Intracellular polyamines are responsible for inward rectification of Ca²⁺-permeable AMPA receptors and, hence, exert a voltage-dependent block upon these channels. In a recently described mechanism, neuronal activation modulates the synthesis of polyamines to regulate the amount of Ca²⁺ flux and the excitability threshold at developing synapses that contain polyamine-sensitive AMPA receptors.

The polyamines putrescine, spermidine and spermine are present in almost all cells. These organic polycations appear to play important roles in protein synthesis, cell growth and cell differentiation, and their synthesis and degradation are tightly controlled by several enzymes that are regulated by cellular activity [1]. Polyamines are protonated at physiological pH and can interact with several intracellular targets, including nucleic acids and proteins. In the past few years, the specific interactions between polyamines, in particular spermine, and several functionally diverse ion channels have been described [2]. Spermine blocks the channel pore of inward-rectifier K⁺ channels from the intracellular side, controlling the resting membrane potential and excitability in neurons and cardiac myocytes. By contrast, spermine acts at extracellular sites in neurons to potentiate the activity of NMDA receptors. Similar to the situation with K⁺ channels, intracellular spermine has also been shown to control rectification and the total amount of current flow in some subtypes of AMPA and kainate receptors.

Spermine determines inward rectification in Ca²⁺-permeable AMPA receptors

AMPA-type glutamate receptors are responsible for fast excitatory neurotransmission in the CNS. They are heteromeric ligand-gated channels composed of four possible subunits (GluR1–GluR4). Most AMPA receptors...
are permeable to Na\(^+\) but impermeable to Ca\(^{2+}\) and have \(I-V\) relationships that are linear (Fig. 1). This reflects the presence in receptor assemblies of at least one GluR2 subunit \([3,4]\), the mRNA of which is edited such that the glutamine residue found in the pore-lining region (at the so-called Q/R site) of the other subunits is replaced with an arginine residue \([5]\). This positively charged residue disrupts the interactions between Ca\(^{2+}\) ions and the channel pore, hindering the flow of the cation through the channel. Under certain conditions, Ca\(^{2+}\)-permeable AMPA receptors lacking the GluR2 subunit can also form. Ca\(^{2+}\)-permeable AMPA receptors are expressed by certain types of mature neurons (especially by hippocampal and cortical interneurons) or glial cells \([6]\). In addition, AMPA receptors transiently lacking GluR2 can be expressed in immature neurons at certain developmental stages \([7]\).

Ca\(^{2+}\)-permeable AMPA receptors display inward rectification (i.e. they exhibit a reduced outward current at depolarizing membrane potentials; Fig. 1), which arises from fast voltage-dependent channel block by intracellular polyamines. Positively charged spermine (and, to a lesser extent, spermidine), as well as polyamine spider toxins such as argiotoxin and Joro spider toxin, selectively block GluR2-lacking receptors. They do so because they are attracted by a negatively charged ring of carbonyl-oxygen groups provided by the glutamine residues of the GluR1, GluR3 or GluR4 subunits but are repelled by the positively charged arginine residue in the GluR2 subunit. It has been calculated that 100 \(\mu\)M intracellular spermine blocks AMPA-receptor channels by 18–36\% at resting potentials, and as the cells depolarize the polyamine block becomes larger \([8]\). In some cases, Ca\(^{2+}\)-permeable AMPA receptors display a ‘double rectification’: inward current at negative potentials, very low conductance between \(-20\) and 30 mV and an outward current at potentials +40 mV (Fig. 1). This recovery of ion flow at very positive potentials appears to be due to the fact that polyamines pass right through the channel from the inside to the outside when the driving force is large enough.

Visual activity regulates the synthesis of spermine and AMPA-receptor currents in immature tectal neurons

The synthesis of polyamines in the CNS can be stimulated by diverse neuronal insults, including seizures \([9]\) or traumatic and ischemic injury \([10]\), but the significance

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Fig. 1. Spermine is responsible for inward rectification of Ca\(^{2+}\)-permeable AMPA receptors. (a) AMPA receptors containing the GluR2 subunit are impermeable to Ca\(^{2+}\) and have electrically linear current–voltage (\(I-V\)) relationships. GluR2 limits Ca\(^{2+}\) gating and blockade by intracellular spermine, owing to the presence of an edited positively charged arginine residue (R) rather than a glutamine residue (Q) in a crucial region lining the pore of the channel (the Q/R site). (b) AMPA receptor channels lacking the GluR2 subunit and composed of GluR1, GluR3 and GluR4 assemblies are permeable to Ca\(^{2+}\) and display inward rectification, which arises from voltage-dependent-channel block by intracellular spermine. Both Ca\(^{2+}\) and spermine are attracted into the channel by negative charges on the glutamine residues. (c) Aizenman et al. \([11]\) have shown that neuronal activity induces expression of ornithine decarboxylase (ODC), resulting in additional synthesis of spermine and, as a result, double rectification of the channel. Functional consequences of the activity-dependent regulation of spermine synthesis are a more pronounced voltage-dependent block by polyamines (PA) and relief of the PA block by repetitive stimulation, leading to facilitation of synaptic responses.
of this regulatory mechanism upon glutamate transmission is unknown. A recent study has now demonstrated that visual stimulation, a more physiological type of stimulus, is also able to increase the synthesis of polyamines in developing Xenopus optic tectal neurons and that this, in turn, modulates synaptic transmission via Ca$_{2+}$-permeable AMPA receptors [11]. Inwardly rectifying Ca$_{2+}$-permeable AMPA receptors, as detected by Ca$_{2+}$ labelling and whole-cell patch clamp, are expressed by immature neurons in the optic tectum, in a gradient that parallels the developmental gradient along the rostrocaudal axis. Because addition of 100 µM spermine in the patch pipette was able to further increase the inward rectification of AMPA receptors, polyamines in these cells were not at saturating concentrations and were, therefore, subject to regulation by activity. When tadpoles were placed in a light chamber and visually stimulated for no more than 4 h, the rectification of Ca$_{2+}$-permeable AMPA receptors increased in immature tectal neurons. This effect was abolished by inhibitors of polyamine synthesis, indicating that neuronal activity can modulate the synthesis of intracellular polyamines and, thereafter, alter the conductance properties of a subset of AMPA receptors that are expressed in developing neurons.

Functional implications

Neuronal activity can modulate AMPA receptor responses by other known mechanisms, including phosphorylation [12], regulation of receptor trafficking [13] and switch in subunit composition [14,15]. Activity-dependent modulation of intracellular polyamine levels could operate in parallel with some of them. For example, when cells express AMPA receptors that are more or less Ca$_{2+}$-permeable (depending on the expression levels of GluR2 subunits), they should also become more or less susceptible, respectively, to modulation by activity-driven changes in polyamine synthesis.

In their study, Aizenman et al. underscore two possible consequences of the activity-dependent spermine block of Ca$_{2+}$-permeable AMPA receptors [11]. On the one hand, they confirm that AMPA-mediated responses can be blocked by elevated spermine levels [8] and show that both spermine and visual stimulation reduce the amplitude of miniature EPSCs in tectal immature neurons – thus suggesting a possible neuroprotective mechanism of action for polyamines. In models of cerebral ischemia, an accumulation of putrescine (which has no effect on AMPA receptors) and a depletion of spermine and spermidine can be observed in injured tissue [10]. Exogenous spermine and several polyamine derivatives have been proposed as neuroprotective agents, and an antioxidant mechanism has recently been claimed to underlie their effects [16]. The results of this study, however, suggest that spermine could be neuroprotective because it blocks Ca$_{2+}$-permeable AMPA receptors, which have been shown to be predominantly expressed in vulnerable regions following global ischemia [7]. On the other hand, Aizenman et al. show that the voltage-dependent block of Ca$_{2+}$-permeable AMPA receptors by spermine can be relieved by repetitive stimulation, leading to facilitation of synaptic transmission. This use-dependent mechanism should make the cell more responsive to repetitive synaptic inputs rather than to single stimuli, facilitating the detection of coincident activity when synaptic transmission is especially intense. In particular, facilitation of inwardly rectifying AMPA receptors could be important during activity-dependent development of the topographic retinotectal map. Bursting retinal ganglion inputs should result in use-dependent relief from the polyamine block, permitting the stronger visual inputs to excite the cell and become part of a newly formed neural circuit.

In conclusion, the activity-dependent synthesis of spermine can be regarded as a novel mechanism to modulate glutamate synaptic transmission mediated by Ca$_{2+}$-permeable AMPA receptors. Because these receptors are expressed in the CNS only by specific neurons or glial cells and under particular conditions, this mechanism is expected to have fundamental repercussions in brain physiology and pathology.

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