Inwardly Rectifying and Ca$^{2+}$-Permeable AMPA-Type Glutamate Receptor Channels in Rat Neocortical Neurons

SHUN-ICHI ITAZAWA,1 TADASHI ISA,2 AND SEJI OZAWA1
1Department of Physiology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371; and
2Department of Integrative Physiology, The National Institute for Physiological Sciences, Myodaiji, Okazaki 444, Japan

Itazawa, Shun-Ichi, Tadashi Isa, and Seiji Ozawa. Inwardly rectifying and Ca$^{2+}$-permeable AMPA-type glutamate receptor channels in rat neocortical neurons. J. Neurophysiol. 78: 2592–2605, 1997. Current-voltage (I-V) relations and Ca$^{2+}$ permeability of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor channels were investigated in neurons of rat neocortex by using the whole cell patch-clamp technique in brain slices. To activate AMPA receptor channels, kainate was used as a non-desensitizing agonist. A patch pipette was filled with solution containing 100 μM spermine to maintain the inward rectification of Ca$^{2+}$-permeable AMPA receptor channels. Three types of responses to kainate were observed: type I response with outwardly rectifying I-V relation, type II response with I-V relation of marked inward rectification, and intermediate response with I-V relation of weaker inward rectification. Neurons with type I, type II and intermediate I-V relations were referred to as type I, type II, and intermediate neurons, respectively. Of a total of 223 recorded cells, 90 (40.4%) were type I, 129 (57.8%) intermediate, and 4 (1.8%) type II neurons. Properties of AMPA receptor channels were examined in the former two types of neurons. The value of P$_{Ca}$/P$_{Cs}$, the ratio of the permeability coefficients of Ca$^{2+}$ and Cs$^+$, was estimated from the reversal potentials of kainate responses in the outside-out patches bathed in Na$^+$-free solution containing 100 mM Ca$^{2+}$ according to the constant-field equation. They ranged from 0.05 to 0.10 (0.08 ± 0.02, mean ± SD, n = 8) for type I neurons and from 0.14 to 1.29 (0.60 ± 0.37, n = 11) for the intermediate neurons. There was a close correlation between the inward rectification and the Ca$^{2+}$ permeability in AMPA receptor channels in these neurons. Intermediate neurons stained with biocytin were nonpyramidal cells with ellipsoidal-shaped somata. Type I neurons had either triangular- or ellipsoidal-shaped somata. Excitatory postsynaptic currents (EPSCs) recorded in both type I and intermediate neurons had 6-cyano-7-nitroquinoxaline-2,3-dione-sensitive fast and d-2-amino-5-phosphonvalerate-sensitive slow components. The I-V relation of the fast component exhibited inward rectification in the intermediate neuron, whereas that in the type I neuron (to 78 ± 6% of the control, n = 6) by bath application of 1 mM spermine. These results indicate that inwardly rectifying and Ca$^{2+}$-permeable AMPA receptor channels are expressed in a population of neurons of rat neocortex and are involved in excitatory synaptic transmission.

INTRODUCTION

Glutamate receptor channels of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type mediate fast excitatory synaptic transmission in most synapses in the CNS (Hestrin et al. 1990; Keller et al. 1991; Ozawa et al. 1991a). AMPA receptor channels are composed of subunits taken from a set of four proteins; GluR1 (GluR-A), GluR2 (GluR-B), GluR3 (GluR-C), and GluR4 (GluR-D). Expression studies have shown that functional properties of AMPA receptor channels depend on their subunit composition: receptors possessing the GluR2 subunit exhibit either a linear or outwardly rectifying I-V relation and little Ca$^{2+}$ permeability, whereas those lacking GluR2 show strong inward rectification and high Ca$^{2+}$ permeability (Burnashev et al. 1992; Hollmann and Heinemann 1994; Hume et al. 1991; Seeburg 1993).

It was found that AMPA receptor channels exhibited marked inward rectification and high Ca$^{2+}$ permeability in a population of cultured rat hippocampal neurons (type II neurons), whereas they exhibited an outwardly rectifying current-voltage (I-V) relation and little Ca$^{2+}$ permeability in the majority of neurons (type I neurons) (Iino et al. 1990; Ozawa and Iino 1993; Ozawa et al. 1991b). Using a patch-clamp reverse transcription-polymerase chain reaction (RT-PCR) technique, Bochet et al. (1994) have demonstrated that only GluR1 and GluR4 subunits are expressed, but no GluR2 subunit is detected in type II cultured hippocampal neurons. In contrast, the abundant expression of the GluR2 subunit has been detected in type I neurons. It is therefore likely that AMPA receptor channels in CNS neurons regulate their rectification properties and Ca$^{2+}$ permeability by changing GluR2 expression.

In rat neocortical neurons, it has been shown that the Ca$^{2+}$ permeability of AMPA receptor channels is much higher and that the level of GluR2 subunit expression is significantly lower in nonpyramidal cells in layer IV of the occipital cortex than in layer V pyramidal cells (Geiger et al. 1995; Jonas et al. 1994). However, it has been reported that the Ca$^{2+}$ permeability is not coupled with the inward rectification in AMPA receptor channels in the neocortical nonpyramidal cells. The Ca$^{2+}$-permeable AMPA receptor channels showed only weak rectification in these neurons (Geiger et al. 1995; Jonas et al. 1994). More recent studies have shown that intracellular polyamines such as spermine mediate the inward rectification of Ca$^{2+}$-permeable AMPA receptor channels (Bowie and Mayer 1995; Donevan and Rogawski 1995; Isa et al. 1995; Kamboj et al. 1995; Koh et al. 1995a). Therefore, the dissipation of internal polyamines in the outside-out patch configuration may have caused the dissociation between Ca$^{2+}$ permeability and rectification properties in Ca$^{2+}$-permeable AMPA receptor channels in the experiment by Jonas et al. (1994). This possibility can be examined by using intracellular solution containing spermine. In the present study, we searched for neurons expressing in-
wardly rectifying and Ca\(^{2+}\)-permeable AMPA receptor channels in rat occipital cortex using patch pipettes containing spermine. We found that they are expressed in non-pyramidal cells in layer II/III of the cortex and mediate excitatory synaptic transmission in these neurons.

**METHODS**

**Patch-clamp recording in neocortical brain slices**

Tight-seal patch-clamp recording was performed as described by Edwards et al. (1989). Briefly, 6- to 15-day-old rats were decapitated under ether anesthesia, and frontal slices of the occipital cortex 180–300 \(\mu\)m in thickness were prepared with a microslicer (DTK-2000, Dosaka EM, Kyoto, Japan). In the recording chamber, the slices were mounted on a stage of an upright microscope (Axiolab FS, Zeiss, Oberkochen, Germany). Morphologically identified neurons were approached with patch pipettes under visual control, with positive pressure applied to the patch pipette; no cleaning pipette was used. Patch pipettes had a resistance of 3–10 M\(\Omega\) when filled with internal solution. The access resistance during the recording was 11–50 M\(\Omega\) and was compensated by 50–70\%. An EPC-7 patch-clamp amplifier (List, Darmstadt, Germany) was used for recording, and pClamp system (Axon Instruments, Foster City, CA) was used for data acquisition and analysis.

**I-V curves** were obtained by ramping the membrane potential from a holding potential at a rate of 50 mV/s in the positive direction by 120 mV. **I-V relations** of responses mediated by AMPA receptors were constructed by subtracting **I-V curves** in the control solution from those obtained during application of the agonist. The average of four such trials was presented routinely as an **I-V** relation. We used kainate as a non-nonsensitizing agonist of AMPA receptor channels in most experiments (Bochet et al. 1994; Isa et al. 1996b). We occasionally used 100 \(\mu\)M AMPA together with 60 \(\mu\)M cyclothiazide (CTZ), which prevents fast desensitization of AMPA receptor channels (Partin et al. 1993), instead of kainate to activate AMPA receptor channels. The **I-V** relations of responses to kainate were virtually the same as those obtained with AMPA plus CTZ. However, the simultaneous application of AMPA and CTZ appeared to be toxic to cortical slices. Thus we used kainate in the present experiments.

When the intracellular solution contained CsCl or Cs-glucuronate, the liquid-junction potential was estimated as −4 or −10 mV, respectively, and the actual membrane potential was corrected by these values. Whole cell current traces were filtered at 3 kHz.

Conductance-voltage (**G-V**) relations for responses to kainate were obtained by calculating the conductance values using the equation

\[
G = \frac{I}{V - E_{rev}}
\]

where \(I\) is the amplitude of the agonist-induced current and \(E_{rev}\) is the reversal potential. Data points near the reversal potential were masked. In the **G-V** relations, relative values to that at −64 mV were plotted. The volume of the bath was 0.7 ml, and complete replacement of the external solution was performed by the addition of 4.0 ml of new solution. The flow rate of the bathing solution was 4 ml/min. Because the dead space was 6 ml, responses to agonists occurred ~90 s after switching to the new solution and reached steady state responses within another 60 s.

**Estimation of Ca\(^{2+}\) permeability**

In the experiments to estimate Ca\(^{2+}\) permeability of AMPA receptor channels, a large nucleated outside-out patch (Sather et al. 1992) was obtained by applying slight negative pressure to the patch pipette before pulling away from the somata. Kainate was applied to the outside-out patch by iontophoresis using high-resistance (100–200 M\(\Omega\)) micropipettes containing 100 mM kainate solution. Kainate was applied using current pulses of 500–1,000 nA intensity. The pulse duration was altered between 5 and 20 ms to obtain current responses adequate for analysis. Kainate responses were recorded in both the control external solution and the Na\(^{+}\)-free, 100 mM Ca\(^{2+}\) solution at various membrane potentials. The Ca\(^{2+}\) permeability of the AMPA receptor channel was estimated from the reversal potential of the kainate responses recorded in the Na\(^{+}\)-free, 100 mM Ca\(^{2+}\) solution by using the constant-field equation (Iino et al. 1990).

**Recording of excitatory postsynaptic currents**

Excitatory postsynaptic currents (**EPSCs**) were elicited by applying a voltage pulse of 5–40 V (80- to 200-\(\mu\)s duration) to a glass pipette containing the control external solution (tip diameter was ~5 \(\mu\)m, resistance was 1–2 M\(\Omega\)) placed on the surface of the slice 50–200 \(\mu\)m away from the recorded neuron. In the **EPSCs** shown, 5–12 records were averaged. Currents were filtered at a 3 kHz bandwidth with a 3-pole Bessel filter and the sampling frequency was 5 kHz. Data are expressed as means ± SD. Statistical evaluation of the results was done with unpaired \(t\)-test (Welch’s \(t\)-test).

**Solutions and drugs**

The slices were superfused continuously with the control external solution containing (in mM) 120 NaCl, 2.5 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 26 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), and 25 glucose bubbled with 95\% O\(_2\)-5\% CO\(_2\). Tetrodotoxin (0.5 \(\mu\)M) was added in the experiments to investigate **I-V** relations of responses to agonists for AMPA receptors. In the experiments to measure the Ca\(^{2+}\) permeability, isotonic Ca\(^{2+}\)-solution containing (in mM) 100 CaCl\(_2\), 10 glucose, 5 N-2-hydroxyethylpiperazine-N’-ethanesulfonic acid (HEPES) and 24 N-methylglucamine (pH adjusted to 7.4 with HCl) was used. The internal solution of patch pipettes contained (in mM) either 122.5 Cs-glucose, 25.5 CsCl, 0.2 ethylene glycol-bis(\(\beta\)-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 10 HEPES, 2 MgGTP, and 0.1 spermine, or 150 CsCl, 5 EGTA, 10 HEPES, and 0.1 spermine (in either case, the pH was adjusted to 7.2 with CsOH). In these internal solutions, the CsOH used to adjust the pH raised the concentration of Cs\(^{+}\) by ~15 mM. In the experiments to investigate **I-V** relations of **EPSCs**, 5 mM lidocaine N-ethyl bromide quaternary salt (QX-314) was added to the internal solution to prevent generation of Na\(^{+}\)-dependent action potentials caused by a poor control of the membrane potential (Stuart and Sakmann 1994). In all experiments, spermine was added to the internal solution to maintain the inward rectification of Ca\(^{2+}\)-permeable AMPA receptors during the patch-clamp recording (Donovan and Rogawski 1985; Isa et al. 1995; Kamboj et al. 1995; Koh et al. 1995a). Kainate and t-2-amino-5-phosphonovalerate (APV) were purchased from Tocris Cookson (Bristol, UK). Other chemicals were obtained from Sigma (St. Louis, MO).

**Intracellular staining**

For intracellular staining, whole cell recording was performed using pipettes containing biocytin (Horikawa and Armstrong 1988). Staining with biocytin was performed as described previously (Isa et al. 1996b). The slices containing stained neurons were counterstained with Nissl and coverslipped. Stained neurons were traced using camera lucida at a \(\times 1,000\) magnification.

**RESULTS**

**Rectification properties of kainate-activated currents**

Figure 1, top, shows three different types of **I-V** relations of responses to kainate, a non-nonsensitizing agonist for
constructed by subtracting I-V curves in the control solution from those obtained during applications of the agonist. Membrane potential was ramped from −64 to +56 mV. Bottom panel (2) shows conductance-voltage (G-V) relations for kainate-activated conductances that were obtained from the I-V relations shown top. Normalized values with respect to the chord conductance value at −64 mV are plotted from −64 to +56 mV.

AMPAs receptor channels, in neurons in layer II/III of the occipital cortex of rat brain slices. I-V curves were constructed by ramping the membrane potential from −64 to +56 mV during bath application of 200 μM kainate, and the I-V relations of the kainate responses were obtained by subtracting the I-V curves in the control solution. The I-V relation in Fig. 1A1 exhibited slight outward rectification through the whole voltage range, whereas that in Fig. 1C1 showed marked inward rectification at potentials less than +30 mV. Although the I-V relation exhibited inward rectification in Fig. 1B1, the degree of the rectification was less prominent than that in Fig. 1C1. The reversal potentials were close to 0 mV in all these neurons. To quantify the degree of inward rectification of current responses of AMPAs receptor channels in cultured rat hippocampal neurons, we previously introduced the following value as a rectification index (RI)

\[ RI = \frac{I_{+40}(40 - E_{rev})}{I_{-60}(-60 - E_{rev})} \]

where \( I_{+40} \) and \( I_{-60} \) were the amplitudes of the kainate-induced currents at +40 and −60 mV, respectively, and \( E_{rev} \) was the reversal potential (Iino et al. 1994; Ozawa et al. 1991b). When these values were >1.0 and <0.25 in the control solution, we assigned the responses to type I and type II categories, respectively. In the case of 0.25 ≤ RI ≤ 1.0, the response was classified as an intermediate type. Accordingly, neurons with \( RI > 1.0, RI < 0.25, \) and \( 0.25 \leq RI \leq 1.0 \) were referred to as type I, type II, and intermediate neurons, respectively. We used the same designation for neurons in neocortical slices in this study. The RI values of the I-V relation in Fig. 1. A1, B1, and C1, were 1.13, 0.62 and 0.13, respectively. Thus these neurons were classified as type I, intermediate, and type II neurons, respectively. The reversal potentials of type I, type II, and intermediate neurons were −2.1 ± 4.1 mV (mean ± SD, n = 84), −3.9 ± 3.2 mV (n = 4,), and −2.9 ± 3.8 mV (n = 116), respectively.

The type I, type II, and intermediate neurons were almost equally sensitive to N-methyl-D-aspartate (NMDA). The shapes of I-V relations of responses to 100 μM NMDA showed similar outward rectification at potentials more positive than −20 mV in all three types of neurons (data not shown). This indicates that the inwardly rectifying I-V relation of kainate responses in the type II and intermediate neurons is not due to a poor control of the membrane potential. Conductance-voltage (G-V) relations for kainate-activated conductances are shown in Fig. 1, bottom. These relations clearly reveal marked differences in the voltage dependence of kainate-activated conductances among the three types of neurons.

The I-V relations of the whole cell kainate responses were obtained in a total of 223 neurons located in layer II/III of the occipital cortex. Type I, type II, and intermediate neurons comprised 40.4% (90/223), 1.8% (4/223) and 57.8% (129/223), respectively. The proportion of type II neurons was quite small in this brain region. This contrasts with the previous observation that a considerable number of type II neurons is found in the rat hippocampal slices (14.4% of nonpy-
and the Na\(^+\) free, 100 mM Ca\(^{2+}\) and the kainate response in the outside-out patch bathed in the control solution.

In this section, we estimated the Ca\(^{2+}\) permeability of AMPA receptor channels expressed in type I and intermediate neurons. In Fig. 2A, a type I neuron was identified by constructing the I-V relation of the whole cell response to 200 µM kainate applied to the bath. The I-V relation exhibited a clear outward rectification, the RI value being 1.67. A large nucleated outside-out patch was excised from this neuron, and current responses to iontophoretic applications of kainate were recorded at various membrane potentials between −80 and +60 mV in a large nucleated outside-out patch excised from the neuron, which whole cell I-V relation of the kainate response, is shown in A. B1: current responses recorded in the control solution. B2: current responses recorded in the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution. B3: I-V relations of the peak amplitude of the current responses in the control solution (●) and the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution (○).

**Correlation between rectification properties and Ca\(^{2+}\) permeability of AMPA receptor channels**

In this section, we estimated the Ca\(^{2+}\) permeability of AMPA receptor channels expressed in type I and intermediate neurons. In Fig. 2A, a type I neuron was identified by constructing the I-V relation of the whole cell response to 200 µM kainate applied to the bath. The I-V relation exhibited a clear outward rectification, the RI value being 1.67. A large nucleated outside-out patch was excised from this neuron, and current responses to iontophoretic applications of kainate were recorded at various membrane potentials between −80 and +60 mV both in the control (Fig. 2B1) and the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solutions (Fig. 2B2). The peak amplitude of the current response is plotted against the membrane potential in Fig. 2B3. The I-V relation of the kainate response in the outside-out patch bathed in the control solution also showed an outward rectification (RI = 1.34), and the reversal potential was close to 0 mV (Fig. 2B3, ●). In all eight type I cells tested, the RI values in the outside-out patches were >1.0, indicating that AMPA receptor channels in the outside-out patch derived from the type I neuron also display type I properties. As shown in Fig. 2B2, only a small inward current was observed even at −80 mV in the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution in the outside-out patch excised from the type I neuron. The reversal potential was −45.5 mV in this case. The value of P_{Ca/P_{Cs}}, the ratio of the permeability coefficients of Ca\(^{2+}\) and Cs\(^+\), was calculated to be 0.08 according to the constant-field equation. The reversal potentials in the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution ranged from −56.0 to −41.5 mV (−46.6 ± 5.1 mV, n = 8). The value of P_{Ca/P_{Cs}} ranged from 0.05 to 0.10 (0.08 ± 0.02). This indicates that AMPA receptor channels expressed in type I neurons are only slightly permeable to Ca\(^{2+}\).

Figure 3 shows the I-V relations of the whole cell responses to bath-applied kainate in an intermediate neuron and the current responses to iontophoretic applications of kainate in a large nucleated outside-out patch excised from the neuron. The whole cell kainate response showed inward rectification at potentials less than +40 mV, and the RI value was 0.57 (Fig. 3A). The kainate response in the outside-out patch in the control solution also showed slight inward rectification at potentials less than +40 mV (Fig. 3B, I and 3, ●). The RI value was 0.73 in this case. In all 11 intermediate neurons tested, the RI values in the outside-out patches were 0.25 ≤ RI ≤ 1.0, thus being assigned to the intermediate category. A substantial inward current was detected at negative potentials in the kainate response in the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution (Fig. 3B2). The reversal potential was −3.4 mV in this case, the value of P_{Ca/P_{Cs}} being 0.68. The reversal potentials of intermediate neurons in the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution ranged from −34.0 to +7.4 mV (−10.7 ± 15.4 mV, n = 11). The value of P_{Ca/P_{Cs}} calculated

**Fig. 2.** Ca\(^{2+}\) permeability of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor channels in a type I neuron. A: I-V relation of the whole cell kainate (200 µM) responses between −60 and +60 mV obtained in the control solution. B: current responses to iontophoretic applications of kainate at various membrane potentials between −80 and +60 mV in a large nucleated outside-out patch excised from the neuron, of which whole cell I-V relation of the kainate response, is shown in A. B1: current responses recorded in the control solution. B2: current responses recorded in the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution. B3: I-V relations of the peak amplitude of the current responses in the control solution (●) and the Na\(^+\)-free, 100 mM Ca\(^{2+}\) solution (○).
FIG. 3. $\mathrm{Ca}^{2+}$ permeability of AMPA receptor channels in an intermediate neuron. A: $I-V$ relation of the whole cell kainate (200 µM) responses between $-60$ and $+60$ mV obtained in the control solution. B: current responses to iontophoretic applications of kainate at various membrane potentials between $-80$ and $+60$ mV in a large nucleated outside-out patch excised from the neuron, of which whole cell $I-V$ relation of the kainate response, is shown in A. B1: current responses recorded in the control solution. B2: current responses recorded in the Na$^+$-free, 100 mM Ca$^{2+}$ solution. B3: $I-V$ relations of the peak amplitude of the current responses in the control solution (●) and the Na$^+$-free, 100 mM Ca$^{2+}$ solution (●).

from the reversal potentials ranged from 0.14 to 1.29 (0.60 ± 0.37). Thus the Ca$^{2+}$ permeability of AMPA receptor channels was seven to eight times higher in intermediate neurons than in type I neurons.

Figure 4 shows scatter plots between the $RI$ in the control solution and the reversal potential value of kainate responses in the Na$^+$-free, 100 mM Ca$^{2+}$ solution in the outside-out membrane patches. These plots indicate a close correlation between the degree of inward rectification and the Ca$^{2+}$ permeability of AMPA receptor channels in the neocortical neurons (correlation coefficient $-0.93, P < 0.0001$), as shown previously in hippocampal neurons both in cultures (Iino et al. 1994) and slices (Isa et al. 1996b).

**Morphological properties of neurons expressing inwardly rectifying AMPA receptor channels**

A total of 33 recorded neurons were stained with biocytin. Among them, 25 and 8 were type I and intermediate neurons, respectively. Figure 5, A and B, shows camera lucida drawings of biocytin-stained type I and intermediate neurons in layer II/III of the occipital cortex, respectively. The arrow in each inset indicates the location of the soma of the stained neuron. The $RI$ values were 1.75 and 1.25 in the type I neurons in Fig. 5A, 0.37 and 0.72 in the intermediate neurons in Fig. 5B, respectively. Type I neurons had either triangular- or ellipsoidal-shaped somata (Fig. 5A). Long dendrites and descending axons were detected in some type I neurons. Intermediate neurons had ellipsoidal-shaped somata and well-branched short dendrites in all directions in the proximity of the somata (Fig. 5B). Somatic diameters of stained type I and intermediate neurons were $(13.1 ± 2.5 \mu \text{m}) \times (10.3 ± 1.7 \mu \text{m}) (n = 25)$, and $(12.6 ± 2.8 \mu \text{m}) \times (10.0 ± 3.0 \mu \text{m}) (n = 8)$, respectively. There was no significant difference between their somatic diameters (for longer axis, $P = 0.64$; for shorter axis, $P = 0.79$).

**Involvement of inwardly rectifying AMPA receptor channels in excitatory synaptic transmission**

To examine whether the inwardly rectifying AMPA receptor channels are involved in excitatory synaptic transmission,
sured at 14.8 ms after the stimulus (Fig. 6, B). Components: the fast and slow components (Fig. 6, C). The EPSCs in this neuron consisted of two distinct components: the fast and slow components (Fig. 6, A and B). The slow component was more prominent at more positive membrane potentials, and the I-V relation of currents measured at 14.8 ms after the stimulus (Fig. 6, A and C) showed a negative slope conductance at potentials more negative than −40 mV. The slow component was abolished by application of 50 μM APV. The amplitude of the fast components of EPSCs measured at 3.0 ms after the stimulus in the control solution (Fig. 6, A and C) was virtually the same as that measured in the presence of 50 μM APV (Fig. 6, B and C), indicating that the fast component was almost unaffected by APV. However, the fast component was abolished completely by 5 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; data not shown). These results indicate that the fast and slow components were generated by activation of AMPA and NMDA receptor channels, respectively. The AMPA component of the EPSCs showed slight outward rectification (RI = 1.05; Fig. 6C). The AMPA component of the EPSC also showed outward rectification in five other type I neurons.

Figure 7 shows the EPSCs recorded at various membrane potentials in an intermediate neuron. The I-V relation of the whole cell response to kainate exhibited inward rectification (Fig. 7D), the RI value being 0.67. The EPSCs also consisted of both CNQX-sensitive fast and APV-sensitive slow components. The I-V relation of the slow component measured at 15.4 ms after the stimulus (Fig. 7, A and C) showed a J-shape with a negative slope conductance, indicating that this component is due to activation of NMDA receptor channels. After the slow component had been abolished by 50 μM APV, the amplitude of the fast component, due to activation of AMPA receptor channels, was plotted against the membrane potential (Fig. 7, B and C, ○). The I-V plot of this AMPA component displayed a clear inward rectification, the RI value being 0.33. In six other intermediate neurons, the I-V relation of the AMPA component of the EPSC also displayed inward rectification. It was noted occasionally that the peak amplitude of the fast EPSC was reduced slightly by 50 μM APV at positive holding potentials. There could be a small contribution of the NMDA component to the peak amplitude of the fast EPSC. APV at this concentration had no effect on I-V relations of kainate responses in intermediate neurons.

In all seven intermediate neurons tested, the I-V plot of the NMDA component invariably showed an outward rectification of the EPSCs were investigated in both type I and intermediate neurons. The EPSCs were evoked by electrical stimulation of a point ~80 μm distant from the soma in control solution containing 10 μM bicuculline methobromide (Bic).

Figure 6 shows EPSCs recorded at various membrane potentials in a type I neuron located in layer II/III. The I-V relation of the whole cell response to kainate exhibited outward rectification (Fig. 6D), the RI value being 1.35. The EPSCs in this neuron consisted of two distinct components: the fast and slow components (Fig. 6, A and B). The slow component was more prominent at more positive membrane potentials, and the I-V relation of currents measured at 14.8 ms after the stimulus (Fig. 6, A and C) showed a negative slope conductance at potentials more negative than −40 mV. The slow component was abolished by application of 50 μM APV. The amplitude of the fast components of EPSCs measured at 3.0 ms after the stimulus in the control solution (Fig. 6, A and C) was virtually the same as that measured in the presence of 50 μM APV (Fig. 6, B and C, ○), indicating that the fast component was almost unaffected by APV. However, the fast component was abolished completely by 5 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; data not shown). These results indicate that the fast and slow components were generated by activation of AMPA and NMDA receptor channels, respectively. The AMPA component of the EPSCs showed slight outward rectification (RI = 1.05; Fig. 6C). The AMPA component of the EPSC also showed outward rectification in five other type I neurons.
FIG. 7. EPSCs recorded in an intermediate neuron. A: EPSCs recorded at various membrane potentials between -60 and +60 mV in 20-mV steps in the control solution containing 10 μM Bic. Stimulation was applied at a point ~80 μm distant from the soma. B: EPSCs recorded during addition of 50 μM APV. C: I-V relations of EPSCs. ● and ○, amplitudes of EPSCs at 3.6 and 15.4 ms after the stimuli, respectively, in A. ●, mainly EPSCs generated by activation of NMDA receptor channels; ○, amplitude of EPSCs at 3.6 ms in B, represent the EPSCs generated by activation of AMPA receptor channels. D: I-V relation of the whole cell response to kainate (100 μM) obtained in the same neuron before recording EPSCs.

Effects of spermine on EPSCs

We have shown previously that spermine suppresses kainate responses mediated by AMPA receptor channels specifically in type II cultured rat hippocampal neurons (IC₅₀ = 170 μM), but not in type I neurons (Isa et al. 1996a). Furthermore, in nonpyramidal cells in rat hippocampal slices, EPSCs mediated by inwardly rectifying AMPA receptor channels were suppressed markedly by 1 mM extracellular spermine, whereas those mediated by outwardly rectifying AMPA receptor channels were much less affected (Isa et al. 1996a). In this study, we examined the effect of spermine on EPSCs mediated by AMPA receptor channels in type I and intermediate neurons in the occipital cortex.

Figure 8A shows the AMPA components of EPSCs recorded in a type I neuron at -80 mV in the solution containing 10 μM Bic and 50 μM APV. In this neuron, the RI values of whole cell kainate responses and EPSCs were 1.11 and 1.18, respectively. When 1 mM spermine was added to the extracellular solution, the amplitude of the EPSC was reduced to 75% of the control response (Fig. 8A). The EPSC recovered almost to the control level after spermine had been washed out. The average amplitude of EPSCs at -80 mV in the presence of 1 mM spermine was 78 ± 6% (n = 6) of the control in type I neurons.

Spermine more markedly suppressed EPSCs mediated by AMPA receptor channels in intermediate neurons. Figure 8B shows the AMPA components of EPSCs recorded at -80 mV in an intermediate neuron. In this neuron, the RI values of whole cell kainate responses and EPSCs were 0.55 and 0.43, respectively. Spermine at 1 mM reversibly reduced the amplitude of the EPSC to 43% of the control in this case. The average amplitude of EPSCs at -80 mV in the presence of 1 mM spermine was 56 ± 15% (n = 12) of the control in intermediate neurons.

Figure 8C shows correlation between the RI value of the EPSC and the degree of suppression by 1 mM spermine of the EPSC recorded at -80 mV. The plots indicate a close correlation between these two values (correlation coefficient -0.89, P < 0.001).
**Ca$$^{2+}$$-permeable AMPA receptors in neocortical neurons**

In expression studies of GluR1–GluR4 subunits of AMPA receptor channels, the inward rectification was linked invari-
ably to high Ca$$^{2+}$$ permeability (Hollmann and Heinemann 1994; Seeburg 1993). This was also true for AMPA recep-
tors in rat hippocampal neurons both in cultures and slices (Iino et al. 1990; Isa et al. 1996b). In rat neocortical ne-
urons, Jonas et al. (1994) have shown that the Ca$$^{2+}$$ permeability of AMPA receptor channels is markedly higher in layer IV nonpyramidal cells than layer V pyramidal cells in the occip-
tal cortex and that the relative abundance of GluR2 mRNA is signifi-
cantly lower in the former than in the latter cells. The value of P_{Ca}/P_{K} in layer IV nonpyramidal cells has been esti-
imated to be 0.63, which is in the range of intermediate neurons in our classification. In the study of Jonas et al. (1994), however, the I-V relation of AMPA receptor channels from the nonpyramidal cells was only weakly rectifying, and Ca$$^{2+}$$ permeability was not necessarily coupled with inward rectification. This result raises a possibility that these neurons express an unidentified or modified subunit, because Ca$$^{2+}$$-permeable and outwardly rectifying AMPA receptor channels can be produced by site-directed mutagenesis (Din-
gledine et al. 1992). More recently, however, it has been shown that the inward rectification of both native and recom-
binant Ca$$^{2+}$$-permeable AMPA receptor channels is lost quickly in cell-free membrane patches due to dissipation of intracellular polyamines, and the loss of the rectification is prevented by adding spermine to the internal solution used to fill the patch pipette (Bowie and Mayer 1995; Donevan and Rogawski 1995; Isa et al. 1995; Kamboj et al. 1995; Koh et al. 1995a). Because the Ca$$^{2+}$$ permeability was esti-
minated in the outside-out patch configuration in the cortical nonpyramidal neurons (Jonas et al. 1994), the apparent uncoupling of the Ca$$^{2+}$$ permeability with the inward rectifica-
tion could be due to the dissipation of the intracellular poly-
amines mediating the inward rectification of Ca$$^{2+}$$-permeable AMPA receptor channels. In the present study, we ex-
amined the relationship between the Ca$$^{2+}$$ permeability and the inward rectification in neocortical neurons using patch pipettes containing spermine and found a close corre-
lation. Thus it is most likely that the relative abundance of GluR2 mRNA determines not only Ca$$^{2+}$$ permeability but also rectification properties in AMPA receptor channels in neocortical neurons.

**Neocortical neurons expressing Ca$$^{2+}$$-permeable AMPA receptor channels**

The patch-clamp RT-PCR technique has revealed that nonpyramidal cells carrying Ca$$^{2+}$$-permeable AMPA recep-
tor channels in the occipital cortex express glutamic acid decarboxylase (GAD) mRNA, and therefore they have been sug-
gested to be GABAergic interneurons (Jonas et al. 1994). In type II cultured hippocampal neurons, the expres-
sion of mRNA for GAD also was detected (Bochet et al. 1994). It has been shown that basket cells in dentate gyrus, which are presumed to be GABAergic neurons, express Ca$$^{2+}$$-permeable AMPA receptor channels (Koh et al. 1995b). Thus it is likely that Ca$$^{2+}$$-permeable AMPA recep-
tor channels are expressed dominantly in GABAergic inter-
neurons both in neocortex and hippocampus.

Very recently, it has been shown that a population of GABAergic nonpyramidal cells contain parvalbumin (Kaw-
guchi and Kubota 1996). Furthermore, an immunohisto-
chemical and in situ hybridization study has demonstrated that very little GluR2 is expressed in parvalbumin-containing nonpyramidal neurons in layers II/III, IV, V, and VI of the rat neocortex (Kondo et al. 1997). These results suggest that the intermediate neurons in the present study are GABAergic interneurons containing parvalbumin. A patch-clamp RT-
multiplex PCR technique developed by Cauli et al. (1997) would be useful to address this issue.

Jonas et al. (1994) have shown that nonpyramidal cells carrying Ca$$^{2+}$$-permeable AMPA receptor channels in the occipital cortex are fast-spiking neurons. It also has been shown that GABAergic nonpyramidal cells containing par-
valbumin in the frontal cortex are fast-spiking neurons (Kaw-
guchi and Kubota 1996). Unfortunately, we could not examine the firing pattern of neurons tested in this study for the following two reasons. First, we filled the patch pipette with Cs$$^{+}$$-rich internal solution, because our experiments were designed to classify the type of neurons according to

**FIG. 8. Effects of extracellular spermine on EPSCs. A: EPSCs recorded in a type I neuron at −80 mV. Three current traces were obtained in the control solution containing 10 μM Bic and 50 μM APV, during the bath-application of 1 mM spermine and after washing out spermine. B: EPSCs recorded in an intermediate neuron at −80 mV. Three current traces were obtained in the control solution containing 10 μM Bic and 50 μM APV, during the bath-application of 1 mM spermine and after washing out spermine. C: scatter plots of the degree of suppression of EPSC at −80 mV by 1 mM spermine, against R_{EPSC}. Degree of suppression correlated negatively with R_{EPSC}. Correlation coefficient was −0.89 (P < 0.001).**

**DISCUSSION**

**Correlation between Ca$$^{2+}$$ permeability and inward rectification in AMPA receptor channels in neocortical neurons**

The value of PCa:PK in layer IV nonpyramidal cells has been esti-
mated to be 0.63, which is in the range of intermediate neurons. The value of PCa:PK in layer IV nonpyramidal cells has been esti-
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lation. Thus it is most likely that the relative abundance of GluR2 mRNA determines not only Ca$$^{2+}$$ permeability but also rectification properties in AMPA receptor channels in neocortical neurons.
the rectification properties of the whole cell kainate responses. To obtain the I-V relation in the positive potential range, a Cs⁺-rich rather than a K⁺-rich solution was used to limit the contribution of outward current passing through the voltage-dependent K⁺ channels. Second, QX-314 was added to the internal solution in most experiments to prevent the contamination of EPSCs by Na⁺-dependent action potentials generated in the unclamped region.

**Effects of spermine on fast EPSCs**

The present study has shown that spermine suppresses fast EPSCs mediated by AMPA receptor channels more markedly in intermediate neurons than in type I neurons in the occipital cortex. This result is in good agreement with our previous findings that spermine specifically suppresses inwardly rectifying AMPA receptor channels in cultured rat hippocampal neurons and that EPSCs mediated by these receptor channels are more sensitive to spermine than those mediated by outwardly rectifying ones in nonpyramidal neurons in rat hippocampal slices (Isa et al. 1996a). However, it should be noted that the fast EPSC of type I in in nature was suppressed by as much as 20% by 1 mM spermine in both neocortical (this study) and hippocampal (Isa et al. 1996a) neurons. This is probably due to some presynaptic effect of spermine, because spermine at this concentration had no effect on type I responses to kainate in neocortical neurons in slices (unpublished data). It has recently been shown that millimolar concentrations of spermine affect Ca²⁺ channels that function at the presynaptic terminals (Sutton et al. 1993). In accordance with this, DiScenna et al. (1994) have reported that 2 mM spermine reduces population EPSPs mediated by non-NMDA receptors in the CA1 area of rat hippocampal slices, which is attributed largely to outwardly rectifying AMPA receptor channels, to ~40%.

In the present study, the degree of suppression by spermine correlates closely with the value of RI in each neuron. This observation suggests that fast EPSCs in intermediate neurons are generated by activations of two distinct populations of AMPA receptor channels, the outwardly and inwardly rectifying receptor channels, the proportions of which vary from neuron to neuron. It is most likely that the RI value decreases and the EPSC becomes more sensitive to spermine when the contribution of the latter component to the EPSC is increased.

**Functional significance of Ca²⁺-permeable AMPA receptor channels in the neocortex**

The present study has shown that inwardly rectifying and Ca²⁺-permeable AMPA receptor channels are involved in the excitatory synaptic transmission in intermediate neurons. It has been shown that Ca²⁺-permeable AMPA receptor channels play a role in causing the long-term changes in synaptic functions by regulating intracellular Ca²⁺ concentrations in spinal dorsal horn neurons in culture (Gu et al. 1996). Brusa et al. (1995) have disrupted partially the edited form of the GluR2 subunit and overexpressed its unedited form in mice. They have found that principal neurons such as CA1 hippocampal subunit and expressed AMPA receptor channels with increased Ca²⁺ permeability in the mutant mice. These mice developed seizures and died within 3 wk (Brusa et al. 1995). Very recently, Jia et al. (1996) have generated mice that lack the GluR2 subunit. They have reported a ninefold increase in the Ca²⁺ permeability of AMPA receptor channels in CA1 hippocampal neurons in the mutant mice. Furthermore, long-term potentiation in the CA1 region was enhanced markedly and became nonsaturating in these mice. These results strongly suggest an important role of Ca²⁺-permeable AMPA receptor channels in regulating synaptic plasticity and brain functions. However, the functional significance of the finding that Ca²⁺-permeable AMPA receptor channels are expressed preferentially in some populations of nonpyramidal neurons remains to be elucidated.

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Address for reprint requests: S.-I. Itazawa, Dept. of Physiology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371, Japan.

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