM) 100 cesium methanesulfonate, 10 cesium-bis-(α-aminophenoxo)-N,N,N',N’-tetraacetic acid, 40 Hepes, and 5 QX-314, adjusted to pH 7.3 with cesium hydroxide. Unless indicated, the intracellular patch pipette solution contained 50 or 100 μM spermine.

Series resistance was monitored on-line using pClamp7.0 software (Axon Instruments) and also by delivery of a −5–mV voltage step before each evoked current. The average series resistance was 10.65 ± 1.07 MΩ. Data were not corrected for this error. Data were not included from recordings in which the series resistance was >20 MΩ or changed by >20%.

Chemicals were obtained from the following suppliers: PhTx-343, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f][1]oxazine-7-sulfonamide (NBQX), bicuculline methchloride, and GYKI 52466, Re-
search Biochemicals, Natick, MA; spermine, 2-amino-5-phosphono-valeric acid (APV), Sigma.

Lucifer yellow and 2-photon microscopy
To determine the morphology of cells displaying rectification and synaptic facilitation, patch pipettes were filled with 0.1% Lucifer yellow, and filled cells were imaged using a 2-photon laser-scanning microscope (Denk et al. 1990). Briefly, a Ti:Sapphire laser (710–1,000 nm, 76 MHz, 200 fs pulse width, Mira Model 900-F, Coherent Laser Group, Santa Clara, CA) that was excited by a 5-W solid-state diode-pumped, frequency-doubled, Nd:Vanadate laser (532 nm, Verdi, Coherent Laser Group) was directly coupled to a LSM510 Laser scanning Axioplan 2 microscope or Axioskop 2 (Carl Zeiss Mikroskopie). Lucifer yellow was excited at 890 nm and LSM510 software was used to reconstruct filled neurons in three dimensions and measure the diameter of each cell body.

RESULTS
GluR2 immunoreactivity
Western blot analysis (n = 2) using a rabbit anti-GluR2 antibody revealed a single band of protein at an approximate molecular weight of 101 kDa as reported previously (108 kDa, Petralia et al. 1998; 102 kDa, Vissavajjhala et al. 1993) (see Fig. 1A). As shown in Fig. 1A, GluR2-immunoreactive protein was detected in most areas of the rat CNS but appeared to be most abundant in the hippocampus, striatum, and neocortex. Immunocytochemistry for GluR2 using the same polyclonal antibody (n = 2; Fig. 1, B–H) revealed dense immunoreactive neuronal somata and dendrites throughout most areas of the CNS. Figure 1B shows an example of the distribution of GluR2 immunoreactivity that was observed in 50-μm parasagittal sections through the rat brain. Figure 1, C–F, shows examples of the GluR2 immunoreactivity that was observed in 25-μm coronal sections through the hippocampus and neocortex. Despite the widespread abundance of GluR2 immunoreactivity, no detectable GluR2-immunoreactive neurons were observed in the midline area of the medial septum (Fig. 1, G and H). However, GluR2-immunoreactive neurons were found in the lateral zone of the medial septum.

Specifically, co-localization studies (n = 2) using triple immunofluorescence and confocal microscopy revealed that the ChAT-containing and PARV-containing neurons of the medial septum had no detectable co-localization of the GluR2 immunoreactivity that was observed in 25-μm sections through the hippocampus and neocortex. (NMDA) receptor-mediated current (Fig. 3, C and D). The remaining bicuculline- and APV-insensitive currents were completely blocked by NBQX (20 μM; Fig. 3, E and F).
FIG. 2. The principal neurons of the MS are not immunoreactive for the AMPA receptor subunit GluR2. A: a low-power (×1.25) photomicrograph of parvalbumin (PARV, black) and anti-choline acetyltransferase (ChAT, brown) immunoreactivity throughout a coronal section of the rat brain at the level of the MS. B: a higher-power (×2.5) photomicrograph showing the distribution of PARV- and ChAT-immunoreactive neurons throughout the basal forebrain of the rat. Note that both types of neurons were found throughout the midline region of the medial septum/diagonal band complex, an area that showed little GluR2 immunoreactivity (see Fig. 1H). C: high-power (×40) photomicrograph of PARV- and ChAT-immunoreactive neurons in the basal forebrain of the rat. D–F: confocal micrographs of PARV (green)- and ChAT (red)-labeled neurons in the MS. D: a low-power (×20) confocal image showing that PARV and ChAT immunocytochemistry resulted in the labeling of 2 separate populations of MS neurons. E and F: higher-power (×63 and ×100) confocal images of PARV- and ChAT-immunoreactive neurons in the MS. Note that both neurons were relatively large with very similar morphological features. G–N: labeling of PARV (green)-, ChAT (red)-, and GluR2 (purple)-immunoreactive neurons in the MS of the rat. Note that none of the PARV- and ChAT-labeled neurons were positive for GluR2. However, a small unidentified population of neurons in the medial septum expressed the GluR2 AMPA receptor subunit. Scale bars = 2.50 mm (A), 1.25 mm (B), 78.125 μm (C), and 50 μm (D–N).
When the polyamine spermine (50 or 100 μM) was included in the patch pipette, synaptically evoked NBQX-sensitive currents could be classified as either rectifying or nonrectifying (Fig. 4) based on a best-fit polynomial (1st vs. 2nd order) regression analysis of the current-voltage relationship. First, 30% (8/28) of the patched cells displayed synaptically evoked currents that were best fit with a first-order polynomial regression analysis. Thus there was a linear or nonrectifying relationship between peak current and holding potential from a range of −80 to +80 mV (Fig. 4, A and B). The currents evoked in these cells were unaffected by external application of PhTx-343 (97.0 ± 8.0% of FIG. 3. Whole cell voltage-clamp recordings revealed three distinct currents evoked by stimulation of midline afferents. Currents were measured from −80 to +80 mV in 20-mV steps. Data presented in this figure were generated from a single cell. A: an example of the complex currents evoked by stimulation of midline afferents. B: the I-V relationship of the inhibitory, bicuculline-sensitive (30 μM), GABA<sub>α</sub> receptor-mediated component of the synaptic current (mean ± SE). This relationship was determined by subtracting the currents observed in bicuculline from the currents observed in control conditions. C: an example of the current evoked in the presence of 30 μM bicuculline. D: the I-V relationship of the excitatory, APV-sensitive (100 μM), NMDA receptor-mediated component of the synaptic current (mean ± SE). This relationship was determined by measuring the amplitude of the evoked current 30 ms following stimulation at the various potentials. E: an example of the current evoked in the presence of 30 μM bicuculline and 100 μM APV. F: the high-affinity AMPA receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (20 μM), successfully blocked the bicuculline- and APV-resistant current.
control), a potent, high-affinity antagonist of Ca²⁺-permeable, GluR2-lacking AMPA receptors (2/2; Fig. 4C). In contrast, NBQX-sensitive currents evoked in the remaining (70%, 20/28) neurons were best fit by a second-order polynomial regression analysis and therefore displayed “inward” rectification of the relationship between peak current and holding potential from a range of −80 to +80 mV (Fig. 4, D and E). The currents evoked in these cells were blocked (19.85 ± 1.45% of control) by external application of PhTx-343 (50 μm), demonstrating that rectifying, NBQX-sensitive currents were mediated by AMPA receptors that contain very few or no GluR2 AMPA receptor subunits (3/3; Fig. 4F). The inward rectification observed in the present study was similar to the rectification that has been described in previous reports of AMPA receptor-mediated currents in cells that lack GluR2 mRNA (Washburn et al. 1997).

Cell size

We examined the morphology of neurons to determine whether there was a correlation between synaptic rectification and cell size. Whole cell voltage-clamp recordings were made to characterize AMPA receptor-mediated currents, and medial septal neurons were subsequently filled and imaged using 2-photon laser scanning microscopy. The neurons that displayed current rectification were significantly larger in diameter [25.4 ± 1.9 (SE) μm; n = 5] than the neurons that displayed nonrectification [10.3 ± 1.2; n = 3; F(1,6) = 31.98, P < 0.01; see Fig. 5]. Previous reports have demonstrated that medial septal ChAT- and PARV-containing neurons are larger than the non-PARV-containing GABAergic neurons (see Jakab and Leranth 1995). These results are consistent with the conclusion that the GluR2-lacking AMPA synaptic responses are found in the cholinergic- and PARV-containing GABAergic projection neurons.

Facilitation

Next we investigated the response of AMPA receptor-mediated synaptic currents to repetitive stimulation. First, the relationship between the amplitude of the peak synaptic current and membrane potential was obtained to determine if the AMPA receptor-mediated synaptic response rectified as described in the preceding text. The isolated AMPA receptor-mediated synaptic currents were then evoked five times at rates ranging from 1 to 20 Hz (n = 26). All of the rectifying AMPA receptor-mediated currents showed amplitude facilitation during repetitive synaptic activation. Both the amount of facilitation and the frequency that produced the maximum facilitation varied somewhat for each cell. However, the largest relative increases were observed when currents were evoked at frequencies of 6–10 Hz (25 ± 10% at 1 Hz vs. 62 ± 14% at 6 Hz and 55 ± 24% at 10 Hz; currents were normalized to the first in each series of 5 evoked currents; n = 16 for facilitation). Figure 6, A and C, shows an example of facilitation at 6 Hz in a cell that displayed a rectifying AMPA receptor-mediated synaptic response.

In contrast to these facilitating currents, AMPA receptor-mediated currents evoked in neurons that displayed nonrectifying I-V curves depressed when stimulated at frequencies ranging from 1 to 20 Hz (n = 10). Figure 6, B and D, shows an example of the type of synaptic depression observed at 6 Hz in a neuron that contained a nonrectifying AMPA receptor-mediated response.

Next we examined the sensitivity of both types of synaptic responses to the potent AMPA receptor antagonist, GYKI
GYKI 52466 (Donevan and Rogawski 1993; Paternain et al. 1995; Zorumski et al. 1993) blocked both the rectifying, facilitating synaptic currents (Fig. 6E; 93.6 ± 4.6% depression, n = 5) and the linear, depressing synaptic currents (Fig. 6F; 95.4 ± 1.2% depression, n = 5). The synaptic currents were also evoked at 6 Hz before and after GYKI 52466 to ensure that the facilitating currents were also AMPA-receptor mediated (Fig. 6G).

Finally, to determine the relationship between rectification and the change in current amplitude when stimulated at 6 Hz, we quantified the degree of rectification by dividing the sum of the peak current amplitude evoked at +60, +40, and +20 mV by the sum of the peak current amplitude evoked at −60, −40, and −20 mV. Using this measure of rectification, cells that displayed AMPA receptor-mediated currents with little current amplitude at membrane potentials >0 mV (rectification) had a small rectification ratio. There was a statistically significant negative correlation between rectification ratio and the change in current amplitude at 6 Hz (Fig. 7A; Pearson r = −0.507, P < 0.05). This significant correlation came about because the rectification ratio of the currents that displayed facilitation were significantly smaller (0.38 ± 0.05) than the rectification ratio of the currents that displayed synaptic depression [0.77 ± 0.09; Fig. 7B; F(1,15) = 15.821, P < 0.05].

**DISCUSSION**

In the present study, we have demonstrated that the AMPA receptor-mediated responses recorded in the principal projection neurons of the medial septum display rapid facilitation following theta-like stimulation of midline afferents. These synaptic currents were mediated by AMPA receptors because they were blocked by either PhTx-343 or GYKI 52466. Our triple immunocytochemistry indicated that the large principal medial septal projection neurons containing either ChAT or PARV were not GluR2 immunoreactive. We suggest that this form of rapid, reversible AMPA receptor-mediated synaptic plasticity may provide medial septal neurons with a unique postsynaptic mechanism for controlling synaptic gain because
Our conclusion that neurons within the medial septum contain Ca\(^{2+}\)-permeable, GluR2-lacking AMPA receptors is consistent with previous reports where fura-2 measurements (Schneegenburger et al. 1993a, b) or voltage clamping (Water and Allen 1998) demonstrated substantial AMPA receptor-mediated Ca\(^{2+}\) influx in these neurons. Previous studies of the distribution of AMPA receptor subunits GluR1–4 within the basolateral forebrain of the rat demonstrated that GluR2/3 immunoreactivity was present within 15% of the cholinergic neurons (Page and Everitt 1995). Until recently the rigorous determination of the distribution of GluR2-lacking AMPA receptors in the septum was hindered by the lack of a GluR2-specific antibody that did not cross-react with GluR3. In the present study, we used a GluR2-selective antibody that did not cross-react with GluR3 to demonstrate the lack of detectable GluR2 immunoreactivity in the large neurons of the medial septum. The main AMPA receptor subunit expressed in medial septal neurons is reported to be GluR4 (Page and Everitt 1995).

Several other regions of the brain also possess neurons that express GluR2-lacking AMPA receptors. For example, electrophysiological data indicate that a subpopulation of GABAergic interneurons in both the hippocampus and neocortex possess Ca\(^{2+}\)-permeable, inwardly rectifying AMPA receptors (Jonas and Burnashev 1995; McBain et al. 1999). Generally, it is believed that most principal neurons possess AMPA receptors with low Ca\(^{2+}\) permeability while some interneurons possess AMPA receptors with high Ca\(^{2+}\) permeability (Seeburg 1996). However, Ca\(^{2+}\)-permeable AMPA receptors have also been found in the principal projection neurons of the cochlear nucleus (Otis et al. 1995), the medial nucleus of the trapezoid body (Geiger et al. 1995), and projection neurons in the dorsal horn of the spinal cord (Kyrozis et al. 1995).

Rozov et al. (1998) previously demonstrated that facilitation of currents through a recombinant AMPA receptor that contains unedited GluR2 AMPA receptor subunits depend on intracellular polyamines and occurs entirely postsynaptically. Our study suggests that a similar mechanism contributes to facilitation at the native GluR2-lacking synapse in medial septum. In the present study, facilitation was only observed in PhTx-343-sensitive AMPA receptors that displayed rectification. The responses were clearly mediated by AMPA receptors because the facilitating synaptic currents were blocked by GYKI 52466, the potent AMPA-receptor antagonist.

AMPA receptor-mediated, low-frequency facilitation may play an important role in the normal functioning of the septo-hippocampal circuit. ChAT- and PARV-containing neurons account for all of the medial septal neurons that project to the hippocampus and are therefore considered the “principal” neurons of the medial septum (Jakab and Leranth 1995). These neurons are critical for the generation of hippocampal theta activity (Bland 1986; Smythe et al. 1992) and hippocampal-dependent learning and memory (Eichenbaum et al. 1990; Packard and McGaugh 1992; Sutherland and Rodriguez 1989; Whishaw and Maaswinkel 1998; Whishaw and Tomie 1997; Whishaw et al. 1995). Medial septal ChAT- and PARV-containing neurons are part of a large ascending midline pathway that generates hippocampal theta activity (Bland 1986). This pathway originates in the rostral pons and ascends through the medial hypothalamus to the medial septum/vertical limb of the diagonal band of Broca (Kocsis and Vertes 1994; Oddie et al. 1994; Smythe et al. 1991). A portion of the ascending midline input to the medial septum arises from glutamatergic neurons...
in the supramammillary area of the hypothalamus (Leranth and Kiss 1996).

The remaining medial septal neurons that are not ChAT or PARV immunoreactive appear to be small GABAergic interneurons that occupy the lateral zones of the MS (Jakab and Leranth 1995). These neurons do not project to the hippocampus (Freund 1989). They are the neurons in present study that contained nonrectifying, GluR2-containing AMPA receptors, and these neurons displayed synaptic depression when stimulated at 6 Hz.

In conclusion, our study suggests that differential localization of GluR2 AMPA receptor subunit within the principal and nonprincipal cells of the medial septum may result in the potent enhancement of glutamatergic synaptic inputs to the septohippocampal pathway at low frequencies.

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