Oliver D, Hahn H, Antz C, Ruppersberg JP, Fakler B (1998) Interaction of permeant and blocking ions in cloned inward-rectifier K+ channels. Biophys J 74:2318–2326

Parker I, Sumikawa K, Miledi R (1986) Messenger RNA from retina induces kainate and glycine receptors in Xenopus oocytes. Proc. R. Soc. Lond. B 225:99–106

Priestley T, Woodruff GN, Kemp JA (1989) Antagonism of responses to excitatory amino acids on rat cortical neurons by spider toxin, argiotoxin636. Br J Pharmacol 97:1315–1323

Ragsdale D, Gant DB, Anis NA, Eldefrawi AT, Eldefrawi ME, Konno K, Miledi R (1989) Inhibition of rat brain glutamate receptors by philanthotoxin. J Pharmacol Exp Ther 251:156–163

Randle JCR, Vernier P, Garrigues AM, Brault E (1988) Properties of the kainate channel in rat brain mRNA injected Xenopus oocytes: ionic selectivity and blockage. Mol Cell Biochem 80:121–132

Rozov A, Zilberter Y, Wollmuth LP, Burnashev N (1998) Facilitation of currents through Ca2+-permeable AMPAR channels by activity-dependent relief from polyamine block. J Physiol (Lond) 511.2:361–377

Sands SB, Barish ME (1992) Neuronal nicotinic acetylcholine receptor currents in phaeochromocytoma (PC12) cells: dual mechanisms of rectification. J Physiol (Lond) 447:467–487

Silver MR, DeCoursey TE (1990) Intrinsic gating of inward rectifier in bovine pulmonary artery endothelial cells in the presence or absence of Internal Mg^{2+}. J Gen Physiol 96:109–133

Sommer B, Köhler M, Sprengel R, Seeburg PH (1991) RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell 67:11–19

Sugiyama S, Matsuo Y, Maenaka K, Vassylyev DG, Matsushima M, Kashiwagi K, Igarashi K, Morikawa K (1996) The 1.8-Å X-ray structure of the *Escherichia coli* PotD protein complexed with spermidine and the mechanism of polyamine binding. Protein Science 5:1984–1990

Swanson GT, Feldmeyer D, Kaneda M, Cull-Candy SG (1996) Effect of RNA editing and subunit co-assembly on single-channel properties of recombinant kainate receptors. J Physiol (Lond) 492:129–142

Swanson GT, Kamboj SK, Cull-Candy SG (1997) Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. J Neurosci 17:58–69

Tabor H, Tabor CW (1965) Spermidine, spermine, and related amines. Pharmacol Rev 245–300

Tóth K, McBain CJ (1998) Afferent specific innervation of two distinct AMPA receptor subtypes on single hippocampal interneurons. Nat. Neurosci 1:572–577

Traub RD, Whittington MA, Stanford IM, Jeffreys JGR (1996) A mechanism for generation of long-range synchronous fast oscillations in the cortex. Nature 383: 621–624

Usherwood PNR, Blagbrough IS (1991) Spider toxins affecting glutamate receptors: polyamines in therapeutic neurochemistry. Pharmac Ther 52:245–268

Vandenberg CA (1987) Inward rectification of a potassium channel in cardiac ventricular cells depends on internal magnesium ions. Proc Natl Acad Sci USA 84: 2560–2564

Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B (1991) Structural determinants of ion flow through recombinant glutamate receptor channels. Science 252:1715–1718

Washburn MS, Dingledine R (1996) Block of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. J Pharmacol Exp Ther 278:669–678

Washburn MS, Numberger M, Zhang S, Dingledine R (1997) Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. J Neurosci 17:9393–9406

Watanabe S, Kusama-Eguchi K, Kobayashi H, Igarashi K (1991) Estimation of polyamine binding to macromolecules and ATP in bovine lymphocytes and rat liver. J Biol Chem 266:20803–20809

Wollmuth LP, Sakmann B (1998) Different mechanisms of Ca^{2+}-transport in NMDA and Ca^{2+}-permeable AMPA glutamate receptor channels. J. Gen. Physiol. 112: 623–636

Yang J, Jan YN, Jan LY (1995) Control of rectification and permeation by residues in two distinct domains in an inward rectifier K+ channel. Neuron 14:1047–1054

Burnashev N, Villarroel A, Sakmann B (1996) Dimensions and ion selectivity of recombinant AMPA and kainate receptor channels and their dependence on Q/R site residues. J Physiol (Lond) 496:165–173
Burrill JD, McCullum CB, Hume RI (1993) Mechanism of rectification in the current-voltage relationship of non-NMDA receptor channels. Soc Neurosci Abstr 19:279(Abstract)
Buzsáki G, Chrobak JJ (1995) Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. Curr Opin Neurobiol 5:504–510
Cui C, Bähring R, Mayer ML (1998) The role of hydrophobic interactions in binding of polyamines to non NMDA receptor ion channels. Neuropharm 37:1381–1391
Curutchet P, Bochet P, Prado de Carvalho L, Lambolez B, Stinnakre J, Rossier J (1992) In the GluR1 glutamate receptor subunit a glutamine to histidine point mutation suppresses inward rectification but not calcium permeability. J Neurosci 182: 1089–1093
Dingledine R, Hume RI, Heinemann SF (1992) Structural determinants of barium permeation and rectification in non-NMDA glutamate receptor channels. J Neurosci 12:4080–4087
Donevan SD, Rogawski MA (1995) Intracellular polyamines mediate inward rectification of Ca^{2+}-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. Proc Natl Acad Sci USA 92:9298–9302
Draguhn A, Jahn W, Witzemann V (1991) Argiotoxin636 inhibits NMDA-activated ion channels expressed in Xenopus oocytes. Neurosci Lett 132:187–190
Dudley HW, Rosenheim O, Starling WW (1927) The constitution and synthesis of spermidine, a newly discovered base isolated from animal tissues. Biochem J 21:97–103
Fakler B, Brändle U, Glowatzki E, Weidemann S, Zenner HP, Ruppersberg JP (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 BA, Henley CM, Brown AM (1994) Spermine and spermidine as gating molecules for inward rectifier K+ channels. Science 266:1068–1072
Geiger JRP, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P, Monyer H (1995) Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principle neurons and interneurons in rat CNS. Neuron 15:193–204
Geiger JRP, Lübke J, Roth A, Frotscher M, Jonas P (1997) Submillisecond AMPA receptor-mediated signaling at a principal neuron-interneuron synapse. Neuron 18:1009–1023
Gilbertson TA, Scobey R, Wilson M (1991) Permeation of calcium ions through non-NMDA glutamate channels in retinal bipolar cells. Science 251:1613–1615
Haghighi AP, Cooper E (1998) Neuronal nicotinic acetylcholine receptors are blocked by intracellular spermine in a voltage-dependent manner. J Neurosci 18:4050–4062
Haverkampf K, Lübke J, Jonas P (1997) Single-channel properties of native AMPA receptors depend on the putative subunit composition. Soc Neurosci Abstr 478.23(Abstract)
Herlitze S, Raditsch M, Ruppersberg JP, Jahn W, Monyer H, Schoepfer R, Witzemann V (1993) Argiotoxin detects molecular differences in AMPA receptor channels. Neuron 10:1131–1140
Higuchi M, Single FN, Köhler M, Sommer B, Sprengel R, Seeburg PH (1993) RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Cell 75:1361–1370
Hille B, Schwarz W (1978) Potassium channels as multi-ion single pores. J Gen Physiol 72:409–442
Hille, B (1992) Ionic channels of excitable membranes, Sunderland: Sinauer Associates, Ed. 2
Howe JR (1996) Homomeric and heteromeric ion channels formed from kainate-type subunits GluR6 and KA2 have very small, but different, unitary conductances. J Neurophysiol 76:510–519

Hume RI, Dingledine R, Heinemann SF (1991) Identification of a site in glutamate receptor subunits that controls calcium permeability. Science 253:1028–1031
Ifune CK, Steinbach JH (1990) Rectification of acetylcholine-elicited currents in PC12 phaeochromocytoma cells. Proc. Natl. Acad. Sci. USA 87:4794–4798
Ifune CK, Steinbach JH (1991) Voltage-dependent block by magnesium of neuronal nicotinic acetylcholine receptor channels in rat phaeochromocytoma cells. Proc. Natl. Acad. Sci. USA 87:4794–4798
Ifune CK, Steinbach JH (1992) Inward rectification of acetylcholine-elicited currents in PC12 phaeochromocytoma cells. J Physiol (Lond) 457:143–165
Iino M, Ozawa S, Tsuzuki K (1990) Permeation of calcium through excitatory amino acid receptor channels in cultured rat hippocampal neurons. J Physiol (Lond) 424:151–165
Iino M, Koike M, Isa T, Ozawa S (1996) Voltage-dependent blockage of Ca^{2+})-permeable AMPA receptors by joro spider toxin in cultured rat hippocampal neurons. J Physiol (Lond) 496:431–437
Isa T, Iino M, Itazawa S, Ozawa S (1995) Spermine mediates inward rectification of Ca^{2+})-permeable AMPA receptor channels. Neuroreport 6:2045–2048
Jackson H, Usherwood PNR (1988) Spider toxins as tools for dissecting elements of excitatory amino acid transmission. Trends Neurosci 11:278–283
Jackson H, Parks TN (1989) Spider toxins: recent applications in neurobiology. Ann Rev Neurosci 12:405–414
Jefferys JGR, Traub RD, Whittington MA (1996) Neuronal networks for induced '40kHz' rhythms. Trends Neurosci 19:202–208
Jonas P, Racca C, Sakmann B, Seeburg PH, 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
Jonas P, Burnashev N (1995) Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. Neuron 15:987–990
Jones MG, Anis NA, Lodge D (1990) Philanthotoxin blocks quisqualate-, AMPA- and kainate-, but not NMDA-, induced excitation of rat brainstem neurons in vivo. Br J Pharmacol 101:968–970
Kamboj SK, Swanson GT, Cull-Candy SG (1995) Intracellular spermine confers rectification on rat calcium-permeable AMPA and kainate receptors. J Physiol (Lond) 486:297–303
Katz B (1949) Les constantes électriques de la membrane du muscle. Arch Sci Physiol 2:285–299
Khan NA, Quemener V, Moulinoux JP (1994) In: Carter C (ed) The Neuropharmacology of Polyamines. Academic Press, London, pp 37–60
Koh DS, Burnashev N, Jonas P (1995) Block of native Ca^{2+}-permeable AMPA receptors in rat brain by intracellular polyamines generates double rectification. J Physiol (Lond) 486:305–312
Ladenburg A, Abel J (1888) Ueber das Aethylenimin (Spermin?). Ber dtsch chem Ges 21:758–766
Lewenhoeck A (1678) Observationes D. Anthonii Lewenhoeck, de Natis è semine genitali Animalculis. Phil Trans R Soc Lond 12:1040–1042
Lopatin AN, Makhina EN, Nichols CG (1994) Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. Nature Lond 372:366–369
Marton LJ, Pegg AE (1995) Polyamines as targets for therapeutic intervention. Ann Rev Pharmacol Toxicol 35:55–91
Mathie A, Colquhoun D, Cull-Candy SG (1990) Rectification of currents activated by nicotinic acetylcholine receptors in rat sympathetic ganglion neurones. J. Physiol (Lond.) 427:625–655
Matsuda H, Saigusa A, Irisawa H (1987) Ohmic conductance through the inwardly rectifying K channel and blocking by internal Mg^{2+}. Nature Lond 325:156–159
Neher E, Steinbach JH (1978) Local anaesthetics transiently block currents through single acetylcholine-receptor channels. J Physiol (Lond) 277:153–176

applied polyamine-amide toxins contained in the venoms of spiders and wasps are employed as offensive agents to kill or paralyze other insect prey via their actions on invertebrate ion channels (Usherwood and Blagbrough 1991). Although these toxin molecules are aimed at peripheral targets, such as the invertebrate glutamatergic neuromuscular junction and cholinergic synapses, polyamine amide toxins have subsequently been shown to block GluRs in the mammalian CNS (Usherwood and Blagbrough 1991; Jackson and Usherwood 1988; Jackson and Parks 1989). To date, three main toxin molecules and their synthetic analogues have been used by a variety of investigators to block vertebrate GluRs at nanomolar concentrations: argiotoxin (ATX) and joro spider toxin (JSTX) from orb-web and joro spiders, respectively, and PhTX from the digger wasp, *Philanthus triangulum*. Early studies of native GluRs suggested that ATX was selective for NMDA receptors (Priestley et al. 1989; Draguhn et al. 1991), whereas PhTX was selective for non-NMDA receptors (Jones et al. 1990). Subsequent analysis of recombinant AMPA receptors revealed, however, that both toxins have similar affinities and that, similar to external Spm block, toxin affinity is dependent on editing at the Q/R site (Herlitze et al. 1993; Washburn and Dingledine 1996; Blaschke et al. 1993; Brackley et al. 1993). In retrospect, the selectivity of ATX for native NMDA versus non-NMDA receptors probably reflects widespread expression of the highly edited GluR-B subunit. In view of the cell-specific expression of edited and unedited AMPA receptors in the CNS, polyamine-amide toxins have since proved useful pharmacological tools in determining the subunit composition of native receptors (Iino et al. 1996; Tóth and McBain 1998; Haverkampf et al. 1997).

Polyamine-amide toxins block AMPA- and kainate receptors primarily by a use- and voltage-dependent mechanism (Herlitze et al. 1993; Brackley et al. 1993; Bähring and Mayer 1998); however, some studies have also reported a weak potentiating action (Brackley et al. 1990; Ragsdale et al. 1989). Bähring and Mayer (1998) have shown that the use- and voltage-dependent action of external PhTX is due to an open-channel block mechanism, where access into and out of the blocking site in the pore is possible only when the channel is in the open state. The open state, however, becomes less stable if PhTX remains bound to the channel, and this allosteric mechanism has been proposed to account for closing and subsequent trapping of PhTX in the channel (Bähring and Mayer 1998).

D. Physiological Implications of Cytoplasmic Polyamine Block

Recent studies suggest that polyamines also access the pore in the closed conformation (Bowie et al. 1998; Rozov et al. 1998). Such a blocking mechanism may provide the molecular basis for novel activity regulation of calcium-permeable AMPA and kainate receptors in the CNS. Evidence suggesting that

cytoplasmic polyamines may access closed AMPA and kainate receptors was proposed from the observation that the rise times of responses to rapid application of Glu were slowed in a voltage-dependent manner in the presence of Spm (Bowie et al. 1998; Rozov et al. 1998), and that paired responses to brief applications of Glu showed facilitation, reflecting relief from closed-channel block (Rozov et al. 1998). Bowie et al. (1998) suggested that the slowing of rise times reflected voltage-dependent re-equilibration of Spm binding with the open state of the channel, since rise times were faster in high-permeation solutions, which lower Spm's affinity and, thus, curtail its residency time on the binding site. Furthermore, these observations were faithfully repro-

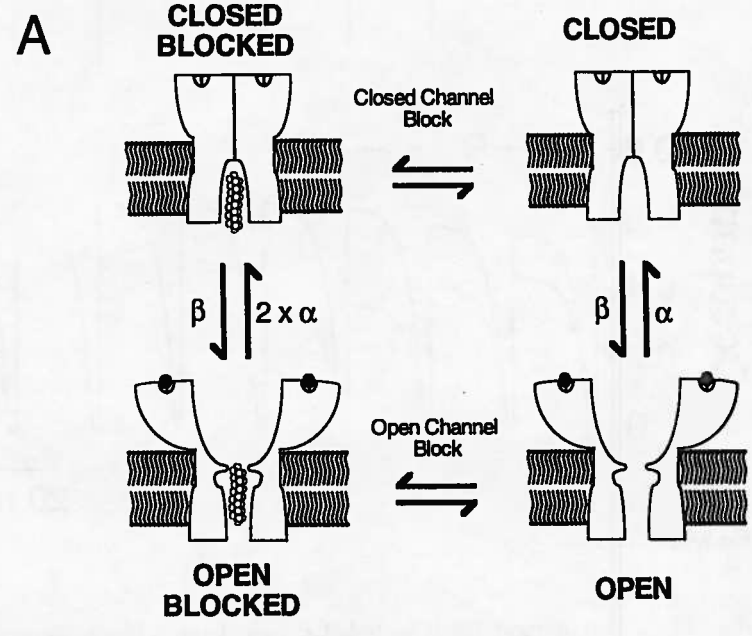


Fig. 7A,B. Schematic diagram outlining open- and closed-channel block by cytoplasmic polyamines. **A** Prior to receptor activation, cytoplasmic polyamines reside in a water-filled cavity that is accessible to the cytoplasm in the closed conformation; this represents the closed–blocked state. When channels open in response to L-glutamate (Glu) application, the conformational steps associated with channel activation expose a polyamine-binding site within the membrane electric field. The rate of binding and dissociation of spermine from the open state of the channel underlies the slow voltage dependence of rise times observed experimentally. The rate of decay of 1-ms responses to 10 mM Glu and, presumably, the rate of decay of excitatory postsynaptic currents at synapses with calcium-permeable non-*N*-methyl-D-aspartate receptors reflects primarily the closing rate of open and open–blocked channels as they relax into closed and closed–blocked states, respectively. **B** Simulations from a kinetic model show that repetitive stimulation leads to potentiation of the Glu-response amplitude as more channels are recruited from the closed–blocked state (*RB*) into the closed state (*R*). Taken from Bowie et al. (1998) with permission

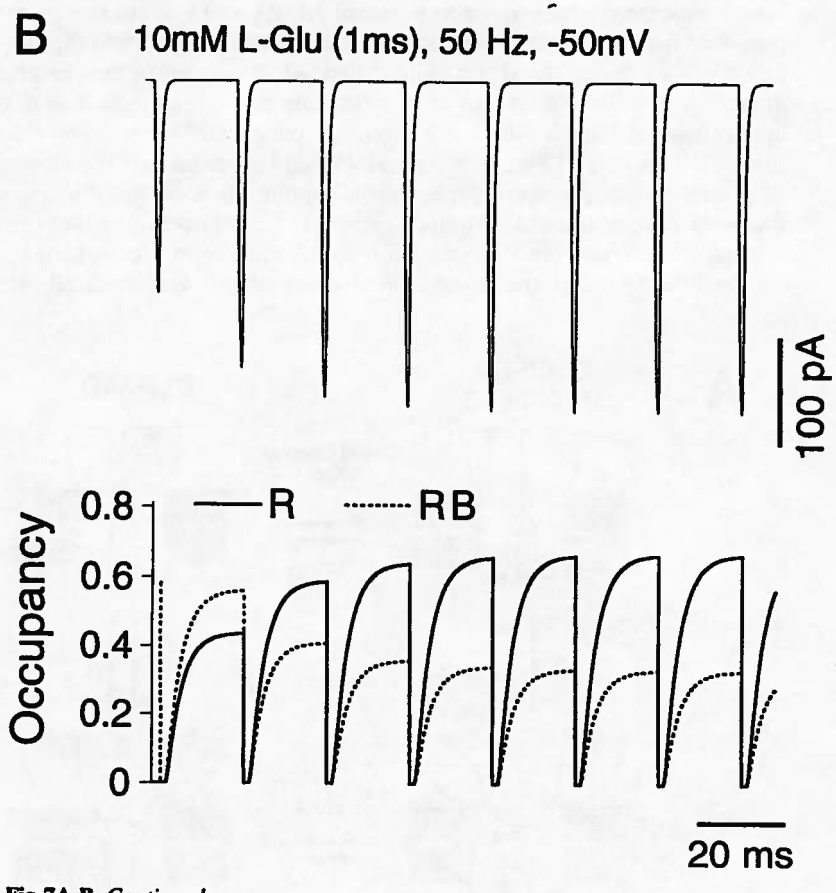


Fig. 7A,B. *Continued*

duced in a kinetic model that included closed-channel block (Bowie et al. 1998). Similar experimental observations were made by Rozov et al. (1998) when desensitization of AMPA receptors was removed by cyclothiazide. Occupancy of closed states by Spm appeared to be insensitive to membrane potential, which may suggest that polyamines bind to a water-filled cavity in the channel and are, therefore, shielded from the potential drop across the membrane (Fig. 7A) (Bowie et al. 1998). In contrast, re-block of closed channels is voltage-sensitive, following first-order kinetics with a time constant (at –60 mV) of 375 ms (Rozov et al. 1998). The relatively slow re-block of closed AMPA receptors by polyamines accounts for the facilitation of response amplitude observed following a train of pulses to 10 mM Glu (Rozov et al. 1998); this effect can be reproduced in a kinetic model of open- and closed-channel block of kainate receptors (Fig. 7A, B) (Bowie et al. 1998). It is not

yet known whether activity-dependent modulation of polyamine block may represent a novel form of short-term plasticity in the CNS. Basket cells of the dentate gyrus, for example, which express Ca^{2+}-permeable AMPA receptors, exhibit rapid gating characteristics (Geiger et al. 1995; Geiger et al. 1997). These characteristics have been proposed to be pivotal in defining the functional roles fulfilled by basket cells in hippocampal-network activity, such as oscillations (Jefferys et al. 1996) and feed-forward and feedback inhibition (Buzsáki and Chrobak 1995). It is interesting that interneuron-doublet firing, which permits long-range synchronization of gamma oscillations in the cortex, is strongly dependent on the unitary AMPA-receptor conductance of basket cells (Traub et al 1996). In view of this, one attractive goal of future studies would be to determine whether the short-term increase in AMPA-receptor amplitude, due to polyamine unblock, modulates the generation of long-range, synchronous oscillations in the cortex. Although Ca^{2+} permeability and gating properties are undoubtedly important factors in sculpting neuronal behavior, these recent findings may suggest that cytoplasmic polyamines also fulfill a novel role in synaptic plasticity.

References

Armstrong CM (1969) Inactivation of the potassium conductance and related phenomena caused by quarternary ammonium ion injected in squid axons. J Gen Physiol 54:553–575

Bähring R, Bowie D, Benveniste M, Mayer ML (1997) Permeation and block of rat GluR6 glutamate receptor channels by internal and external polyamines. J Physiol (Lond) 502:575–589

Bähring R, Mayer ML (1998) An analysis of philanthotoxin block for recombinant rat GluR6(Q) glutamate receptor channels. J Physiol (Lond) 509: 635–650.

Baukrowitz T, Yellen G (1996) Use-dependent blockers and exit rate of the last ion from the multi-ion pore of a K+ channel. Science 271:653–656

Blaschke M, Keller BU, Rivosecchi R, Hollmann M, Heinemann S, Konnerth A (1993) A single amino acid determines the subunit-specific spider toxin block of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor channels. Proc Natl Acad Sci USA 90:6528–6532

Bowie D, Smart TG (1989) Properties of a kainate receptor expressed in Xenopus laevis oocytes with bovine brain mRNA. J Physiol (Lond) 418:196P(Abstract)

Bowie D, Mayer ML (1995) Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. Neuron 15:453–462

Bowie D, Lange GD, Mayer ML (1998) Activity-dependent modulation of glutamate receptors by polyamines. J Neurosci 18:8175–8185

Brackley P, Goodnow R, Jr, Nakanishi K, Sudan HL, Usherwood PN (1990) Spermine and philanthotoxin potentiate excitatory amino acid responses of Xenopus oocytes injected with rat and chick brain RNA. Neurosci Lett 114:51–56

Brackley PT, Bell DR, Choi SK, Nakanishi K, Usherwood PN (1993) Selective antagonism of native and cloned kainate and NMDA receptors by polyamine-containing toxins. J Pharmacol Exp Ther 266:1573–1580

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

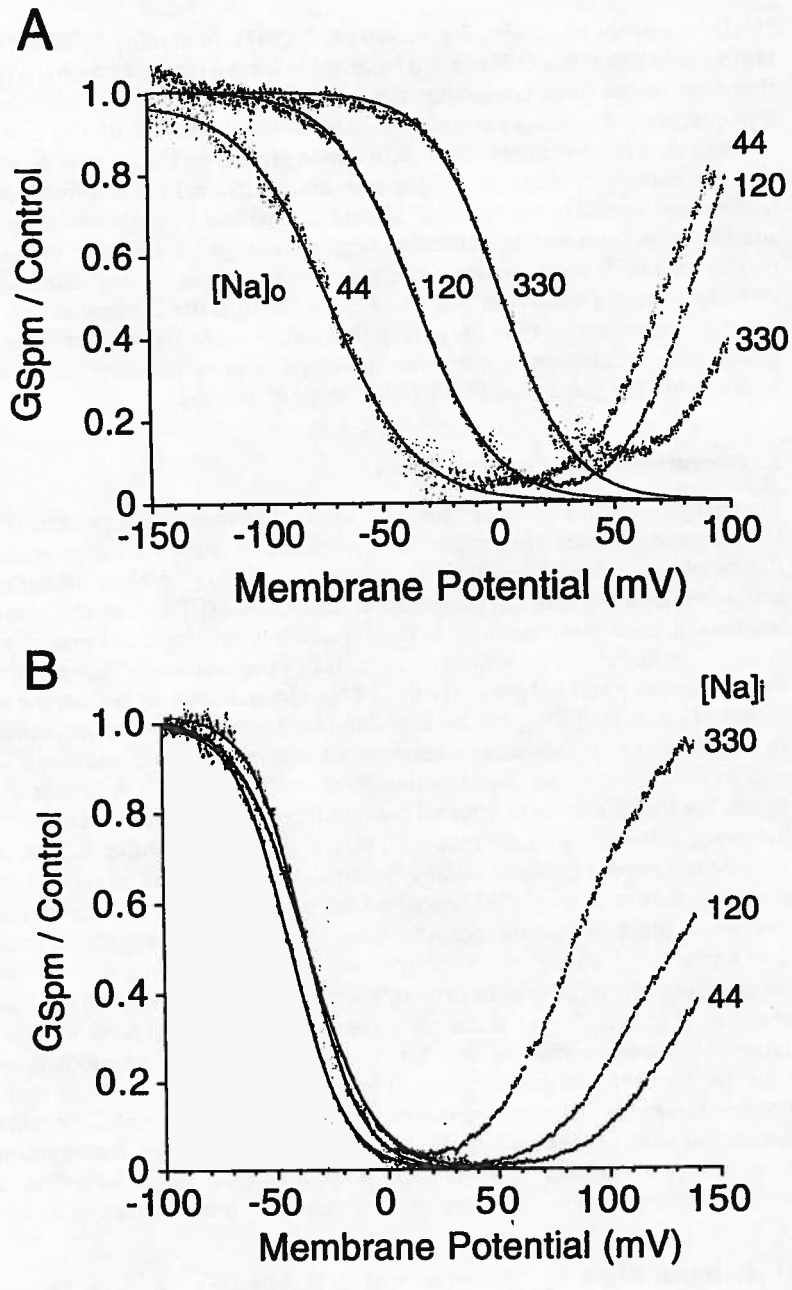


Fig. 6A–B.

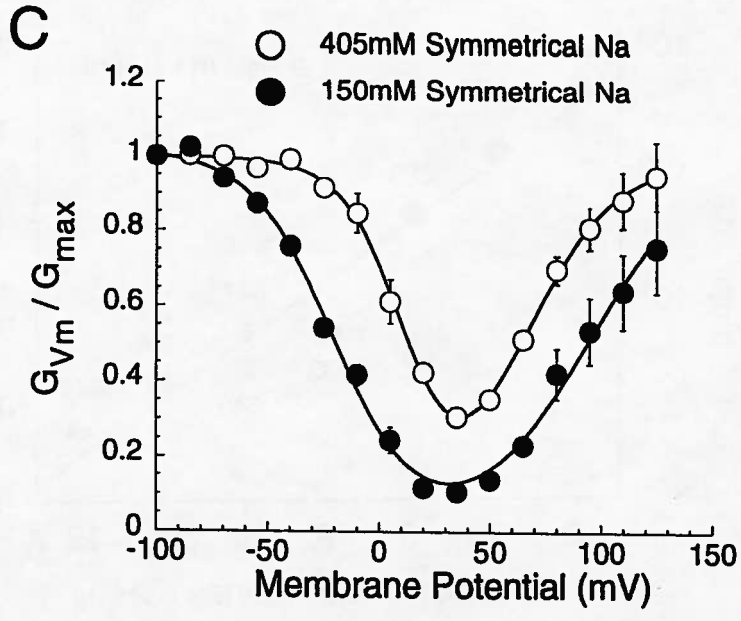


Fig. 6A–C. External and internal permeant ions strongly influence block by internal spermine (Spm). **A** Conductance–voltage relationship for block by 30 μM internal Spm is shifted to the right, with increasing external sodium concentration reflecting a decrease in Spm affinity at negative potentials. **B** In contrast, increases in internal sodium concentration have almost no effect on block at negative potentials, but increase relief from block at positive potentials. **C** Symmetrical increases in external and internal Na from 150 mM to 405 mM cause a concomitant decrease in block for 20 μM Spm at negative and positive potentials. Taken from BÄHRING et al. (1997) and BOWIE et al. (1998) with permission

entering the channel from either side of the membrane. In agreement with this prediction, externally applied Spm produces voltage-dependent block of both AMPA and kainate receptors (WASHBURN and DINGLEDINE 1996; WASHBURN et al. 1997; BÄHRING et al. 1997). Block is controlled by editing of the Q/R site (WASHBURN and DINGLEDINE 1996; WASHBURN et al. 1997), as expected if some of the molecular determinants of internal and external block are shared. External block, however, is weaker and much less voltage dependent, which may reflect greater permeation of external polyamines (BÄHRING et al. 1997). At this time, the molecular basis of asymmetry between internal and external block is not fully understood; however, it is likely to be governed by the location of the barriers and wells that control the binding of permeant ions and polyamines (BÄHRING et al. 1997).

Although influx and efflux pathways for polyamines from mammalian cells have been described (KHAN et al. 1994), a clear physiological role for block by external polyamines remains to be elucidated. In contrast, externally

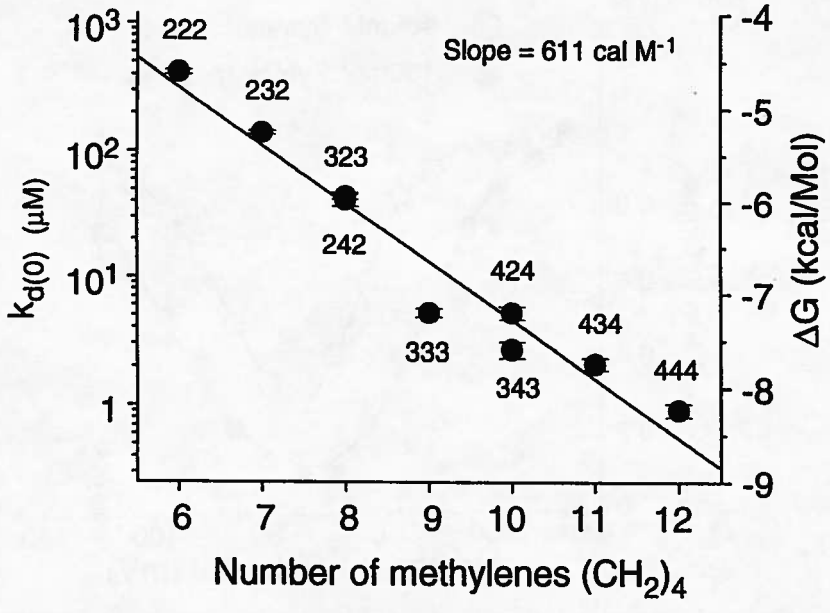


Fig. 5. Plot of the affinity of a series of tetravalent polyamines for glutamate receptor (GluR) 6(Q) block versus the number of methylene groups. The three digits above each data point denote the number of methylenes separating each amine group. A linear fit through the data suggests that polyamine block varies with the number of methylene groups, corresponding to a 2.9-fold increase in affinity per methylene group. Taken from Cui et al. (1998)

number of permeation properties, including block by cytoplasmic polyamines (Bowie and Mayer 1995), calcium (Ca^{2+}) permeability (Jonas and Burnashev 1995), anion versus cation permeability (Burnashev et al. 1996) and single-channel conductance (Howe 1996; Swanson et al. 1996, 1997). Nevertheless, some of these properties can be uncoupled from one another, since mutagenesis of the Q/R site has been shown to remove inward rectification whilst preserving divalent permeability (Burnashev et al. 1992; Curutchet et al. 1992; Dingledine et al. 1992). Consistent with this idea, divalent permeability in heteromeric receptors is more sensitive to replacement of glutamine residues by arginines than internal polyamine block (Washburn et al. 1997).

In addition to the Q/R site, a negatively charged aspartate, four amino acid residues downstream, is also important for internal polyamine block and divalent permeability (Dingledine et al. 1992). Replacement of the negative charge of aspartate with a neutral asparagine of similar size reduces internal polyamine block, with no obvious influence on divalent permeability (Dingledine et al. 1992). A model involving the Q/R site and the downstream aspartate residue has been proposed to account for the differential dependence of divalent permeability and internal polyamine block of open non-

NMDA receptor channels (Washburn et al. 1997). In unedited channels, glutamine residues at the Q/R site are believed to form a ring of carbonyl oxygens that contributes to or constitutes the binding site for external divalent ions. Cytoplasmic polyamines entering the channel also interact with this ring and, through electrostatic interactions with amine groups on the blocker molecule, with the carboxyl groups of the downstream aspartate. In heteromeric receptors, the sequential replacement of glutamine residues by arginines at the Q/R site has been proposed to neutralize negative charges in the pore by the formation of salt bridges between the guanidinium group of arginine and the carboxyl group of aspartate. This, apparently, disrupts the divalent ion-binding site to a greater extent than for polyamines but, as more arginines replace each glutamine, the electrostatic environment of the pore becomes increasingly unfavorable for polyamine block (Washburn et al. 1997).

3. Interaction with Permeant Ions

Internal polyamines entering the pore interact strongly with permeant ions entering the channel from either the cytoplasm or from the external side of the membrane (Bähring et al. 1997; Bowie et al. 1998) (Fig. 6). A similar interaction between the external potassium concentration ($[K^+]_{ext}$) and K_{ir} channels has been known for some time; in this interaction, the degree of inward rectification, or the blocker's affinity, was found to depend on $[K^+]_{ext}$ rather than the membrane potential *per se* (Hille 1992). The reduction in polyamine affinity with increasing $[K^+]_{ext}$ can be explained by Eyring rate theory, assuming a strong electrostatic interaction between an external binding site for K^+-ions and an inner site for an impermeable blocker (Hille 1992). A similar explanation for the reduction in internal polyamine block observed when $[Na^+]_{ext}$ is increased is less obvious for non-NMDA receptors since, unlike K_{ir} channels, they do not appear to act as multi-ion pores (Wollmuth and Sakmann 1998). Recently, Bowie et al. (1998) described the effect of membrane potential on polyamine block of kainate receptors linked to ion flux and suggested that the development of strong voltage-dependent block following depolarization results from the reduction in driving force of inward ion flux through open channels (Fig. 6C). As yet, these observations have not been modeled quantitatively; however, unlike the case for conventional permeation models, which treat the blocker as a point charge, it may be necessary to consider that the energy transmitted between permeant ions and blocker molecules is positively correlated with the volume of the ion absorbing the energy. Furthermore, a large part of the voltage dependence of polyamine block might be indirect and may reflect coupling to the movement of permeant ions (Oliver et al. 1998).

II. External Block by Spermine and Arthropod-Toxin Molecules

One prediction from the observation that internal polyamines permeate non-NMDA channels is that their binding site(s) can be accessed by polyamines

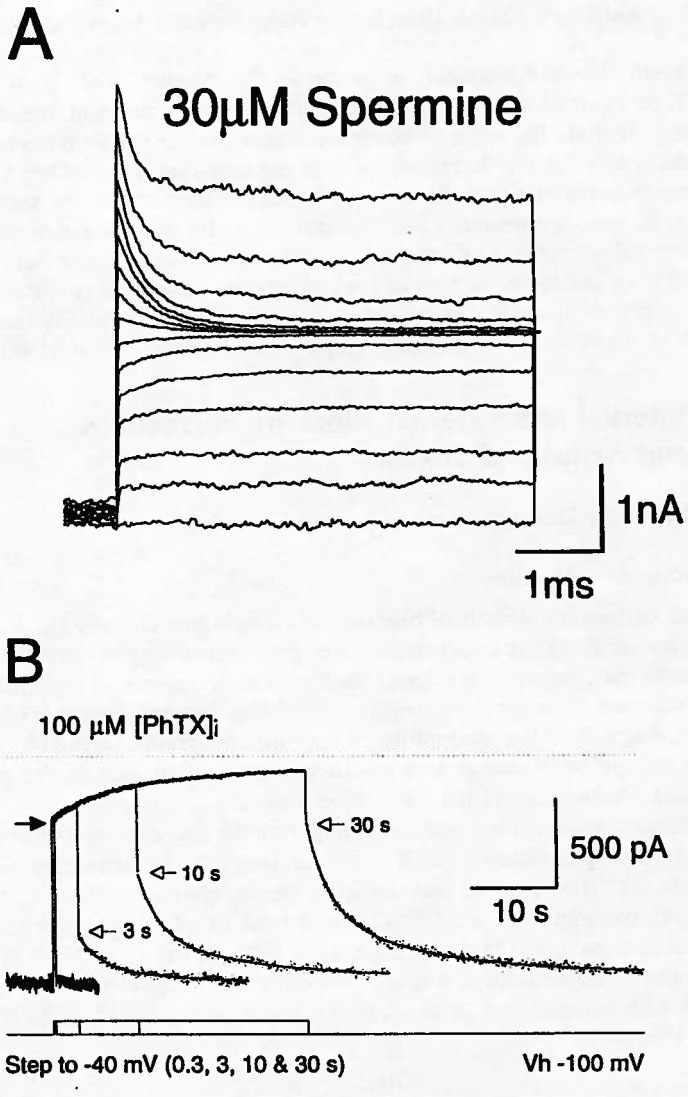


Fig. 4A,B. Open-channel block and trapping mechanisms occur on different time scales. **A** Current relaxations observed following voltage steps from –100 mV to +110 mV (in 15-mV increments), with 30 μM internal spermine, exhibit rapid first order kinetics and reflect the time course of open-channel block for glutamate receptor (GluR) 6(Q). **B** A series of superimposed GluR6(Q) responses following voltage steps from –100 mV to –40 mV in the presence of 100 μM internal philanthotoxin-343 (PhTX); the *black arrow* indicates the level of block predicted by fast ramps like that shown in Fig. 3. Successive voltage steps of increasing duration permit PhTX to remain bound to the channel long enough to destabilize the open state. PhTX is subsequently trapped in the channel, as evidenced by the increasing amplitude of the slow component of block (*open arrows*) and the prolonged period required for complete recovery following repolarization to –100 mV. Taken from Bowie et al. (1998) and Bähring and Mayer (1998) with permission

(Neher and Steinbach 1978), the binding rate for Spm (k_{on} = 48.5 μM $^{-1}$s $^{-1}$) suggests that this process is probably diffusion limited (Bowie et al. 1998). The slower binding rate for PhTX (k_{on} = 14.1 $\mu M^{-1}s^{-1}$) suggests that the toxin must enter the channel in a particular conformation for block to occur. Once bound, however, PhTX resides longer than Spd (k_{on} = 32.6 $\mu M^{-1}s^{-1}$), which is also a trivalent polyamine, suggesting that, in addition, to the polyamine chain, the aromatic moiety of the toxin molecule participates in the stabilizing of the blocked state (Bähring and Mayer 1998; Bowie et al. 1998). As discussed later, in addition to producing low-affinity open-channel block, PhTX is able to trigger entry into a more stable, blocked state, from which recovery requires many seconds.

In addition to blocking the ion-permeation pathway, polyamines also affect gating behavior by destabilizing the open state of the channel (Bähring and Mayer 1998; Bowie et al. 1998). When PhTX, for example, is permitted to remain bound to the open state, the channel enters a conformational state where the toxin molecule becomes trapped, requiring extended recovery periods for complete dissociation (Bähring and Mayer 1998) (Fig. 4B). Spm and Spd may also trigger entry into closed blocked states, since channel closure is accelerated by internal polyamines following the removal of Glu (Bowie et al. 1998). As yet, it is not known if this effect occurs through an allosteric mechanism similar to that reported for PhTX and other sequential channel blockers or if the open state is destabilized by the long, rod-like polyamine structure emptying the pore of permeant ions, similar to the effect of use-dependent blockers on K^+ channels (Baukrowitz and Yellen 1996).

Surprisingly, the role of hydrophobic binding is more important than might be at first anticipated for the strongly voltage-dependent block produced by highly charged, naturally occurring polyamines. A recent comparison of block produced by a series of tetravalent polyamines with different numbers and spacings of methylene groups revealed that hydrophobic interactions strongly influence the blockers' affinities, whereas different spacing, between amine groups had little effect (Fig. 5) (Cui et al. 1998). These results suggest that, in addition to charged residues in the permeation pathway, the hydrophobic environment of the pore may be a major factor in determining the extent of polyamine block.

2. Critical Pore-Lining Residues

Although recent evidence suggests that hydrophobic amino acids in the pore may be important in polyamine binding, most mutagenesis studies have concentrated on two residues: the Q/R site and a conserved ring of negative charges four amino acid residues downstream. Insight into structural elements important for polyamine block followed the identification of RNA editing in M2 at the Q/R site, which contains either a neutral glutamine or a positively charged arginine (Hume et al. 1991; Verdoorn et al. 1991). The Q/R site is believed to contribute to the channel's selectivity filter, since it determines a

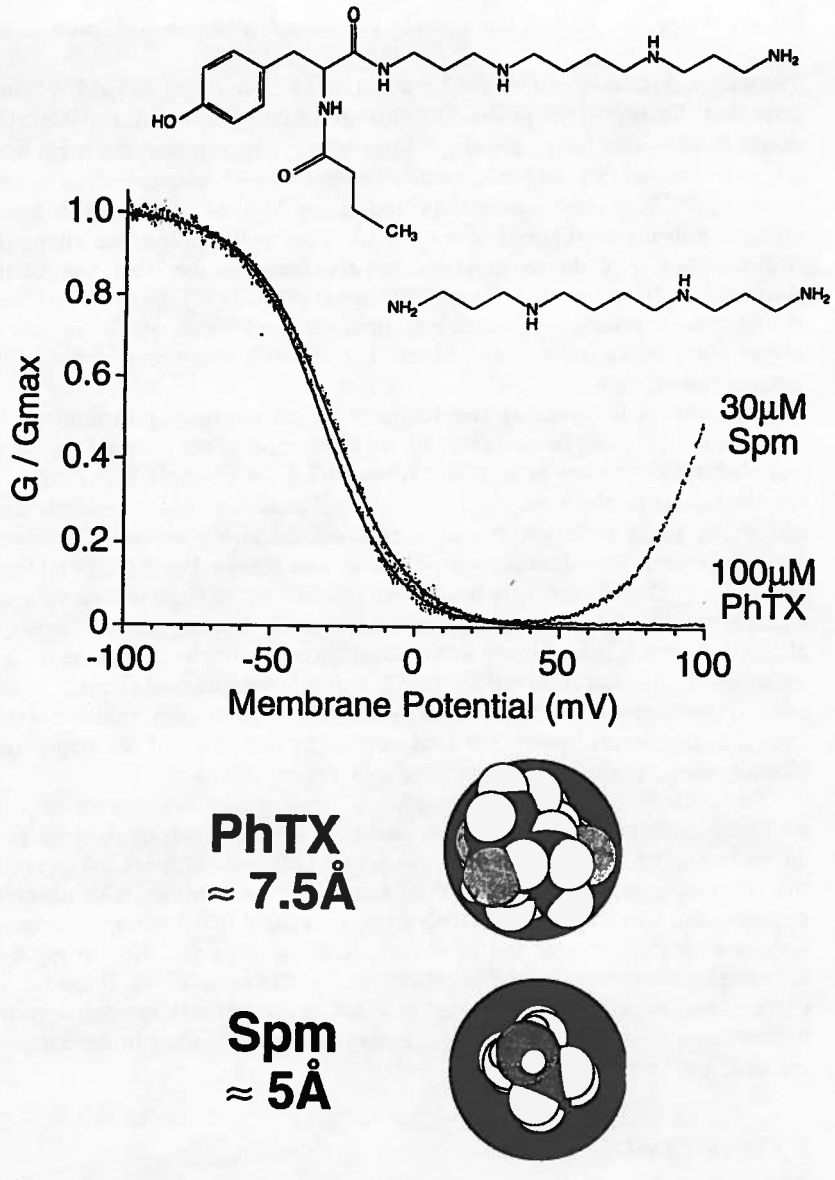


Fig. 3. Relief from block by internal polyamines varies with molecular size. Shown are conductance–voltage plots for internal spermine (Spm; 30 μM) and philanthotoxin-343 (PhTX; 100 μM) with each blocker concentration applied at concentrations giving similar block at negative membrane potentials. Relief from block at positive membrane potentials for Spm was not detectable for PhTX. *Inset* shows the extended structure of PhTX (*upper*) compared with Spm (*lower*). Consistent with these observations, superimposition of Corey–Pauling–Koltum models of PhTX and Spm on *circles* that represent non-NMDA channel dimensions suggest that relief from block is determined by the molecular size of the polyamines. Adapted from BÄHRING et al. (1997) with permission

(5 Å) and, thus, are less likely to permeate the channel. When Spm, PPS and PhTX concentrations were matched to achieve similar block at negative membrane potentials, the relief of block was much greater for Spm than PPS, and immeasurable for PhTX, consistent with the idea that relief of block for Spm is due to permeation of the blocker molecule (Fig. 3). In the second case, polyamine-ion permeability was ascertained under bi-ionic conditions where all external permeant ions were replaced by either Spm, Spd or Put (BÄHRING et al. 1997). The measurement of inward currents confirmed that Put, Spd and Spm permeate non-NMDA receptor channels with permeability ratios (relative to Na^+) of 0.42, 0.07 and 0.02, respectively (BÄHRING et al. 1997).

C. Internal and External Block by Polyamines and Arthropod Toxins

I. Molecular Determinants of Internal Block

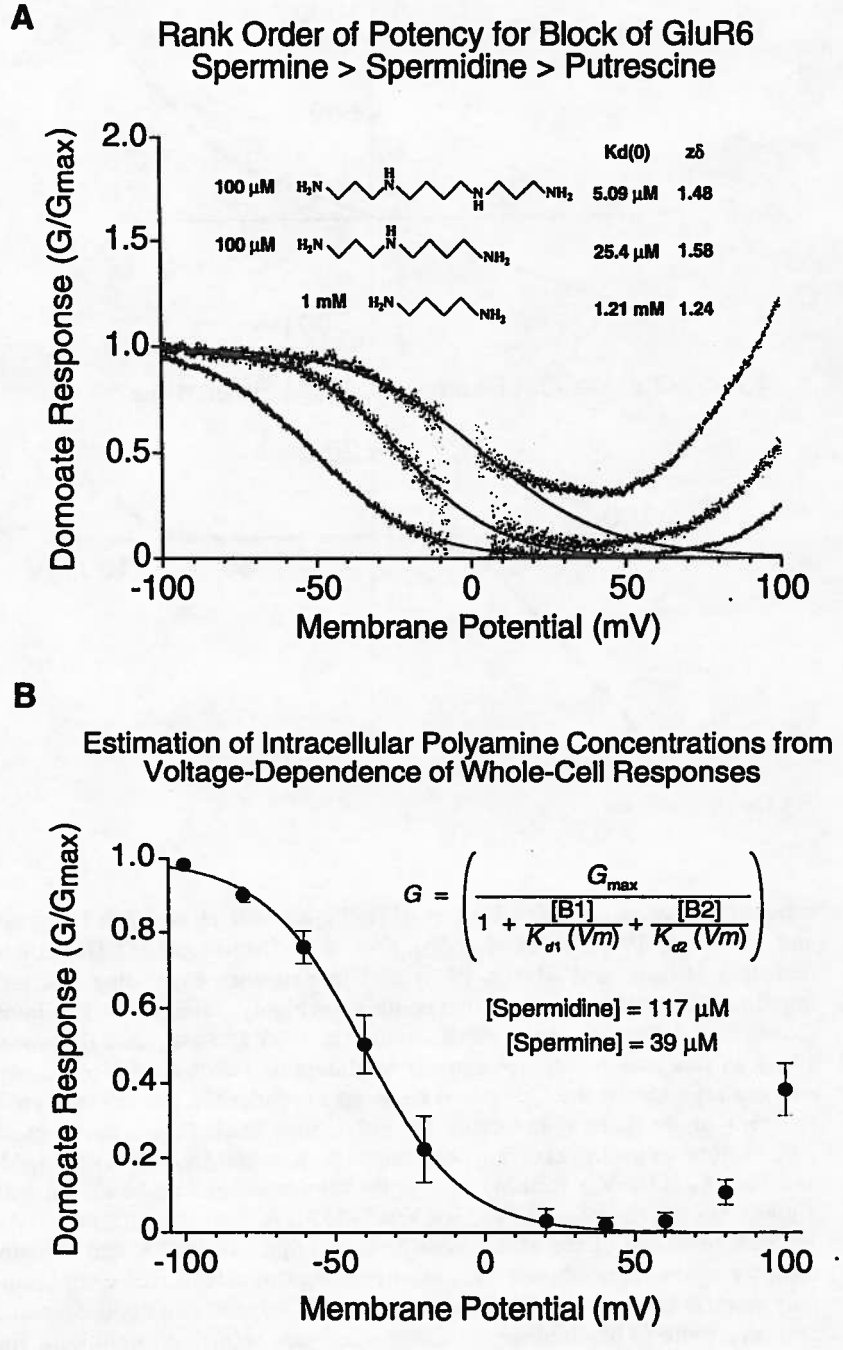
1. Polyamine Structure

Unlike conventional channel blockers, the distributed charges and hydrophobic moieties along the linear structure of polyamines suggests that the blocker molecule may, in fact, interact with multiple contact points on the channel wall as it advances through the permeation pathway. Progress towards identifying which, if not all, of the hydrophilic or hydrophobic groups on the blocker molecule interact with amino acid residues or with other ions in the pore first requires a knowledge of the block mechanism.

Recent voltage-jump-relaxation experiments and dose-response analyses suggest that open-channel block by polyamines exhibits first-order kinetics at a single site. This suggests that, unlike some K_{ir} channels (YANG et al. 1995), the open-channel pore accommodates, at least to a first approximation, only one blocker molecule (BOWIE and MAYER 1995; BOWIE et al. 1998) (Fig. 4A). Transitions into and out of a single blocked state are governed by three molecular events according to the scheme below (BÄHRING et al. 1997; BOWIE et al. 1998):

$$X_{Inside} \underset{k_{off}}{\overset{[B]k_{on}}{\rightleftharpoons}} X_{Site} \overset{k_{perm}}{\rightleftharpoons} X_{Outside}$$

where the rate of binding from the cytoplasm is determined by [B] times the rate constant of binding (k_{on}) and the exit rates are dependent on the rate constant for the return of the blocker to the cytoplasm (k_{off}) or, as described above, the rate constant for the permeation of blocker (k_{perm}). Fitting data with this model for Spm, Spd and PhTX has yielded estimates for their rates of binding and dissociation from the open channel. These estimates indicate that the voltage dependence of block is governed largely by the exit or permeation of the blocker rather than its entry into the channel (BOWIE et al. 1998). Similar to other open-channel blockers, such as local anesthetics at muscle nAChRs



voltage dependences of block observed with Spm ($z\delta$ = 1.48) and Spd ($z\delta$ = 1.58) are similar to that observed in whole-cell recording conditions, while block by Put is weaker ($z\delta$ = 1.24), suggesting that Put does not play a major role (Fig. 2A, inset). The ratio of the free concentrations of Spm to Spd in biochemical estimates from bovine lymphocytes and rat liver cells is 1 :3 (Watanabe et al. 1991). When constrained to this ratio, the free concentrations of Spm and Spd predicted from analysis of the voltage-dependence of whole-cell responses in HEK 293 cells are 40 μM and 120 μM, respectively (Fig. 2B). which is in good agreement with biochemical estimates (Watanabe et al. 1991). As yet, it is not known if the regulation of free-polyamine concentration is a dynamic process; extrusion and uptake mechanisms may be important to consider in this context (Khan et al. 1994). As discussed in Sect. D, however, the nature of polyamine block is clearly dynamic, but this process relies instead on the activation frequency of the receptor.

III. Relief of Block by Polyamine Permeation

A major difference between polyamine block of K_{ir}-channels and non-NMDA receptors is that relief of block is observed at extreme positive membrane potentials in non-NMDA receptors (Fig. 3). An early explanation for relief of block was that, at high membrane electric-field strengths, cytoplasmic polyamines permeate non-NMDA receptors. The relief of channel block is observed experimentally as the appearance of outward current flow (Bowie and Mayer 1995; Koh et al. 1995). The different behavior of GluR and K^+ channels is indirectly supported by estimates of their pore dimensions, generally accepted to be smaller for K^+ channels (3-Å diameter) (Hille 1992) than non-NMDA receptors (7.0–7.8 Å in diameter) (Burnashev et al. 1996).

Direct evidence in support of permeation of polyamines through GluR channels has been obtained by comparing internal block by polyamines of differing cross-sectional diameter and in experiments with polyamines as single-charge carriers (Bähring et al. 1997). In the first case, the possibility that relief of Spm block is due to its permeation was realized by comparison of Spm block with block produced by the synthetic toxin analogues *N*-(4-hydroxyphenylpropanoyl)-spermine (PPS) and philanthotoxin-343 (PhTX), which have larger cross-sectional diameters (7 Å and 7.5 Å, respectively) than Spm

Fig. 2A,B. Spermine (*Spm*) and spermidine (*Spd*) are responsible for rectification observed in intact cells. **A** Conductance–voltage plots for 100 μM Spm (*left*), 100 μM Spd (*middle*) and 1 mM putrescine (Put; *right*) with fits of the Woodhull model of channel block for a non-permeant blocker. Each polyamine produces voltage-dependent block of GluR6(Q) channels, but with different affinities. Extended structures of Spm (*upper*), Spd (*middle*) and Put (*lower*) are shown in the *inset*. **B** Analysis of whole-cell responses, assuming that Spm and Spd are present in the cytoplasm at a ratio of 1:3, show that the rectification observed in intact HEK 293 cells is due to 40 μM Spm and 120 μM Spd. Adapted from Bowie and Mayer (1995) with permission

patches but restored by the inclusion of Spm in the patch pipette (Fig. 1) (Bowie and Mayer 1995; Koh et al. 1995; Kamboj et al. 1995; Donevan and Rogawski 1995; Isa et al. 1995). Spm block is concentration- and voltage-dependent and occurs selectively in M2 (Q) unedited recombinant AMPA and kainate receptors (Bowie and Mayer 1995; Kamboj et al. 1995) and in neurones possessing calcium-permeable AMPA receptors which lack GluR-B

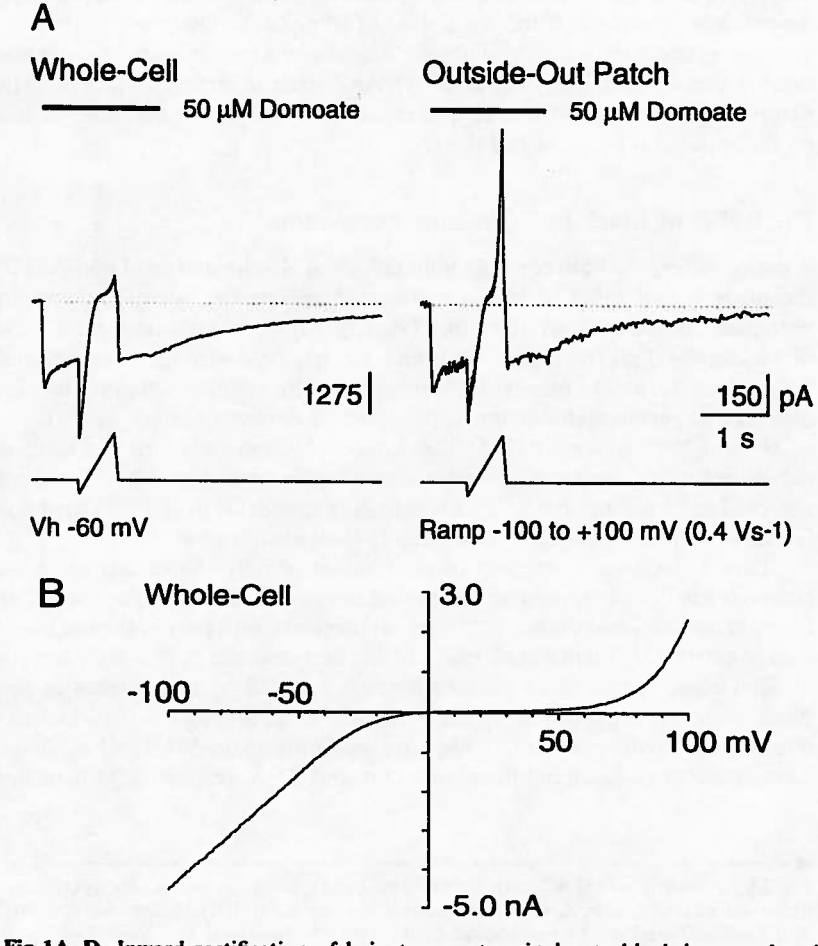


Fig. 1A–D. Inward rectification of kainate receptors is due to block by cytoplasmic polyamines. Strong inward rectification observed under whole-cell (**A**, *left panel*, and **B**) recording conditions is lost in a patch excised from the same HEK 293 cell expressing GluR6(Q) channels (**A**, *right panel*, and **C**). **D** The inclusion of 100 μM spermine to the patch pipette solution restored inward rectification similar to that of whole-cell recordings. Taken from Bowie and Mayer (1995) with permission

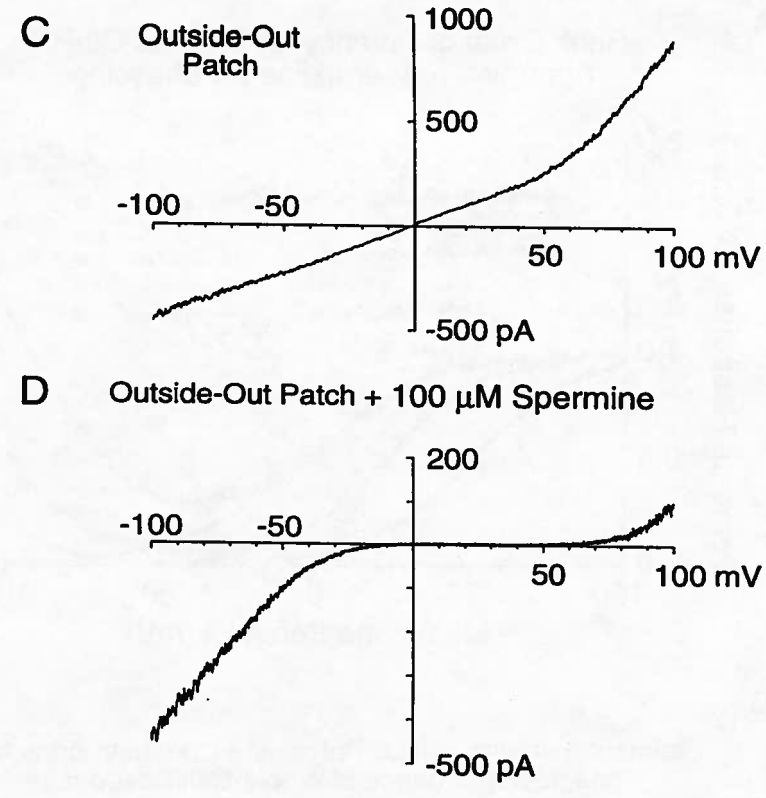


Fig. 1A–D. *Continued*

subunits (Jonas et al. 1994; Koh et al. 1995; Kamboj et al. 1995; Donevan and Rogawski 1995; Isa et al. 1995). Spm is ineffective on M2 (R) edited receptors (Bowie and Mayer 1995) and in neurones expressing calcium-impermeable AMPA receptors that contain the highly edited GluR-B subunit (Jonas et al. 1994; Koh et al. 1995; Kamboj et al. 1995; Donevan and Rogawski 1995). In this latter case, replacement of glutamine residues with positively-charged arginines at the Q/R site is believed to render the electrostatic environment of the pore unfavorable for polyamine block (Dingledine et al. 1992). Other naturally occurring polyamines, such as Spd (K_d at 0 mV = 25 μM) and Put (K_d at 0 mV = 1.2 mM), also cause voltage-dependent block but with a lower affinity than Spm (K_d at 0 mV = 5 μM) (Fig. 2A) (Bowie and Mayer 1995). Knowledge of the affinities of Spm and Spd for AMPA and kainate-receptor channels, combined with biochemical estimates of free cytoplasmic polyamine concentrations (Watanabe et al. 1991), suggests that both Spm and Spd contribute to block observed under whole-cell recording conditions. The

occurring polyamines, spermidine (Spd) and putrescine (Put), were later discovered in ox pancreas and decomposing tissues, respectively (Dudley et al. 1927), and, like Spm, were shown to be ubiquitous cell components that had changed little throughout evolution, each being essential for normal growth of eukaryotes as well as prokaryotes (Tabor and Tabor 1965).

Ornithine decarboxylase, the rate-limiting enzyme for biosynthesis of polyamines, catalyses the formation of Put, which, together with *S*-adenosylmethionine, is the substrate for synthesis of Spd and Spm (Marton and Pegg 1995). Spd and Spm are found at millimolar levels in cells, the majority complexed with nucleic acids, proteins and phospholipids. This association has underscored the traditionally accepted role of polyamines in cell growth and differentiation (Marton and Pegg 1995). More recently, however, rectification of inwardly rectifying potassium (K_{ir}) channels as well as kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and neuronal nicotinic acetylcholine receptors (nAChR; Haghighi and Cooper 1998) has been shown to be due to voltage-dependent blocking by micromolar levels of polyamines in the cytoplasm. These apparently disparate roles for polyamines in cells can be partly accounted for by their positively charged nature at physiological pH, which endows them with a high affinity for a variety of cellular polyanions that exert pronounced effects even at low concentrations. The specificity for interaction with target macromolecules is probably conveyed by the long, rod-shaped polyamine structure that is interspersed with multiple amine and methylene groups that are likely to favor multiple contact points through electrostatic and hydrophobic interactions, respectively (Sugiyama et al. 1996; Cui et al. 1998). In view of their chemical properties, it is not surprising that the electrostatic environment of cation-permeable pores, such as that found in AMPA and kainate-receptor channels, is particularly attractive to polyamine ions, which can enter and block the narrow pathway taken by permeating ions. However, the mechanisms that render AMPA and kainate-receptor channels more sensitive to polyamine block than *N*-methyl-D-aspartate (NMDA) receptors are not yet well understood.

In this review, we will focus on the nature and mechanism of voltage-dependent block of AMPA and kainate receptors by cytoplasmic polyamines. During their evolution, the venoms of spiders and wasps have evolved toxins that capitalize on specific chemical properties of polyamines. Although designed to disarm their prey by binding to peripheral sites, such as glutamate receptors (GluRs) at the neuromuscular junction, polyamine-conjugated toxins have selective effects on L-glutamate (Glu)-activated receptors in the vertebrate central nervous system (CNS). We will discuss the use of arthropod toxins as selective pharmacological tools to study AMPA and kainate receptors and their mechanism of block. Finally, recent studies suggest that cytoplasmic polyamines block both closed and open non-NMDA receptor channels (Bowie et al. 1998; Rozov et al. 1998). We will discuss the possibility that activity-dependent modulation of polyamine block may impart

short-term plasticity on neurones expressing calcium-permeable non-NMDA receptors.

B. Emergence of Polyamines as Ubiquitous Channel Blockers

I. Historical Perspective

Strong inward rectification was first described for K^+ conductance in skeletal muscle (Katz 1949). Sir Bernard Katz described the conductance as an "anomalous" rectifier, the anomaly being that, unlike previously studied K^+ conductances, the membrane conductance decreased with depolarization. Progress in understanding the molecular nature of inward rectification was stimulated by the observation that block by internal tetraethylammonium of delayed rectifier K^+ channels resembled the apparent intrinsic gating behavior of K_{ir}-channels (Armstrong 1969). Although the identity of the blocking molecule(s) was still unknown, Hille and Schwarz (1978) proposed that an impermeant blocker in a permeation model based on Eyring rate theory could adequately account for strong inward rectification, including its strict coupling with external K^+ ions. Taken together, these observations provided a framework for ongoing studies aimed at determining the identity of the blocking molecule.

Rectification of K_{ir} channels and nAChRs was first proposed to be a composite of voltage-dependent block by Mg^{2+} with an as yet unidentified but strongly voltage-dependent gating mechanism (Matsuda et al. 1987; Vandenberg 1987; Silver and DeCoursey 1990; Ifune and Steinbach 1990, 1991, 1992; Mathie et al. 1990; Sands and Barish 1992). Rectification of non-NMDA receptors was also described in early studies (Parker et al. 1986; Randle et al. 1988; Bowie and Smart 1989; Iino et al. 1990; Gilbertson et al. 1991); however, a structural basis for the mechanism emerged only with the identification of an amino acid residue, the Q/R site (Hume et al. 1991; Verdoorn et al. 1991), which, through RNA editing, was shown to control rectification as well as Ca^{2+} permeability (Sommer et al. 1991; Higuchi et al. 1993). Although, a report that rectification was lost in outside-out patches suggested that a diffusible, cytoplasmic factor may account for non-NMDA receptor rectification (Burrill et al. 1993), it was not until cytoplasmic polyamines were shown to block K_{ir} channels in a voltage-dependent manner that a likely candidate was identified (Fakler et al. 1995; Ficker et al. 1994; Lopatin et al. 1994).

II. Linking Inward Rectification to Polyamine Block

Consistent with the idea that rectification of non-NMDA receptors is also due to a freely diffusible modulator in the cytoplasm, the complex rectification observed under whole-cell recording conditions is lost in excised outside-out

CHAPTER 7

Block of AMPA and Kainate Receptors by Polyamines and Arthropod Toxins

D. Bowie, R. Bähring, and M.L. Mayer

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATX	Argiotoxin
Ca^{2+}	Calcium
CNS	Central nervous system
Glu	L-glutamate
GluR	Glutamate receptor
G–V	Conductance–voltage
JSTX	Joro spider toxin
$[K^+]_{ext}$	External potassium concentration
K_{ir} channels	Inwardly rectifying potassium channels
k_{off}	Rate constant of unbinding
k_{on}	Rate constant of binding
k_{perm}	Rate constant of permeation
Mg^{2+}	Magnesium
$[Na^+]_{ext}$	External sodium concentration
nAChR	Neuronal nicotinic acetylcholine receptor
NMDA	*N*-methyl-D-aspartate
PhTX	Philanthotoxin-343
PPS	*N*-(4-hydroxy-phenylpropanoyl)-spermine
Put	Putrescine
Spd	Spermidine
Spm	Spermine
$z\delta$	Charge times electrical distance from the cytoplasmic face of membrane for blocking

A. Introduction

In a letter describing spermatozoa to the Royal Society in 1678, Anthonii Lewenhoeck noted crystal formation in drying semen and, in doing so, rather unwittingly made the earliest formal identification of polyamines (Lewenhoeck 1678). Although others subsequently confirmed his findings, it was not until 1888 that the familiar term "spermine" (Spm) was used to describe these crystals (Ladenburg and Abel 1888). The other naturally