REVIEW ARTICLE

Interactions of polyamines with ion channels

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Endogenous polyamines, in particular spermine, have been found to cause block and modulation of a number of types of ion channel. Intracellular spermine is responsible for intrinsic gating and rectification of strong inward rectifier K^+ channels by directly plugging the ion channel pore. These K^+ channels control the resting membrane potential in both excitable and non-excitable cells, and control the excitability threshold in neurons and muscle cells. Intracellular spermine causes inward rectification at some subtypes of Ca^{2+}-permeable glutamate receptors in the central nervous system, again by plugging the receptor channel pore, and spermine can even permeate the ion channel of these receptors. Extracellular spermine has multiple effects at the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor, including stimulation that increases the size of NMDA receptor currents, and voltage-dependent block. A number of polyamine-conjugated arthropod toxins and synthetic polyamine analogues are potent antagonists of glutamate receptors, and represent new tools with which to study these receptors. Interactions of polyamines with other types of cation channels have been reported. This area of research represents a new biology and a new pharmacology of polyamines.

INTRODUCTION

The polyamines putrescine, spermidine and spermine (Figure 1) are present in almost all cells and have important roles in protein synthesis, cell division and cell growth. Much is known about the biosynthesis and degradation of polyamines and about the molecular properties of some of the enzymes involved in these processes. These areas of polyamine biology have been extensively reviewed [1–5]. Putrescine is synthesized from L-ornithine in a reaction catalysed by ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis. Putrescine and decarboxylated *S*-adenosylmethionine are substrates for the synthesis of spermidine, which is a precursor of spermine. Polyamines are organic polycations that are protonated at physiological pH and can potentially interact with a variety of cellular targets including nucleic acids and proteins. In recent years, specific interactions of polyamines with a number of different types of ion channels have been reported. These interactions include the block of some types of K^+ channels and glutamate receptors by intracellular polyamines, and the modulation of other types of glutamate receptors by extracellular polyamines. Some of these effects undoubtedly have considerable physiological significance, whereas others remain experimental curiosities that, nonetheless, are providing new and useful information about the properties and regulation of receptors and ion channels.

BLOCK OF INWARD RECTIFIER K^+ CHANNELS (KIR CHANNELS)

Different forms of K^+-selective ion channels have been identified, based on their biophysical, pharmacological and molecular properties [6]. These include Kir channels [6,7]. The term 'inward rectifier' refers to the ability of the channel to conduct ions in the inward direction at negative membrane potentials, but to show a greatly decreased outward conductance at membrane potentials positive to the potassium equilibrium potential (E_k; shown schematically in Figure 2c). Kir channels, which are present in both excitable and non-excitable cells, are crucial for maintaining the resting membrane potential close to E_k [6]. In excitable cells such as neurons and muscle cells, Kir channels are important for controlling excitability thresholds and the shape of the action potential. Different types of Kir channels have been identified, including strong and mild rectifiers. The strong rectifiers gate little or no outward current (solid line in Figure 2c), whereas mild rectifiers gate some outward current. The genes encoding a number of subunits of Kir channels have been cloned, and these include strong rectifiers such as Kir2.1 (also called IRK1), Kir3.1 (GIRK1) and Kir2.3 (HRK1), and mild rectifiers such as Kir1.1a (ROMK1) [7].

Part of the mechanism of inward rectification of Kir channels involves block of the ion channel by intracellular Mg^{2+} [8]. However, block by Mg^{2+} cannot account for all of the gating and rectification properties of Kir channels, and may be only a minor component of rectification in strong Kir channels. Mg^{2+} causes an almost instantaneous block of Kir channels, but there is another component of rectification, termed 'intrinsic gating', which is a major contributor to rectification in some channels [6,9]. Intrinsic gating is characterized by a slow (50–100 ms) decrease in current at depolarized membrane potentials.

The characteristic *I–V* relationship seen with strong Kir channels (solid line in Figure 2c) has been observed at recombinant Kir2.1 and Kir2.3 channels expressed in *Xenopus* oocytes when the channels are studied by intracellular microelectrode recording or by 'on-cell' patch-clamp recording. In these experimental paradigms the cell membrane remains intact and the intracellular constituents are largely undisturbed. When Kir channels are studied in small patches of membrane (inside-out patches) that are pulled off oocytes, inward rectification is lost and the shape of the *I–V* relationship changes (broken line in Figure 2c) [10–13]. In this experimental paradigm the cytoplasmic face of the cell membrane is no longer exposed to the intracellular constituents of the oocyte, suggesting that a cytoplasmic factor is responsible for the rectification of Kir channels and that this factor is lost when the membrane patch is removed from the cell.

The intracellular factors that are responsible for intrinsic gating and rectification at strong Kir channels are polyamines, in particular spermine and spermidine [10–14]. Exogenous poly-

Abbreviations used: Kir channel, inward rectifier K^+ channel; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

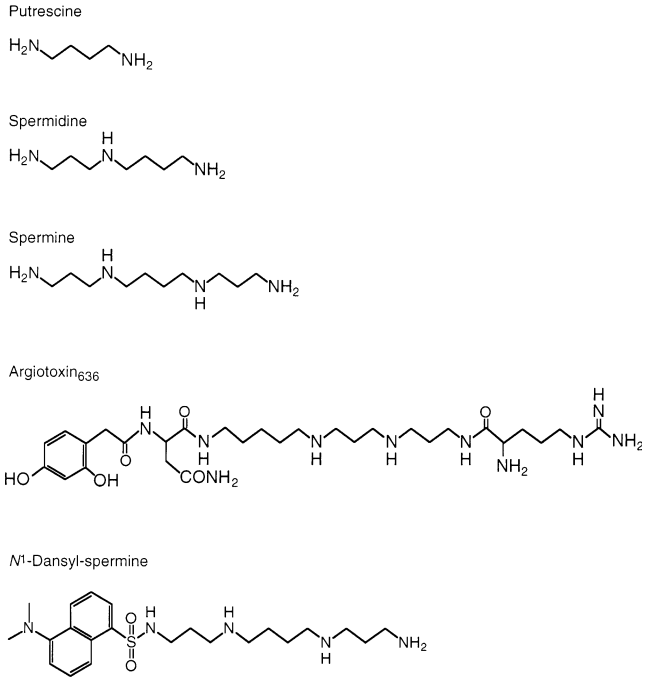

Figure 1 Structures of the endogenous polyamines, the spider toxin argiotoxin$_{636}$ and N^1-dansylspermine

amines, when applied to the intracellular side of patches, can mimic the effects of intracellular medium on these channels. Concentrations of spermine as low as 10–100 nM cause substantial block of Kir2.1 channels, and concentrations of polyamines in oocytes (200–800 μM) are more than sufficient to account for the effects observed after the removal of membrane patches [10,11]. Rectification of Kir channels has been characterized by the expression of recombinant channels in mammalian cells, as well as in amphibian oocytes. Similar to the results seen with oocytes, spermine caused rectification and intrinsic gating of Kir2.1 channels expressed in mouse fibroblast L cells [15] and of Kir2.2 channels expressed in HEK 293T cells [16]. In addition to recombinant Kir channels, effects of spermine have been seen at native muscarinic acetylcholine-gated Kir channels on atrial myocytes [17]. These channels are opened by acetylcholine and contribute to the slowing of the heart rate during vagal stimulation. The channels show steep inward rectification that is lost in excised patches and is mimicked by intracellular spermine, similar to the effects seen at recombinant Kir channels [17]. The weak rectifiers such as Kir1.1a are relatively insensitive to block by spermine compared with strong rectifiers such as Kir2.1 and Kir2.2 (Figure 2b), again consistent with the proposal that endogenous spermine is normally responsible for the rectification of Kir channels.

The affinity of Kir channels for polyamines and the degree of voltage-sensitivity increases with increasing charge on the polyamine. Spermine (a tetra-amine) is more potent than spermidine (a triamine) and considerably more potent than putrescine (a diamine). Polyamines are long, flexible molecules that could enter the channel pore in an extended linear conformation or in a folded conformation. Lopatin et al. [14] have proposed a model in which spermine, in a linear conformation, can enter deep within the ion-channel pore to interact at sites within the membrane-spanning region of the protein. To best account for the experimental results, the model requires that two molecules of spermine block the Kir channel sequentially, i.e. two molecules of spermine line up end-to-end to plug the channel pore and prevent K^+ ions moving through the pore (Figure 2a).

Although there is good evidence that spermine is responsible for intrinsic gating of Kir channels [10–12,14], there may be another component of intrinsic gating that is not mediated by polyamine block [18]. This component of intrinsic gating is sensitive to intracellular pH, whereas block by spermine or Mg^{2+} is not [18]. The physiological significance of this component of gating is not known, but it may affect channel activity during marked changes in pH, for example following ischaemia [18]. Another study, using Kir2.1 channels reconstituted in lipid bilayers, has suggested that intrinsic gating can occur in the absence of spermine (or Mg^{2+}) and that spermine and Mg^{2+} act to amplify this gating process [19]. In that study it was proposed that intrinsic gating is indeed an intrinsic, voltage-dependent property of the channel protein and that the speed of the gating process is affected by the concentrations of free Mg^{2+} and spermine at the cytoplasmic face of the Kir channel [19]. This contrasts with the model in which spermine can enter and plug the channel [14]. In the absence of structural data that unequivocally show spermine bound to a Kir channel, and the location of such a binding site, both models of intrinsic gating should be considered and investigated further.

Exogenous polyamines can faithfully restore the rectification characteristics of Kir channels in excised membrane patches [10]. These and similar results suggest that endogenous polyamines are normally responsible for rectification at Kir channels (Figure 2a). Further evidence for this has come from studies of Kir channels in cells in which the polyamine content was altered pharmacologically [20,21]. Importantly, these studies also indicate that changes in cellular polyamine content can indeed alter the activity of Kir channels and the resting membrane potential. In one study, recombinant Kir2.1 channels were studied in oocytes injected with the polyamine biosynthesis inhibitors α-difluoromethylornithine and methylglyoxal bis-(guanylhydrazone) [21]. In another study, native Kir channels were studied on rat basophil leukaemia (RBL-1) cells after treatment with the *S*-adenosylmethionine decarboxylase inhibitor MDL73811, which inhibits polyamine biosynthesis [20]. In both cases, there was a decrease in rectification and an increase in the outward current through Kir channels, correlating with a decrease in intracellular spermine and spermidine levels, after treatment with inhibitors of polyamine synthesis [20,21]. Another ingenious approach that was used to determine the influence of endogenous polyamines on Kir channels was to study the channels in an ornithine decarboxylase-deficient cell line, O-CHO [21]. Due to a lack of ornithine decarboxylase, these cells require exogenous putrescine to synthesize spermidine and spermine to maintain normal growth. Strong rectifier Kir2.3 channels were transfected into O-CHO cells and studied after withdrawal of putrescine, which leads to a decrease in the cellular polyamine content [21]. Similar to the results of pharmacological manipulations of polyamine levels, withdrawal of putrescine from O-CHO cells led to a decrease in rectification of Kir channels, affecting both the voltage-dependence and the kinetics of channel activity, as would be predicted from a change in intracellular spermine levels [21].

Results from site-directed mutagenesis studies have provided information about the site of interaction of polyamines in Kir channels. The strong inward rectifiers Kir2.1 and Kir2.3, which are highly sensitive to spermine, contain an aspartate residue in the M2 segment (Figure 3a). The mild inward rectifier Kir1.1a, which is insensitive to spermine, contains a neutral asparagine at the equivalent position. Mutation of the aspartate in Kir2.1 or

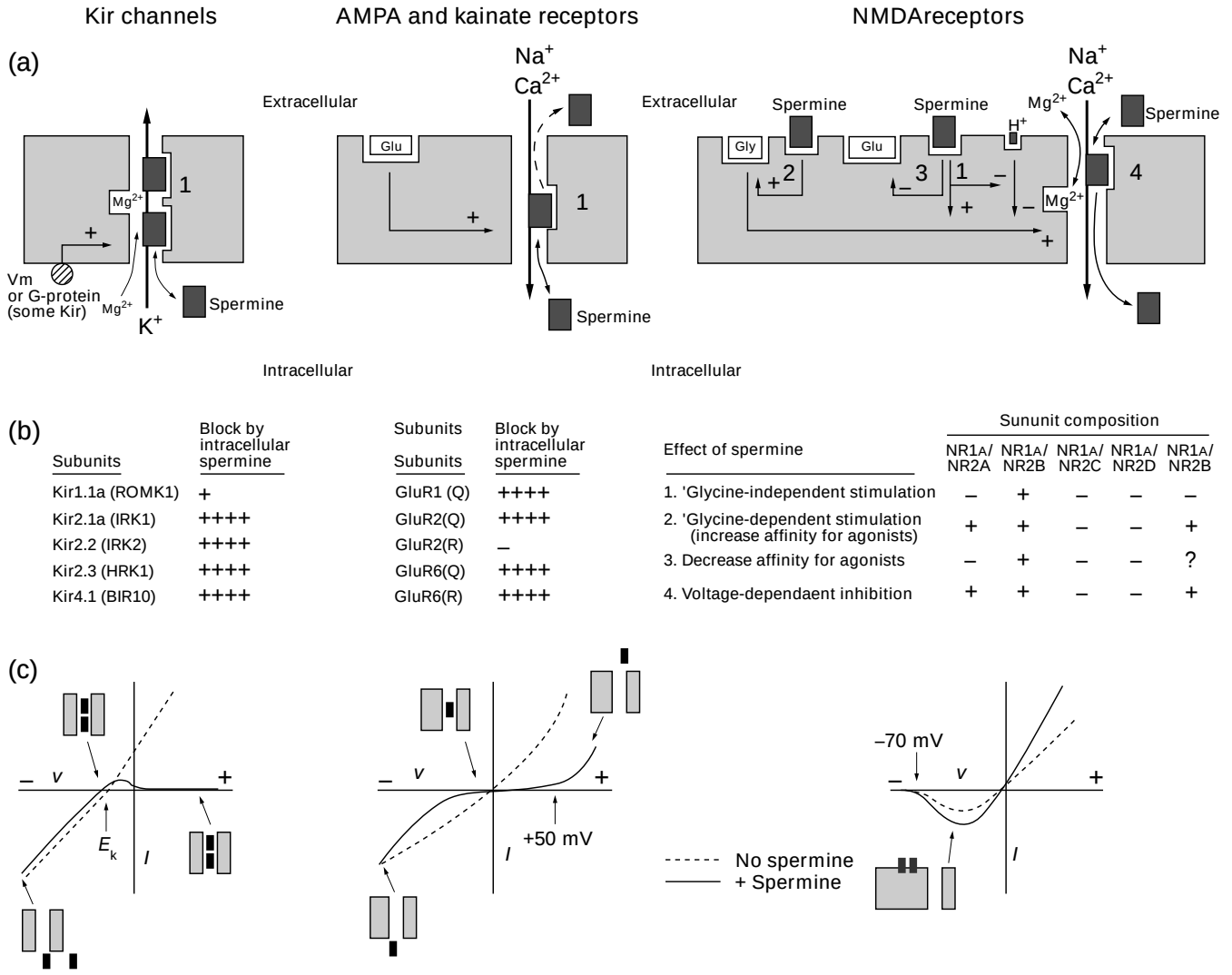

Figure 2 Modulation and block of ion channels by polyamines

(**a**) Schematic models to illustrate the block of Kir channels and AMPA/kainate receptors by intracellular spermine and the modulation and block of NMDA receptors by extracellular spermine. Large grey rectangles represent the ion channel proteins, with the channel pore that gates K^+, Na^+ or Ca^{2+} in the centre. Dark rectangles represent spermine. Outward currents through Kir channels are carried by K^+, whereas inward currents through glutamate receptors are carried by Na^+ and (in some cases) Ca^{2+}. Glutamate receptors also gate K^+, and outward currents through glutamate receptors are carried by K^+ moving from the intracellular side to the extracellular side (not illustrated). In Kir channels, two molecules of spermine may block the channel simultaneously. AMPA and kainate receptors are blocked by intracellular spermine at depolarized potentials and, as the cell is hyperpolarized, spermine unbinds and returns to the cytoplasm (solid arrow). At extreme depolarized potentials (more positive than +50 mV), spermine can permeate the ion channel of AMPA receptors and pass through to the extracellular side (broken arrow). Similarly, extracellular spermine can block NMDA channels at negative membrane potentials (solid arrow), and at extreme negative potentials spermine can permeate the NMDA channel to pass into the cell (broken arrow). (**b**) Effects of spermine at Kir channels and glutamate receptors are dependent on subunit composition. At NMDA receptors, spermine has four macroscopic effects that are differentially controlled by NR2 subunits. (**c**) Schematized current–voltage (I–V) plots for currents through Kir channels and glutamate receptors. Kir channels on intact cells show inward rectification (solid line) because of block by cytoplasmic spermine. This block develops as V becomes more positive, and is relieved as V becomes negative. Similarly, AMPA receptors show inward rectification due to block by intracellular spermine (solid line), but if V is made sufficiently positive spermine can permeate the AMPA channel and ion flux is restored. In patches pulled off cells (i.e. in the absence of cytoplasmic spermine), rectification at Kir channels and AMPA channels is lost (broken lines), but can be restored by the application of spermine. At NMDA receptors the I–V curve is U-shaped in the presence of extracellular Mg^{2+} (broken line), and extracellular spermine potentiates NMDA responses (solid line). Grey rectangles represent the ion channel proteins, and dark rectangles represent spermine.

Kir2.3 to asparagine decreases the spermine sensitivity, whereas mutation of the asparagine in Kir1.1a to aspartate increases the sensitivity to spermine [10,11,22]. Another strong rectifier, Kir4.1, contains a glutamate residue in the M2 region in a position analogous to the aspartate in Kir2.1. Mutation of this glutamate to asparagine decreases the polyamine block at Kir4.1 channels [13]. Thus an acidic residue in the M2 region of strong Kir channels controls the spermine block, and probably contributes directly to the spermine binding site.

In the strong rectifier Kir2.1, a glutamate residue (Glu-224) in the intracellular C-terminal domain (Figure 3a) has also been found to influence the block by spermine [22,23]. The weak rectifier Kir1.1a contains a neutral glycine residue at the equivalent position. Mutation of Glu-224 in Kir2.1 to a neutral amino acid greatly decreased the spermine block, and the effect of this mutant was additive with that of mutations at the aspartate residue (Asp-172) in the M2 region [22,23]. Thus Glu-224 and Asp-172 (Figure 3a) control spermine sensitivity in-

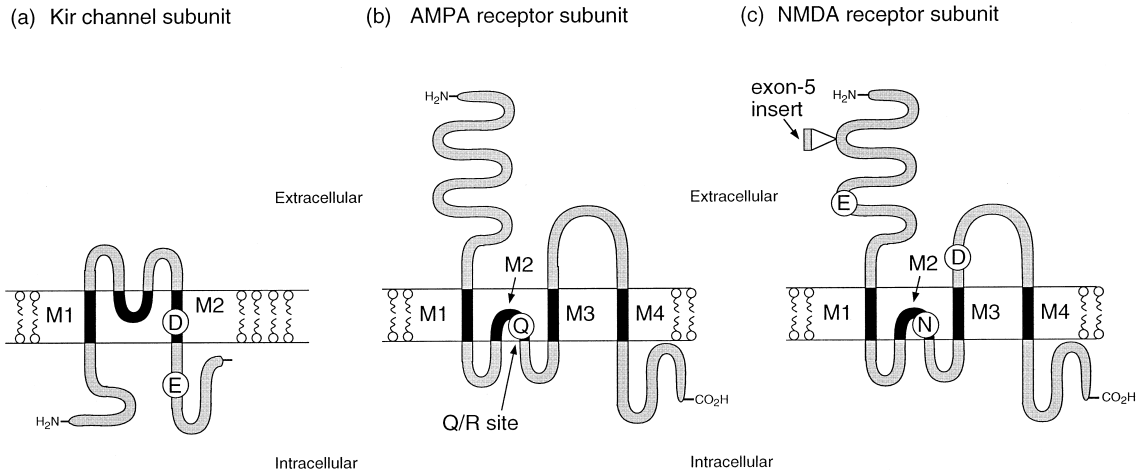

Figure 3 Structural topology of Kir channel and glutamate receptor subunits

Transmembrane or membrane re-entrant segments are shown in black, and extracellular and intracellular regions of the proteins are shaded. Open circles show the positions of Asp (D) and Glu (E) residues that affect the spermine block of Kir channels, the Q/R (Gln/Arg) site in AMPA/kainate receptors and the equivalent Asn (N) site in NMDA receptors, and Glu and Asp residues (Glu-342 and Asp-669) that affect stimulation by spermine in NMDA receptors.

dependently, and may contribute individually to a common polyamine binding site in Kir channels, each acidic residue interacting with an amino or imino group of spermine. The spermine and Mg^{2+} binding sites in Kir channels may be overlapping, and share at least some common residues on the channel protein [10,11,16,22,24].

The physiological significance of the polyamine block of Kir channels is obvious: Kir channels, and thus intracellular polyamines, control the resting membrane potential and the excitability threshold for the initiation of action potentials. An increase in the concentration of intracellular polyamines will tend to increase the rectification of Kir channels and hence increase cellular excitability. Because Kir channels have a high affinity for spermine at depolarized potentials, and polyamines are present at high micromolar concentrations in cells, changes in polyamine levels will have the most marked effects on Kir channels around the resting membrane potential or at hyperpolarized potentials where the affinity for polyamines is decreased. Alterations in polyamine levels in dividing cells or in tumour cells could have effects on the membrane potential through interactions with Kir channels. The magnitude of these effects will depend on the relative levels of spermine, spermidine and putrescine, and on the types of Kir channels (strong or mild) present in a particular cell. It is not known whether changes in intracellular polyamine levels are involved in the aetiology of any pathologies, but increases in the cellular polyamine content have been reported in models of seizure activity and in myocardial hypertrophy [25]. It is conceivable that increased levels of polyamines could contribute to over-excitability in neurons and cardiac myocytes in these pathologies by increasing the block of Kir channels.

BLOCK OF NON-*N*-METHYL-D-ASPARTATE (NON-NMDA) GLUTAMATE RECEPTORS

Glutamate receptors are classified on the basis of agonists that selectively activate them, and include the NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors [26], with the last two classes sometimes referred to as 'non-NMDA receptors'. Glutamate receptors mediate fast excitatory transmission at synapses in the brain and spinal cord. AMPA and kainate receptors mediate much of the fast 'point-to-point' signalling in the nervous system, and these receptors are also involved in some forms of synaptic plasticity and in the induction of neuronal cell death.

Most AMPA and kainate receptors gate Na^+, but are relatively impermeable to Ca^{2+}, and have *I–V* relationships that are close to linear. However, a subset of these receptors has high Ca^{2+} permeability and pronounced inward rectification [27–30]. A large number of subunits of these receptors have been cloned. The topography of glutamate receptor subunits has been proposed to involve an extracellular N-terminal domain, three membrane-spanning domains (M1, M3 and M4) and a re-entrant loop (M2) that contributes to the ion channel pore [31–34] (Figures 3b and 4). AMPA receptor subunits include GluR1–GluR4, and kainate receptor subunits include GluR5–GluR7 and KA-1 and KA-2 [35]. The diversity of GluR subunits is extended by alternative splicing of their mRNAs and by editing of the pre-mRNA [35,36]. One of the most dramatic effects involves RNA editing at a so-called 'glutamine/arginine (Q/R) site' of the GluR2 (AMPA subunit) and GluR6 (kainate subunit) mRNAs. The Q/R site is in the pore-forming (M2) region (Figure 3b) and controls the Ca^{2+} permeability of AMPA and kainate receptors [35–37]. The edited GluR2 subunit, which represents $> 99\,\%$ of native GluR2 subunits, contains an arginine residue at the Q/R site, which makes the channels impermeable to Ca^{2+}. Thus almost all native AMPA receptors that contain GluR2 do not gate Ca^{2+}. Other subunits, such as GluR1, GluR3 and GluR4, contain a glutamine residue at the Q/R site and do gate Ca^{2+}. The Q/R site also controls rectification of AMPA and kainate receptors. Receptors that contain the edited GluR2(R) or GluR6(R) subunits have a nearly linear *I–V* relationship or show outward rectification (similar to the broken line in Figure 2c), whereas receptors that contain the unedited GluR2(Q) or GluR6(Q) subunits show a very pronounced inward rectification (solid line in Figure 2c) [30,37,38]. Thus the Ca^{2+}-permeable AMPA and kainate receptors exhibit inward rectification. In some cases the receptor/channels show a double rectification,

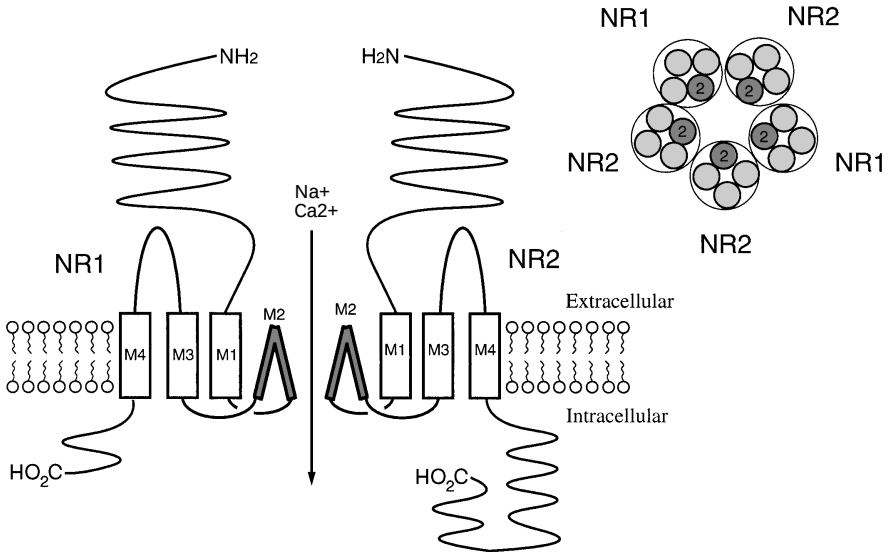

Figure 4 Hypothetical structure of NMDA receptors

NMDA receptor subunits are suggested to have a large extracellular N-terminal domain, three membrane-spanning domains (M1, M3 and M4) and a re-entrant loop (M2) that forms part of the channel pore. A similar topology was first proposed for subunits of AMPA and kainate receptors and for kainate binding proteins [31–34]. NMDA receptors (and AMPA/kainate receptors) may be pentamers, with the M2 region from each of five subunits contributing to the channel pore. Portions of the M1, M3 and M4 domains may also form part of the ion channel (not illustrated). NMDA receptors may each contain two copies of the NR1 subunit together with various combinations of one, two or more different types of NR2 subunit.

passing inward current at negative membrane potentials, a very low or zero conductance between about -10 mV and $+50$ mV, and an outward conductance at potentials more positive than $+50$ mV [30,39] (illustrated schematically in Figure 2c).

Similar to the rectification of Kir channels, inward rectification of Ca^{2+}-permeable AMPA receptors is lost in membrane patches removed from a cell, and rectification is also lost over time in neurons studied in the whole-cell patch-clamp mode [40–44], an experimental paradigm in which the intracellular contents become 'dialysed' by the contents of the patch electrode (a modified saline solution). These results suggest that, as with K^+ channels, a soluble intracellular factor is responsible for the rectification of Ca^{2+}-permeable AMPA and kainate receptors.

The intracellular factors that cause rectification of AMPA and kainate receptors are polyamines, which probably act by blocking the pore of the receptor channel to prevent the flux of Na^+ and Ca^{2+} as the membrane is depolarized (Figure 2) [40–44]. The evidence for this comes from studies of native glutamate receptors expressed on neurons and of recombinant receptors expressed in amphibian and mammalian cells. Native AMPA receptors on Bergmann glial cells and on type II hippocampal neurons are Ca^{2+}-permeable and have a double rectification that is lost when membrane patches are pulled off the cells. Rectification can be completely restored by exposing the intracellular surface of the patch to spermine or by using recording techniques that do not allow dialysis of the cell contents during whole-cell recording [40,42,43]. Effects of spermine have also been seen at native AMPA receptors on dentate gyrus basket cells and on cerebellar granule cells [40–43]. In these two types of cell, the AMPA receptors show only moderate rectification, probably due to the existence of mixed populations of spermine-sensitive and spermine-insensitive AMPA channels.

In studies of recombinant AMPA and kainate receptors, it was found that receptors containing GluR2(Q) or GluR6(Q) showed a pronounced double rectification, and that rectification was due to block by intracellular polyamines [41,44]. Maximal effects of intracellular spermine were seen at concentrations of 1–10 μM. Kainate receptors expressed from GluR6(R) did not show rectification, and spermine had no effect on conductance through GluR6(R) channels [44]. Results of studies on recombinant AMPA and kainate receptors are in accordance with studies of native receptors: spermine causes rectification of Ca^{2+}-permeable receptors that either lack GluR2(R) and GluR6(R) or contain the unedited (Q) forms of these subunits (Figure 2b). A notable feature of the conductance through Ca^{2+}-permeable AMPA and kainate receptors is the double rectification, i.e. the conductance seen at negative membrane potentials is greatly decreased or abolished as the membrane potential is made more positive, but if the membrane is made sufficiently positive ($> +50$ mV) the response begins to recover (illustrated in Figure 2c). Polyamines are responsible for both components of the double rectification [44]. The simplest interpretation of the recovery at very positive membrane potentials is that spermine can actually permeate the ion channel of the receptor under these conditions. Thus, when the electrical driving force is large enough, spermine that has entered the channel pore from the inside can pass right through the channel to the outside, and ion flux is restored (Figure 2). Polyamines can also permeate NMDA channels [45,46], but there is no evidence to suggest that spermine can permeate Kir channels [47].

AMPA and kainate receptors that show inward rectification, controlled by intracellular polyamines, represent a subset of native receptors that are Ca^{2+}-permeable and are expressed on particular types of neurons and glial cells. Calcium entering through these channels may be involved in synaptic plasticity and in neurotoxicity, similar to effects described for NMDA receptors [48]. Changes in intracellular polyamine levels could alter Ca^{2+} flux and the excitability threshold at synapses containing polyamine-sensitive AMPA receptors. A decrease in the concentration of polyamines would reduce the block of AMPA/kainate channels, increase cell excitability and increase Ca^{2+} influx. An increase in the intracellular polyamine concentration

would have the opposite effect. Because AMPA receptors have a somewhat lower affinity for polyamines than do Kir channels, fluctuations of polyamine concentrations in the micromolar range may have a more marked effect on the activity of AMPA/kainate receptors than on strong Kir channels.

MODULATION AND BLOCK OF NMDA RECEPTORS

Activation of NMDA receptors is associated with the induction of various forms of synaptic plasticity, including some forms of long-term potentiation and long-term depression, processes that may underlie learning and memory [26,49]. Excessive or prolonged activation of glutamate receptors, in particular NMDA receptors, leads to neurodegeneration [50,51]. NMDA receptors have a number of characteristics that distinguish them from other glutamate receptors. Activation of NMDA receptors requires binding not only of glutamate but also of a co-agonist, glycine, at a separate site on the receptor (Figure 2a). NMDA channels are blocked in a voltage-dependent manner by extracellular Mg^{2+}, leading to a decreased conductance at negative potentials, with little or no current flow below about -70 mV (Figure 2c) [26,49]. Two families of NMDA receptor subunits, termed NR1 and NR2, have been cloned. The NR1 subunit is the product of a single gene that is transcribed as eight alternatively spliced mRNAs. The NR2 subunits, NR2A–NR2D, are distinct gene products with different regional and temporal patterns of expression [35]. Native NMDA receptors are probably hetero-oligomers composed of combinations of NR1 and NR2 subunits (Figure 4). The number of subunits in each heteromer is still unknown, but NMDA receptors, and AMPA/kainate receptors, may each contain five subunits [52] (Figure 4). NMDA receptors may contain two copies of the NR1 subunit in each receptor complex [53], and at least some NMDA receptors contain two different types of NR2 subunit (e.g. NR2A and NR2B) within a single hetero-oligomer [54–56]. Most studies of recombinant NMDA receptors have focused on 'binary' receptors containing NR1 and one type of NR2 subunit (e.g. NR1/NR2B) (Figure 2b).

Effects of polyamines on NMDA receptors were first reported in 1988 [57], and much of the early work in this area has been reviewed elsewhere [58–61]. Before the cloning of NMDA receptor subunits [62], a number of laboratories studied the effects of extracellular spermine on NMDA-induced whole-cell currents in cultured neurons. Stimulatory effects of spermine were described, although the degree of potentiation by spermine varied widely between individual neurons, with some neurons showing up to 200 % enhancement of whole-cell currents and some showing no enhancement. This suggested that NMDA receptors expressed on different neurons were differentially sensitive to spermine [45,63–67]. It is likely that this variability is due, in part, to the existence of different molecular forms of the NMDA receptor and, in part, to the multiple effects of spermine that have subsequently been uncovered (Figure 2a). In addition to stimulation, voltage-dependent inhibition by extracellular spermine was seen at native NMDA receptors (in the absence of extracellular Mg^{2+}), possibly representing a direct block of the ion channel by spermine [45,64,65,67].

The multiple effects of spermine on NMDA receptors are summarized in Figure 2. Spermine potentiates NMDA currents in the presence of saturating concentrations of glycine (glycine-independent stimulation), an effect that involves an increase in the frequency of channel opening and a decrease in the desensitization of NMDA receptors [45,63,64]. A second effect involves an increase in the affinity of NMDA receptors for glycine (glycine-dependent stimulation) [45,68]. A third effect of spermine, a decrease in affinity for glutamate, has been observed at some recombinant NMDA receptors [69]. The mechanism underlying this effect is not known, but it may reflect an increased rate of dissociation of glutamate from the receptor in the presence of spermine. In this case, spermine could have a marked effect on the time course of NMDA responses at the synapse, where the duration of the response is dependent on the rate of unbinding of glutamate from the receptors.

Inhibition by extracellular spermine (mechanism 4 in Figure 2a) is strongly voltage-dependent and may be due to a fast open-channel block, similar to that by Mg^{2+}. This is conceptually similar to the block of Kir channels and AMPA channels by intracellular polyamines, except that, in the case of NMDA receptors, the channels are blocked by extracellular spermine, although the block is very weak compared with the block of Kir and AMPA channels by intracellular spermine. When applied extracellularly, spermine is also a weak antagonist of Ca^{2+}-permeable AMPA receptors [43,70]. There is no evidence to suggest that intracellular polyamines block NMDA channels, although spermine, applied extracellularly, produces an incomplete block that probably reflects permeation of spermine through NMDA channels (Figure 2a) [45,46]. In addition to a direct channel-blocking effect, spermine may also interact with negatively charged residues on the NMDA receptor, possibly close to the channel mouth, to impede ion flow and thus reduce currents through NMDA channels [64,65]. Voltage-dependent block by extracellular spermine is likely to be negligible under physiological conditions, because the block is weak and develops much more slowly than the block caused by physiological concentrations of extracellular Mg^{2+}. Thus a net stimulatory effect of spermine is shown in Figure 2(c). In addition to its channel-blocking effect, Mg^{2+} can act at an extracellular site to potentiate NMDA receptor activity, similar to spermine [71]. Stimulation by spermine is seen in the presence of 1 mM extracellular Mg^{2+} [72], but not with high (10 mM) concentrations of Mg^{2+} [71]. Mg^{2+} may act at the same stimulatory site as spermine on NMDA receptors, suggesting that spermine may have only modest stimulatory effects in the presence of physiological concentrations of Mg^{2+} [71].

Stimulation by spermine is dependent on the type of NR1 splice variant and the type of NR2 subunit present in NMDA receptors [69,72–76]. Only receptors expressed from splice variants, such as NR1A, that lack a 21-amino-acid insert encoded by exon 5 show glycine-independent stimulation by spermine [73,74]. Furthermore, the various effects of spermine are differentially manifested in heteromeric NR1A/NR2 receptors containing different NR2 subunits [69,72,76] (Figure 2). NMDA receptors are inhibited by protons, with a tonic inhibition of about 50 % at physiological pH (7.3–7.5) [77,78]. Sensitivity to protons, like sensitivity to stimulation by spermine, is influenced by the presence of exon 5 in NR1. Variants that contain the insert (such as NR1B) are less sensitive to protons than are variants that lack the insert [79]. The NR2 subunits, however, control pH sensitivity with a profile that is different from that of spermine sensitivity: only NR2C reduces proton sensitivity of NR1/NR2 receptors [79]. Thus different regions of the NR2 subunits are presumably involved in modulating responsiveness to spermine and to protons. The glycine-independent form of spermine stimulation is influenced by extracellular pH. At NR1A/NR2B receptors, spermine produces a larger fold increase in macroscopic currents at acidic pH than at alkaline pH [79]. Because there is a greater inhibition of NMDA receptors by protons at acidic pH, part of the mechanism of action of spermine may involve relief of tonic proton inhibition (Figure 2a) [79]. The insert encoded by exon 5, which abolishes stimu-

lation by spermine and decreases proton sensitivity, could function to shield the proton sensor or could act as a 'spermine-like' constitutive modulator of NMDA receptors [79].

Results of studies using site-directed mutagenesis, designed to look for residues that may contribute to polyamine binding sites on the NMDA receptor, identified residues that influence both stimulation by spermine and inhibition by protons. Mutations at Glu-342, located in the extracellular N-terminus, or at Asp-669, located in the extracellular loop between regions M3 and M4 of NR1 (Figure 3c), decreased proton inhibition and spermine stimulation [80,81]. Because the change in spermine sensitivity appears to be largely secondary to a change in the proton sensitivity of Glu-342 and Asp-669 mutants, these residues may not contribute directly to a spermine binding site, but may form part of a proton sensor on NR1 or may couple proton and spermine binding to channel gating [80,81].

Differences in sensitivity to voltage-dependent block by spermine at NR1/NR2 receptors are reminiscent of differences in sensitivity to Mg^{2+}. Thus voltage-dependent block by spermine and Mg^{2+} is weak or absent in receptors containing NR2C or NR2D compared with receptors containing NR2A or NR2B (Figure 2b). Sensitivity to Mg^{2+} is affected by an asparagine residue that is present in the pore-forming (M2) loop of NMDA receptor subunits (Figure 3c). This asparagine residue is in a position equivalent to the Q/R site that controls the Ca^{2+} permeability and spermine block of AMPA receptors (Figure 3b). In NMDA receptors, the asparagine residue (Asp-616) in the NR1 subunit has also been found to influence the voltage-dependent block by spermine [82], by the polyamine-conjugated spider toxin argiotoxin$_{636}$ (Figure 1) [83] and by the novel polyamine antagonist N^1-dansylspermine (Figure 1) [82].

If polyamines have modulatory effects on NMDA receptors *in vivo*, this would involve extracellular polyamines. Spermine and spermidine are present in high concentrations in the nervous system, and uptake and depolarization-induced release of polyamines from brain slices has been reported [84–86]. Thus it is possible that polyamines are released from neurons or glia in the brain and can reach concentrations in the synaptic cleft that are sufficient to influence the activation of NMDA receptors, although there is as yet no direct evidence that this happens *in vivo*. The NR2B subunit, which forms receptors that are sensitive to stimulation by spermine, is the predominant NR2 subunit in embryonic and neonatal brain. Therefore NMDA receptors in embryonic and neonatal brain may be particularly sensitive to the stimulatory effects of polyamines. This is the developmental period of cell proliferation and migration in the central nervous system, and a period when levels of polyamines and the activity of ornithine decarboxylase are higher than in adult brain. It is possible that polyamines acting on NMDA receptors could influence neuronal growth, migration and synaptogenesis during development. Excessive activation of NMDA receptors leads to neurodegeneration, and it is conceivable that excessive release of polyamines, for example from injured cells, could exacerbate neuronal injury by potentiating the activity of NMDA receptors.

SPIDER TOXINS AND OTHER POLYAMINE DERIVATIVES: NOVEL TOOLS TO STUDY ION CHANNELS

An area of investigation that has developed concomitant with studies of the effects of natural polyamines on ion channels concerns the properties of polyamine-conjugated toxins, found in spider and wasp venoms, that also block ion channels. A large number of these toxins, which include argiotoxin$_{636}$ (Figure 1), have been described. They are characterized structurally by a polyamine-like tail (often a penta-amine or larger) coupled to an aromatic amino acid head group. There is a large and growing literature on the polyamine-conjugated spider toxins, which is reviewed elsewhere [87–89]. The toxins are potent antagonists of some invertebrate and vertebrate glutamate receptors. Some of the toxins, including argiotoxin$_{636}$, the α-agatoxins Agel-489 and Agel-505, and the philanthotoxins such as philanthotoxin-343, are potent blockers of AMPA and/or NMDA receptors, and in some cases they show selectivity for particular receptor types [70,83,90–95]. Block by these toxins is strongly voltage-dependent, and the toxins probably act as open-channel blockers, binding to sites deep within the ion-channel pore to plug the pore and prevent ion flux.

Because of their novel structures and their potent activities at some glutamate receptors, polyamine-conjugated toxins such as argiotoxin$_{636}$ are useful tools with which to study glutamate-receptor ion channels. However, many of these toxins are unstable and, furthermore, they have to be purified from venom or synthesized by very laborious strategies in the laboratory. A number of other N-substituted polyamines have been examined as potential tools in the study of glutamate channels. Among the most promising compounds are *N*-sulphonylpolyamines such as N^1-dansylspermine (Figure 1), a stable compound whose synthesis is quite straightforward. N^1-Dansylspermine is several-thousand-fold more potent than spermine as a blocker of NMDA channels, and has been used to study block and permeation of recombinant NMDA receptors [82]. It is likely that similar tools, based on polyamine molecules, can be developed with specificities for the various glutamate receptor types.

OTHER ION CHANNELS

Modulation or block by polyamines and polyamine-derived arthropod toxins has been seen at several other types of cation channel. Polyamines may permeate non-selective cation channels in tonoplast membranes of *Arabidopsis thaliana* [96]. High concentrations of spermine (1–5 mM) reduce currents through voltage-gated K^+ channels and through Ca^{2+}-activated K^+ channels in *Aplysia californica* neurons and in pituitary tumour cells [97,98], and at the ryanodine-receptor Ca^{2+} channel of the sarcoplasmic reticulum [99]. At ryanodine-receptor channels, spermine can block and permeate the channel similar to its effects at AMPA receptors [99]. Spermine, argiotoxin$_{636}$ and several low-molecular-mass analogues of spermine inhibit voltage-activated Ca^{2+} currents in neurons when applied extracellularly [100,101]. The philanthotoxins, which block glutamate receptors, also block nicotinic acetylcholine receptors and some Ca^{2+} channels [102,103], and the potentiation of muscle acetylcholine receptors by extracellular spermine has been reported [104]. The physiological significance of these effects is not known. However, even if polyamines do not interact with these channels *in vivo*, such studies provide examples of other channels at which polyamines or polyamine derivatives may be useful pharmacological tools for the study of the structure, function and regulation of ion channels.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Pronounced effects of physiological concentrations of polyamines on some types of K^+ channels and glutamate receptors have been described. These effects include high-affinity block of ion channels by intracellular polyamines and modulation of channel gating by extracellular polyamines. There are obvious questions about whether changes in intracellular or extracellular levels of polyamines alter the activity of glutamate receptors, K^+ channels or

other ion channels in physiological and pathological conditions. Little is known about the subcellular distribution of polyamines, and no specific probes or chelating agents are currently available to measure changes in the levels or distribution of polyamines in real time in intact cells. On the other hand, a good deal is known about the biosynthesis and metabolism of polyamines and the enzymes involved in these processes, and selective inhibitors of some of these enzymes are available. Thus, although a complete understanding of the physiological interactions of polyamines with ion channels will require new and specific tools with which to study polyamines, some experimental tools are already available that can allow manipulation of levels of polyamines in cells, tissues and intact animals.

Because levels of polyamines can be manipulated pharmacologically, it is conceivable that the polyamine systems, through their interactions with ion channels, may provide new therapeutic targets. For example, lowering of polyamine levels in cardiac myocytes would tend to decrease excitability by reducing rectification of strong Kir channels, and may provide a novel route to the treatment of cardiac arrhythmia [25]. Similarly, lowering polyamine levels could decrease excitability in central neurons via effects on Kir channels, an approach that could be used to reduce seizure activity and to aid neuroprotection. However, lowering of polyamine levels could also increase excitability and Ca^{2+} flux through inward-rectifying AMPA/kainate receptors in neurons, a potentially dangerous situation that may lead to Ca^{2+} overload. Clearly, the problem here is one of specificity because of the ubiquitous synthesis and distribution of polyamines in eukaryotic cells. Nonetheless, this is an area worthy of further investigation.

A number of agents that interfere with the enzymes involved in polyamine biosynthesis or with the turnover and metabolism of polyamines are used clinically or are in clinical trials [4]. Inhibitors of polyamine synthesis, and polyamine analogues that may act by binding to nucleic acids, are also being developed as potential anti-tumour agents. It is possible that chronic treatment with enzyme inhibitors and polyamine analogues could change polyamine pools such that the rectification properties of K^+ channels and AMPA receptors or the modulation of NMDA receptors is altered, possibly producing serious clinical side-effects. Furthermore, some polyamine analogues may interact directly with ion channels. For example, the bis(ethyl)polyamine analogue BE4444, which has anti-tumour activity, is a potent blocker of NMDA receptors [46]. Because the anti-tumour activity of bis(ethyl)polyamines depends on their ability to accumulate within cells, side-effects could arise if these analogues have direct effects on Kir channels or other channels that are sensitive to intracellular polyamines.

In addition to questions about the physiological and pathophysiological roles of endogenous polyamines, studies of the polyamine block of ion channels are providing information about the channels themselves and leading to the discovery or development of new tools to study such channels. Two high-affinity glutamate channel blockers, one from spider venom and the other synthesized in a laboratory, are shown in Figure 1. It may be possible to tag these types of compounds with radioisotopes or with affinity labels for use in biochemical studies of the channel proteins. Many of the known polyamine derivatives may serve as lead structures for more potent and selective compounds, and it is anticipated that some of these derivatives will be useful for physiological or biochemical studies of receptors and channels and of their roles in cellular and neuronal signalling. An interesting example was the recent use of philanthotoxin to selectively block Ca^{2+}-permeable AMPA receptors (there are no other classes of compounds that selectively block these channels) to demonstrate a direct role for these receptors in a novel form of synaptic plasticity [48].

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