Block of native Ca^{2+}-permeable AMPA receptors in rat brain by intracellular polyamines generates double rectification

D.-S. Koh, N. Burnashev and P. Jonas*

*Max-Planck-Institut für medizinische Forschung, Abteilung Zellphysiologie, Jahnstraße 29, 69120 Heidelberg, Germany and * Physiologisches Institut der Universität, Hermann-Herder-Straße 7, 79104 Freiburg, Germany*

1. The influence of intracellular factors on current rectification of different subtypes of native α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPARs) was studied in rat brain slices by combining fast application of glutamate with patch pipette perfusion.

2. The peak current–voltage (I–V) relation of the AMPARs expressed in Bergmann glial cells of cerebellum and dentate gyrus (DG) basket cells of hippocampus was weakly rectifying in outside-out patches and nystatin-perforated vesicles, but showed a doubly rectifying shape with a region of reduced slope between 0 and +40 mV in nucleated patches. The I–V relation of AMPARs expressed in hippocampal CA3 pyramidal neurones was linear in all recording configurations.

3. Intracellular application of 25 μM spermine, a naturally occurring polyamine, blocked outward currents in outside-out patches from Bergmann glial cells and DG basket cells in a voltage-dependent manner, generating I–V relations with a doubly rectifying shape which were similar to those recorded in nucleated patches. AMPARs in CA3 pyramidal cell patches were unaffected by 25 μM spermine.

4. The half-maximal blocking concentration of spermine at +40 mV was 0·3 μM in Bergmann glial cell patches and 1·5 μM in DG basket cell patches, whereas it was much higher (⩾100 μM) for CA3 pyramidal cell patches. Spermidine also affected current rectification, but with lower affinity. The block of outward current by polyamines following voltage jumps developed within < 0·5 ms.

5. We conclude that current rectification, rather than being an intrinsic property of the Ca^{2+}-permeable AMPAR channel, is generated by polyamine block.

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPARs) expressed in different types of neurones of the mammalian central nervous system (CNS) are molecularly diverse. Molecular cloning identified four AMPAR subunits, designated as GluR-A, -B, -C, and -D (or GluR1, 2, 3, and 4), from which the native receptors presumably are assembled. Studies on recombinant AMPARs expressed in *Xenopus* oocytes and human embryonic kidney (HEK 293) cells indicate that the subunit composition controls both Ca^{2+} permeability and current rectification. Recombinant AMPARs containing the edited GluR-B subunit show low Ca^{2+} permeability and linear or outwardly rectifying current–voltage (I–V) relations, whereas AMPARs lacking GluR-B exhibit high Ca^{2+} permeability and doubly or inwardly rectifying I–V relations (Hollmann, Hartley & Heinemann, 1991; Burnashev, Monyer, Seeburg & Sakmann, 1992*b*). Both functional properties are determined by the amino acid residue at the Q/R site of the pore-forming region which is either an arginine in the edited GluR-B subunit or a glutamine in the other subunits (Hollmann *et al.* 1991; Burnashev *et al.* 1992*b*).

For native AMPARs, the relation between Ca^{2+} permeability and current rectification is less clear than for recombinant AMPARs. In principal neurones of hippocampus and neocortex, AMPARs exhibit low Ca^{2+} permeability and linear or outwardly rectifying I–V relations (Colquhoun, Jonas & Sakmann, 1992; Jonas, Racca, Sakmann, Seeburg & Monyer, 1994; Geiger *et al.* 1995). In Bergmann glial cells of cerebellum, GABAergic interneurones of hippocampus and neocortex, and auditory relay neurones, AMPARs show high Ca^{2+} permeability; unexpectedly, the degree of current rectification of these Ca^{2+}-permeable AMPARs

* To whom correspondence should be addressed.

This manuscript was accepted as a Short Paper for rapid publication.

varies from linearity to strong inward rectification (Burnashev *et al.* 1992*a*; McBain & Dingledine, 1993; Jonas *et al.* 1994; Koh, Geiger, Jonas & Sakmann, 1995; Geiger *et al.* 1995). The reasons are as yet unclear. Current rectification of recombinant and native AMPARs is more pronounced in the whole-cell configuration than in the excised patch, suggesting that cytoplasmic factors are required to maintain rectification (e.g. Swanson, Feldmeyer & Cull-Candy, 1994).

Recently, it was reported that intracellular polyamines, which occur ubiquitously in neuronal and non-neuronal cells, act as 'gating molecules' of inward rectifier K^+ channels (Ficker, Taglialatela, Wible, Henley & Brown, 1994; Lopatin, Makhina & Nichols, 1994; Fakler, Brändle, Glowatzki, Weidemann, Zenner & Ruppersberg, 1995). We therefore investigated the effects of intracellular polyamines on rectification properties of native AMPARs in three different cell types: in cerebellar Bergmann glial cells distinguished by high Ca^{2+} permeability of AMPARs and lack of GluR-B mRNA (Burnashev *et al.* 1992*a*; Geiger *et al.* 1995), in hippocampal CA3 pyramidal neurones characterized by low Ca^{2+} permeability of AMPARs and high GluR-B mRNA abundance (Colquhoun *et al.* 1992; Geiger *et al.* 1995), and in hippocampal dentate gyrus (DG) basket cells showing intermediate Ca^{2+} permeability of AMPARs and low GluR-B mRNA abundance (Koh *et al.* 1995; Geiger *et al.* 1995). This approach allows us to elucidate the relation between current rectification, Ca^{2+} permeability, and subunit composition of native AMPARs.

METHODS

Patch-clamp recording in brain slices

Wistar rats (13–15 days old) were killed by decapitation. Transverse hippocampal slices or parasagittal cerebellar slices (300 μm thick) were cut using a vibratome (FTB, Bensheim, Germany). Cells were identified visually using infrared differential interference contrast video microscopy (Jonas *et al.* 1994). Cerebellar Bergmann glial cells were distinguished by the location of the cell body (close to that of Purkinje cells), the presence of several glial processes extending into the molecular layer, the absence of action potentials, the low input resistance (< 50 MΩ), and the very negative resting potential (−80 to −90 mV). Hippocampal DG basket cells were identified by their location at the border between the granule cell layer and the hilus, the large size and pyramidal shape of the soma, and the high frequency train of action potentials generated upon sustained membrane depolarization (Koh *et al.* 1995; Geiger *et al.* 1995).

Patch pipettes were fabricated from borosilicate glass tubing (2·0 mm outer diameter, 0·5 mm wall thickness) using horizontal electrode pullers (either Zeitz, Augsburg, Germany, or Flaming/Brown P-97, Sutter, Novato, CA, USA). When filled with a K^+-rich intracellular solution, they had a resistance of 2–4 MΩ (tip diameter about 1 μm). For the voltage-jump experiments, patch pipettes were coated with Sylgard (Dow Corning, Midland, MI, USA). Cells were approached with patch pipettes under visual control while positive pressure was applied to the interior; no cleaning was performed.

Perforated vesicles were obtained using nystatin (Sigma) dissolved in dimethylsulphoxide and diluted in intracellular solution to a final concentration of 120 μg ml^{-1} before the experiment (Horn & Marty, 1988). Seals were formed with nystatin-free solution, and nystatin was applied in the cell-attached configuration by patch pipette perfusion (see below). The patch pipette was withdrawn after an access resistance of 20–40 MΩ had developed. Isolation of nucleated patches was facilitated by removing most of the tissue surrounding the cell soma with a cleaning pipette (3–5 μm tip diameter). Continuous suction was applied during withdrawal of the patch pipette (Sather, Dieudonné, Macdonald & Ascher, 1992). The diameter of nucleated patches was 7–12 μm. Only nucleated patches with AMPAR-mediated peak currents larger than 300 pA (−80 mV, 3 mM glutamate) were analysed.

Fast application of glutamate and patch pipette perfusion

Fast application of glutamate was performed using a double-barrelled application pipette fabricated from theta glass tubing as described previously (Colquhoun *et al.* 1992). The exchange time (20–80%) was 50–150 μs. For patch pipette perfusion, rapidly tapering patch pipettes were used (pulled with the Flaming/Brown puller). Perfusion capillaries with tip diameters of 30–60 μm were pulled from perfluoro-ethylene-propylene tubing (IFK-Isofluor, Neuss, Germany) after heating. The distance between the perfusion capillary opening and the patch pipette tip was 400–600 μm. Polyamines were applied by perfusing polyamine-containing intracellular solutions at a rate of 2–5 μl min^{-1} through the capillary; in some experiments, polyamine-free solution was reapplied to test reversibility. Solution at the intracellular face of the membrane was exchanged (> 95%) within 2–3 min, as judged by the shift of reversal potential of the glutamate-activated current following replacement of K^+-rich by *N*-methyl-D-glucamine$^+$-rich solution ($n = 5$). Measurements in the presence of intracellular polyamines were made 6–10 min after the beginning of perfusion.

Data acquisition and analysis

Membrane current was recorded with an EPC-7 amplifier (List, Darmstadt, Germany); in the nucleated patch configuration, series resistance compensation (> 90%) was used. Signals were filtered with an 8-pole low-pass Bessel filter at a bandwidth (−3 dB) of 3 kHz except in the voltage-jump experiments where a 10 kHz bandwidth was used (Fig. 4*C*). Data were digitized at twice to four times the filter frequency and stored on-line using a VMEbus computer system (Motorola, Tempe, AZ, USA). All recordings were made at room temperature (around 22 °C). Glutamate pulses (100 ms, 3 mM) were applied every 2–3 s. The membrane potential was held at 0 mV and was stepped to the indicated values 100 ms before the glutamate pulse. The traces shown in figures are either individual recordings or averages of 2–14 recordings. *I–V* relations were fitted with fifth- or sixth-order polynomials. The index of rectification (*IR*) was calculated as the ratio of chord conductances at +40 and −80 mV, assuming a reversal potential of 0 mV. Concentration–effect curves were fitted with the Hill equation:

$$f(c) = B[1 + (c / IC_{50})^n]^{-1} + 1 - B,$$

where c denotes the concentration of polyamine added to the intracellular solution (the concentration of free polyamine may be lower by a factor of about 2 in the presence of 2 mM ATP; Watanabe, Kusama-Eguchi, Kobayashi & Igarashi, 1991), B denotes the maximal inhibition (mostly fixed at 1), IC_{50}, the half-maximal inhibitory concentration, and n, the Hill coefficient. Numerical values denote means ± standard error of the mean (S.E.M.). Error bars in figures also denote S.E.M.s.

Solutions and drugs

Physiological extracellular saline solution used for superfusion of slices contained (mM): 125 NaCl, 25 $NaHCO_3$, 25 glucose, 2·5 KCl, 1·25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, bubbled with 95% O_2–5% CO_2 gas. The Na^+-rich extracellular solution used for perfusing the double-barrelled application pipette contained (mM): 135 NaCl, 5·4 KCl, 1·8 $CaCl_2$, 1 $MgCl_2$, 5 Hepes, pH adjusted to 7·2 with NaOH, and 50 μM D-AP5 (Tocris Cookson, Bristol, UK) to block NMDA receptors. The K^+-rich intracellular solution used for filling and perfusing the patch pipette contained (mM): 140 KCl, 10 EGTA, 2 $MgCl_2$, 2 Na_2ATP, 10 Hepes, pH adjusted to 7·3 with KOH. In a few experiments, a Cs^+-rich intracellular solution (containing (mM): 140 CsCl, 10 BAPTA, 2 $MgCl_2$, 2 Na_2ATP, 10 Hepes, pH adjusted to 7·3 with CsOH) was used. The intracellular solution for the nystatin-perforated vesicles contained 120 mM KCl and 20 mM K_2SO_4 instead of 140 mM KCl. For the voltage-jump experiments, 100 μM cyclothiazide (Eli Lilly, Indianapolis, IN, USA) was added to the extracellular solution. Spermine and spermidine were from Sigma. Stock solutions (40 mM) were prepared in intracellular solution (pH adjusted to 7·3 with HCl), stored at −20 °C, and diluted directly before the experiment.

RESULTS

Rectification of AMPAR-mediated currents in different recording configurations

To investigate the influence of intracellular factors on the rectification properties of native AMPARs, we compared I–V relations of glutamate-activated currents in outside-out patches, nystatin-perforated vesicles, and nucleated patches. The results are illustrated for Ca^{2+}-permeable AMPARs expressed in DG basket cells (Fig. 1). In the outside-out patch configuration with Na^+-rich extracellular and K^+-rich intracellular solution, the I–V relation of the glutamate-activated current was only weakly rectifying (Fig. 1*A*). The average index of rectification (IR; see Methods) was 1·12 ± 0·08 ($n = 16$, measured 3–5 min after patch excision). The shape of the I–V relation in nystatin-perforated vesicles was similar to that recorded in outside-out patches (Fig. 1*B*; IR = 0·80 ± 0·12 after 3–5 min; $n = 7$). In contrast, the I–V relation showed a

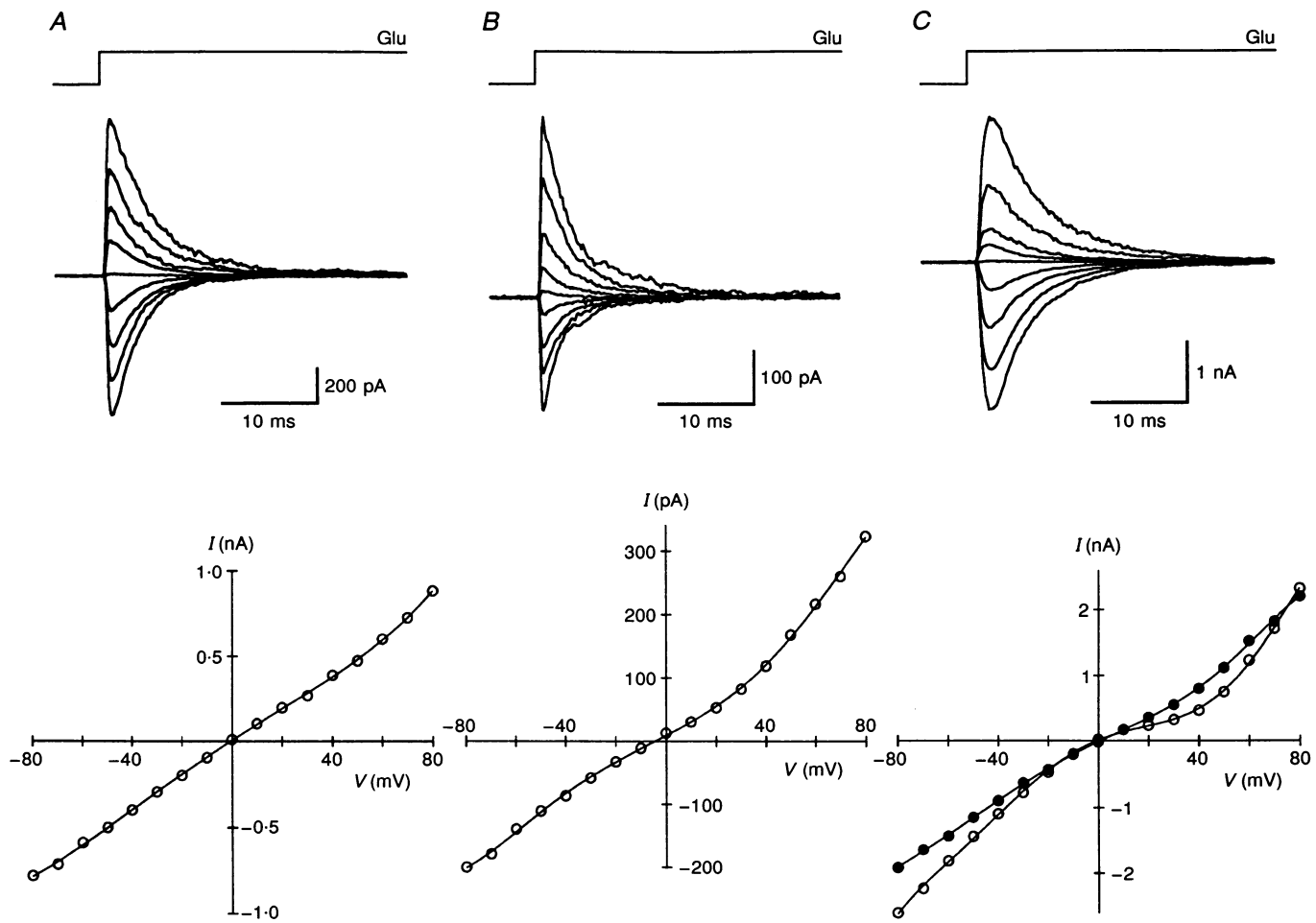

Figure 1. Rectification of AMPAR-mediated currents in different recording configurations

AMPAR-mediated currents in an outside-out patch 5 min after patch excision (*A*), in a nystatin-perforated vesicle 5 min after patch excision (*B*), and in a nucleated patch 5 (○) and 30 min (●) after patch excision (*C*) from DG basket cells of hippocampus. K^+-rich intracellular solution in the patch pipette. Currents activated by 100 ms pulses of 3 mM glutamate (Glu) at membrane potentials between −80 and +80 mV varied in 20 mV steps are shown on top (5 min after patch excision). Corresponding peak I–V relations are illustrated on the bottom.

marked doubly rectifying shape in the nucleated patch configuration (Fig. 1*C*, ○; IR = 0·45 ± 0·07 after 3–5 min; $n = 5$), but became more linear with time (Fig. 1*C*, ●). Since double rectification was strongest in the nucleated patch directly after excision, a diffusible intracellular molecule appeared to be involved in maintaining rectification of Ca^{2+}-permeable AMPARs. As current rectification was not maintained in the nystatin-perforated vesicle, the critical cytoplasmic factor may be of low molecular mass (about 200 Da or less; Horn & Marty, 1988).

Analysis of Ca^{2+}-permeable AMPARs expressed in Bergmann glial cells and Ca^{2+}-impermeable AMPARs in CA3 pyramidal cells revealed cell-specific differences in rectification properties. AMPARs in Bergmann glial cells, like those in DG basket cells, showed a weakly rectifying *I–V* relation in the outside-out patch and perforated vesicle, but strong double rectification in the nucleated patch. AMPARs in CA3 pyramidal neurones, unlike those in DG basket cells, showed weakly rectifying *I–V* relations in all recording configurations (data not shown). *I–V* relations recorded in outside-out patches from Bergmann glial cells with K^+-rich intracellular solution containing EGTA and Cs^+-rich solution containing BAPTA were similar in shape ($n = 3$). This indicates that the *I–V* relation was not influenced by K^+ currents that might become activated following AMPAR-mediated Ca^{2+} influx.

Intracellular polyamines generate double rectification

Guided by recent reports that intracellular polyamines act as 'gating molecules' of inward rectifier K^+ channels (Ficker *et al.* 1994; Lopatin *et al.* 1994; Fakler *et al.* 1995), we investigated whether polyamines applied intracellularly by patch pipette perfusion could alter the shape of the *I–V* relation of AMPAR-mediated currents. In outside-out patches excised from Bergmann glial cells, 25 μM intracellular spermine almost completely blocked the outward current at membrane potentials between 0 and +40 mV, converting the *I–V* relation from an almost linear to a complex, doubly rectifying shape (Fig. 2*A*). In DG basket cell patches the current between 0 and +40 mV was partly reduced by 25 μM spermine (Fig. 2*B*), whereas it remained unaffected in CA3 pyramidal cell patches (Fig. 2*C*). The average rectification index in the presence of 25 μM intra-

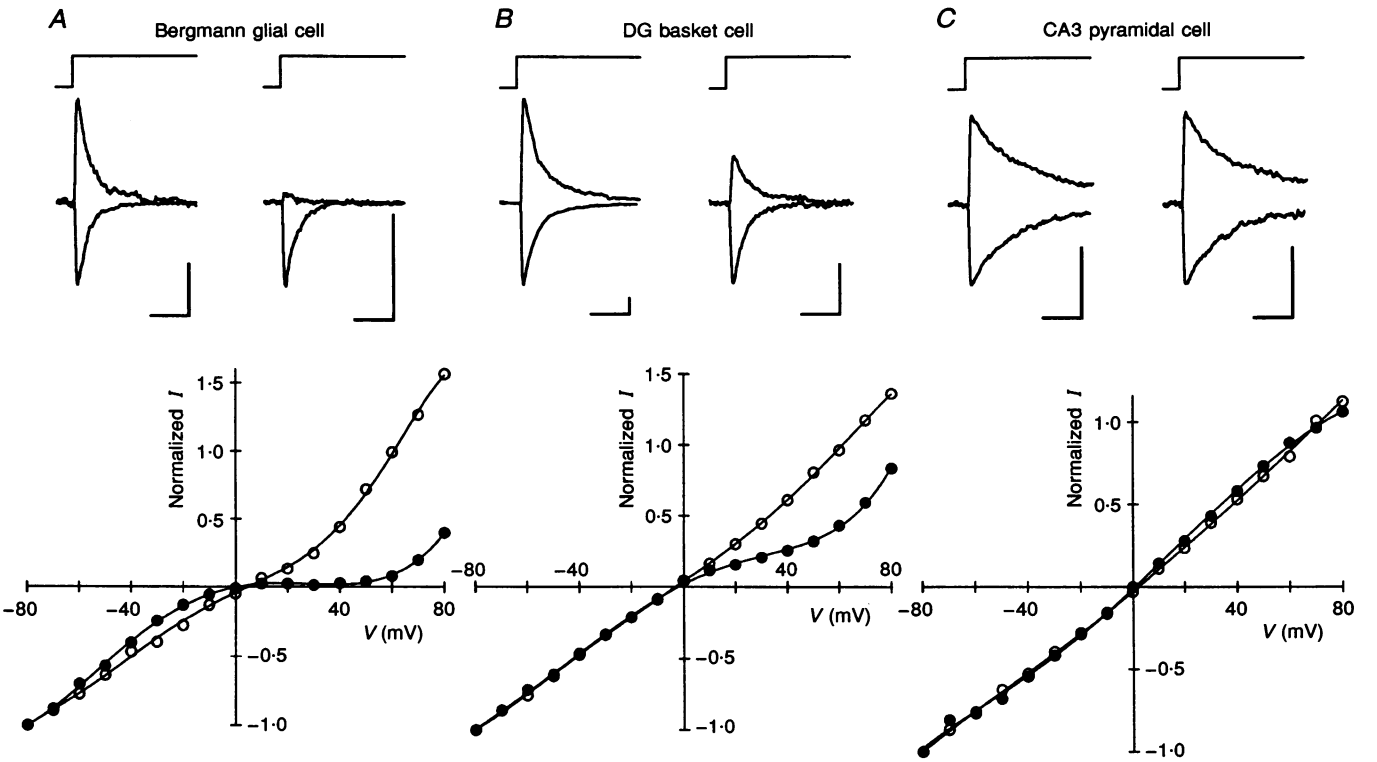

Figure 2. Effects of intracellular polyamines on current rectification of different native AMPAR subtypes

AMPAR-mediated currents in an outside-out patch from a Bergmann glial cell of cerebellum (*A*), a DG basket cell of hippocampus (*B*), and a CA3 pyramidal neurone of hippocampus (*C*). Glutamate-activated currents at −60 and +60 mV are shown on top in the absence (left) and presence of 25 μM spermine (right); the glutamate pulse (100 ms, 3 mM) is indicated above the traces. Spermine was applied to the intracellular face of the membrane by patch pipette perfusion. Scale bars correspond to 200 pA and 10 ms. Corresponding peak *I–V* relations are illustrated on the bottom in the absence (5 min after patch excision, ○) and the presence of 25 μM spermine (●). Peak currents were normalized to the value at −80 mV.

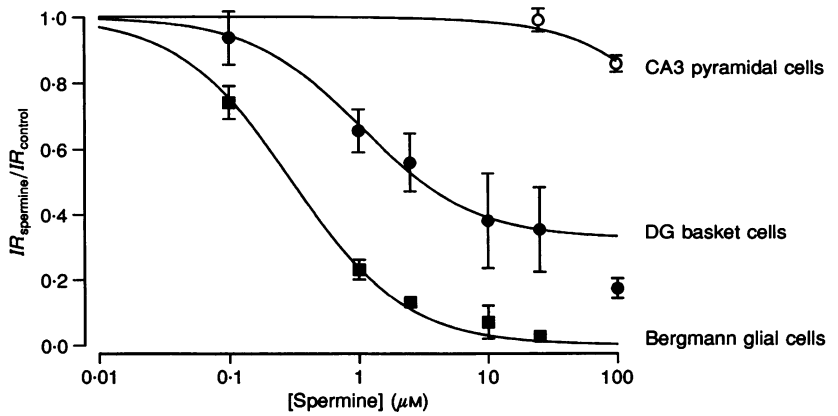

Figure 3. Concentration dependence of block by intracellular spermine

Ratio of IR in the presence ($IR_{spermine}$) and absence of spermine ($IR_{control}$) was plotted against intracellular spermine concentration. Data for outside-out patches excised from Bergmann glial cells (■, $n = 24$), hippocampal DG basket cells (●, $n = 27$), and CA3 pyramidal cells (○, $n = 10$). $IR_{control}$ values measured 20 min after patch excision were 1·63 ($n = 5$), 1·43 ($n = 4$), and 1·09 ($n = 3$), respectively. Points were fitted with the Hill equation. The point at 100 μM for DG basket cell patches was excluded from the fit because, at this concentration, block of CA3 pyramidal cell AMPARs became noticeable. Apparent IC_{50} values were 0·3, 1·5 (maximal inhibition $B = 75\%$), and 646 μM, respectively. The Hill coefficient was fixed as 1; when fitted as a free parameter, values between 0·7 and 1 were obtained. Spermine was applied either by patch pipette perfusion (measurement 6–10 min after beginning of perfusion) or was included into the intracellular solution before seal formation (measurement approximately 15 min after patch excision).

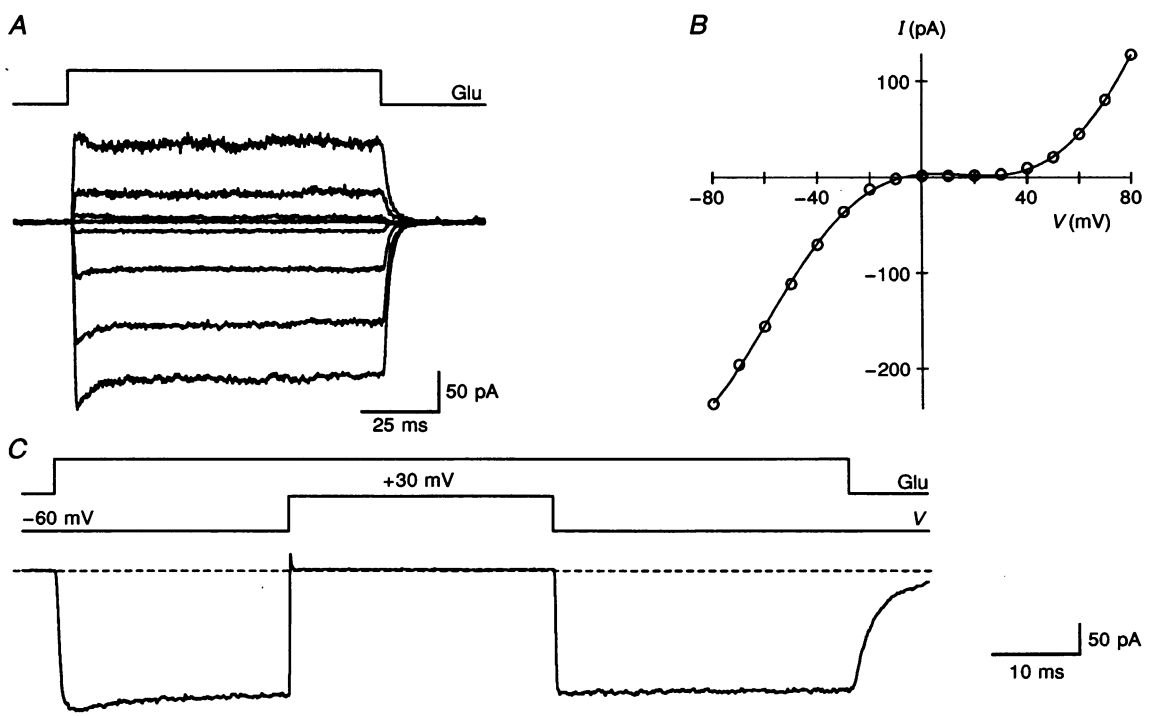

Figure 4. Kinetics of block by intracellular spermine

A, AMPAR-mediated currents activated by glutamate pulses (100 ms, 3 mM) in the presence of 100 μM cyclothiazide (in both barrels of the application pipette). Membrane potential was varied between −80 and +80 mV in 20 mV steps. Spermine (25 μM) was included in the intracellular solution. *B*, corresponding *I–V* relation of the mean glutamate-activated current 26·5–56·5 ms after the beginning of the glutamate pulse. *C*, voltage-jump experiment. The trace shown represents the difference between the current elicited with and without the glutamate pulse. The voltage was stepped from −60 to +30 mV (26·5 ms after the beginning of the glutamate pulse) and back to −60 mV (after 56·5 ms). Spermine (25 μM) was included in the intracellular solution. The dashed horizontal line indicates current baseline. All data were obtained from a Bergmann glial cell outside-out patch.

cellular spermine was $0{\cdot}04 \pm 0{\cdot}01$ ($n = 4$) for Bergmann glial cell patches, $0{\cdot}50 \pm 0{\cdot}18$ ($n = 4$) for DG basket cell patches, and $1{\cdot}12 \pm 0{\cdot}02$ ($n = 4$) for CA3 pyramidal cell patches. Intracellular spermine (100 μM) did not affect the shape of the I–V relation of the NMDA receptor-mediated current recorded in the absence of extracellular Mg^{2+} and in the presence of glycine (CA3 pyramidal cell patches; $n = 3$, data not shown). This suggested that intracellular spermine specifically affects Ca^{2+}-permeable AMPARs. The effects of spermine on current rectification were reversible upon washout.

Unlike the rectification properties, the desensitization time course of glutamate-activated currents in patches from Bergmann glial cells, DG basket cells and CA3 pyramidal cells was not changed by 25 μM spermine in the range of membrane potentials investigated. In Bergmann glial cell patches, for example, the desensitization time constant was $2{\cdot}7 \pm 0{\cdot}2$ ms ($n = 5$) in the absence and $2{\cdot}8 \pm 0{\cdot}2$ ms ($n = 4$) in the presence of 25 μM spermine at −80 mV.

Affinity and kinetics of polyamine block

Analysis of the inhibition of outward current by different intracellular spermine concentrations revealed that Ca^{2+}-permeable AMPARs were very sensitive to polyamines. Plots of the ratio of rectification indices (IRs) in the presence and absence of spermine against concentration revealed a half-maximal inhibitory concentration (IC_{50}) of 0·3 μM in Bergmann glial cell patches and 1·5 μM in DG basket cell patches (Fig. 3). In Bergmann glial cell patches, AMPARs were almost completely inhibited at high concentrations (> 25 μM), while in DG basket cell patches the maximal inhibition was about 75%. In contrast, the IC_{50} value for CA3 pyramidal cell patches was $\gg 100$ μM, about 1000-fold higher than that for Bergmann glial cell patches. Intracellular spermidine, an endogenous polyamine structurally related to spermine, also induced double rectification, but was less potent than spermine. IC_{50} values were 1·4 μM in Bergmann glial cell patches ($n = 17$), 9·9 μM in DG basket cell patches ($n = 18$), and $\gg 100$ μM in CA3 pyramidal cell patches ($n = 3$).

To distinguish whether polyamines affected gating or conductance properties of AMPARs, the time course of block after voltage steps was investigated in the presence of 25 μM intracellular spermine using Bergmann glial cell patches. Since desensitization would have interfered with the measurement of responses to voltage steps, cyclothiazide was added to the extracellular solutions (Koh *et al.* 1995). Cyclothiazide (100 μM) almost completely abolished desensitization, but did not affect the double rectification of the glutamate-activated current (Fig. 4*A* and *B*). When the voltage was stepped from −60 to +30 mV (Fig. 4*C*) after activation of AMPARs, the current showed instantaneous reversal followed by a decay phase of less than 0·5 ms duration, resulting in a transient outward current ($n = 4$). Following a voltage step back to −60 mV, the current rose to its original amplitude within 0·5 ms. This suggests that development and recovery of polyamine block is very rapid, but not instantaneous. In the absence of intracellular spermine, the I–V relation was linear and time-dependent current relaxations were not observed (data not shown).

DISCUSSION

Subunit composition of AMPARs and polyamine concentration determine current rectification

We showed that current rectification of native AMPARs depends on two major factors: the subunit composition and the intracellular concentration of polyamines. AMPARs in Bergmann glial cells, DG basket cells and CA3 pyramidal neurones differ about 1000-fold in spermine sensitivity ($IC_{50} = 0{\cdot}3$, 1·5, and $\gg 100$ μM, respectively). The polyamine sensitivity of the AMPARs expressed in these cells is apparently related to their Ca^{2+} permeability ($P_{Ca}/P_{Na} = 2{\cdot}8$, 1·6, and 0·1, respectively; Geiger *et al.* 1995) and inversely related to the abundance of GluR-B mRNA relative to that of all AMPAR subunit mRNAs (0·03, 0·12, and 0·44, respectively; Geiger *et al.* 1995). This indicates that the GluR-B subunit determines both the Ca^{2+} permeability and the affinity of native AMPARs for polyamines.

Polyamines are thought to be ubiquitously present in neuronal and non-neuronal cell types at concentrations up to 1 mM (Watanabe *et al.* 1991). The exact polyamine level in different neurone types is not known. The degree of rectification of evoked EPSCs in whole-cell recorded DG basket cells (IR = 0·51; D.-S. Koh, unpublished observations), however, is comparable to the rectification observed in the presence of 25 μM spermine in outside-out patches (Fig. 2*B*). Dendritic AMPARs in cultured hippocampal neurones show stronger rectification of the I–V relation than somatic AMPARs (Lerma, Morales, Ibarz & Somohano, 1994), suggesting that either the polyamine concentration or the AMPAR subunit composition is not identical in different regions of a neurone. High polyamine levels were reported in *Xenopus* oocytes (Ficker *et al.* 1994) and proliferating cell lines (Pegg, 1988), which are frequently used as host cells for AMPAR expression. Hence, differences in rectification properties between recombinant AMPAR subtypes have to be reinterpreted as differences in polyamine sensitivity.

Mechanisms of polyamine block

Intracellular polyamines block outward currents through native Ca^{2+}-permeable AMPARs between 0 and +40 mV. The block occurs within 0·5 ms, more rapidly than deactivation or desensitization of AMPARs (Koh *et al.* 1995; Geiger *et al.* 1995), indicating a direct interaction

with the pore. At more positive potentials, the conductance rises again. The most likely explanation is that polyamines permeate AMPARs when the driving force is large. The interaction between polyamines and AMPARs thus resembles the block and permeation of delayed rectifier K^+ channels by intracellular Na^+ (French & Wells, 1977).

Site-directed mutagenesis indicated that the amino acid residue at the Q/R site of the pore-forming region of GluR subunits determines Ca^{2+} permeability and rectification properties of recombinant AMPARs. A positively charged arginine at this position results in low Ca^{2+} permeability and a linear I–V relation, whereas a neutral glutamine leads to high Ca^{2+} permeability and doubly rectifying I–V in the whole-cell configuration. The results shown here allow a straightforward interpretation. Ca^{2+} and polyamines are multivalent cations likely to be repelled by the positively charged arginine due to electrostatic interactions.

Ca^{2+} permeability and current rectification of recombinant AMPARs have been dissociated by mutation. Subunits containing the smaller asparagine residue at the Q/R site form channels which are Ca^{2+} permeable but show linear I–V relations in the whole-cell recording mode (Burnashev *et al.* 1992*b*) and are almost insensitive to polyamines in outside-out patches (IR = 1·8 in the absence and 1·5 in the presence of 25 μM intracellular spermine; N. Burnashev, unpublished observations). This suggests that both charge and size of the residue at the Q/R site of AMPAR subunits determine whether the channel is blocked by intracellular polyamines.

Extracellular spider toxin ($Argiotoxin_{636}$) specifically blocks recombinant Ca^{2+}-permeable AMPARs (Herlitze *et al.* 1993). This toxin consists of a polyamine moiety connected to a phenyl ring, which may get plugged into the outer mouth of the channel (Herlitze *et al.* 1993). This may explain why argiotoxin blocks AMPARs, whereas polyamines show block as well as permeation.

Mechanisms identical to that of polyamine block and permeation of AMPARs may operate in other ligand-gated ion channels. Neuronal nicotinic ACh receptors are also highly Ca^{2+} permeable and show doubly rectifying I–V relations (Mathie, Colquhoun & Cull-Candy, 1990). Although the factors responsible for rectification of ACh receptors have not been identified, it was suggested that a 'strongly voltage-dependent gating particle prevents outward current from flowing' (Mathie *et al.* 1990). Hence, neuronal nicotinic ACh receptors may represent a second example that Ca^{2+} permeability of a channel is linked to polyamine sensitivity.

Burnashev, N., Khodorova, A., Jonas, P., Helm, P. J., Wisden, W., Monyer, H., Seeburg, P. H. & Sakmann, B. (1992*a*). Calcium-permeable AMPA-kainate receptors in fusiform cerebellar glial cells. *Science* **256**, 1566–1570.

Burnashev, N., Monyer, H., Seeburg, P. H. & Sakmann, B. (1992*b*). Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron* **8**, 189–198.

Colquhoun, D., Jonas, P. & Sakmann, B. (1992). Action of brief pulses of glutamate on AMPA/kainate receptors in patches from different neurones of rat hippocampal slices. *Journal of Physiology* **458**, 261–287.

Fakler, B., Brändle, U., Glowatzki, E., Weidemann, S., Zenner, H.-P. & Ruppersberg, J. P. (1995). Strong voltage-dependent inward rectification of inward rectifier K^+ channels is caused by intracellular spermine. *Cell* **80**, 149–154.

Ficker, E., Taglialatela, M., Wible, B. A., Henley, C. M. & Brown, A. M. (1994). Spermine and spermidine as gating molecules for inward rectifier K^+ channels. *Science* **266**, 1068–1072.

French, R. J. & Wells, J. B. (1977). Sodium ions as blocking agents and charge carriers in the potassium channel of the squid giant axon. *Journal of General Physiology* **70**, 707–724.

Geiger, J. R. P., Melcher, T., Koh, D.-S., Sakmann, B., Seeburg, P. H., Jonas, P. & Monyer, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron* (in the Press).

Herlitze, S., Raditsch, M., Ruppersberg, J. P., Jahn, W., Monyer, H., Schoepfer, R. & Witzemann, V. (1993). Argiotoxin detects molecular differences in AMPA receptor channels. *Neuron* **10**, 1131–1140.

Hollmann, M., Hartley, M. & Heinemann, S. (1991). Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science* **252**, 851–853.

Horn, R. & Marty, A. (1988). Muscarinic activation of ionic currents measured by a new whole-cell recording method. *Journal of General Physiology* **92**, 145–159.

Jonas, P., Racca, C., Sakmann, B., Seeburg, P. H. & Monyer, H. (1994). Differences in Ca^{2+} permeability of AMPA-type glutamate receptor channels in neocortical neurons caused by differential GluR-B subunit expression. *Neuron* **12**, 1281–1289.

Koh, D.-S., Geiger, J. R. P., Jonas, P. & Sakmann, B. (1995). Ca^{2+}-permeable AMPA and NMDA receptor channels in basket cells of rat hippocampal dentate gyrus. *Journal of Physiology* **485**, 383–402.

Lerma, J., Morales, M., Ibarz, J. M. & Somohano, F. (1994). Rectification properties and Ca^{2+} permeability of glutamate receptor channels in hippocampal cells. *European Journal of Neuroscience* **6**, 1080–1088.

Lopatin, A. N., Makhina, E. N. & Nichols, C. G. (1994). Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. *Nature* **372**, 366–369.

McBain, C. J. & Dingledine, R. (1993). Heterogeneity of synaptic glutamate receptors on CA3 stratum radiatum interneurones of rat hippocampus. *Journal of Physiology* **462**, 373–392.

Mathie, A., Colquhoun, D. & Cull-Candy, S. G. (1990). Rectification of currents activated by nicotinic acetylcholine receptors in rat sympathetic ganglion neurones. *Journal of Physiology* **427**, 625–655.

WATANABE, S., KUSAMA-EGUCHI, K., KOBAYASHI, H. & IGARASHI, K. (1991). Estimation of polyamine binding to macromolecules and ATP in bovine lymphocytes and rat liver. *Journal of Biological Chemistry* **266**, 20803–20809.

Acknowledgements

We thank Dr B. Sakmann, Dr V. Witzemann, J. Geiger, and A. Roth for helpful discussions and Dr D. Feldmeyer and Dr A. Villarroel for reading the manuscript. We also thank M. Kaiser for technical and H. Spiegel for secretarial assistance. Supported by DFG grant SFB-317/B14 (P.J.).

Received 20 April 1995; accepted 24 May 1995.