Synaptic and Non-synaptic AMPA Receptors Permeable to Calcium

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ABSTRACT—For a long time, alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors permeable to calcium have been considered to be either non-existent or as “atypical”. There is now ample evidence that these receptors exist in numerous regions of the nervous system and in many neuronal as well as non-neuronal cell populations. This evidence has been accumulated by several methods, including electrophysiological recording, calcium imaging and cobalt-loading. Functional AMPA receptors permeable to calcium are already expressed at very early stages of embryonic development, well before the onset of synaptogenesis. They are probably involved in the paracrine signaling necessary for construction of the nervous system before becoming involved in synaptic transmission. In immature cells, cyclothiazide strongly increases the steady-state level of responses not only to AMPA, but also to kainate. Ingestion, during pregnancy, of food or drug substances that can cross the placental barrier and act upon the embryonic receptors may constitute a risk for normal development. In the adult nervous system, synaptic as well as non-synaptic (paracrine) AMPA receptors permeable to calcium are probably widely expressed in both glial and neuronal cells. They may also participate in controlling some aspects related to adult neurogenesis, in particular the migration of newly formed neurons.

Keywords: Paracrine action, Migration, Cell death, Cobalt, Adult neurogenesis

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1. Introduction

Glutamate is well known as the principal excitatory transmitter at synaptic junctions, and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors seem to play a major role in fast neurotransmission. In contrast to N-methyl-d-aspartate (NMDA) receptors, AMPA receptors were longtime thought to be impermeable to calcium, although contradictory evidence has been published as early as 1984 (1). This “dogma” (2) has probably influenced, and possibly biased, physiological studies, principally those devoted to “neurotoxicity” of glutamate (3). Since calcium influx was considered as the key factor triggering cell death and since only NMDA receptors were believed to be permeable to calcium, the role of AMPA receptors in this domain has been investigated only when it became more and more obvious that the borderline between calcium permeability and impermeability is not situated between NMDA and AMPA receptors, but within the AMPA-receptor family (4 – 17).

Soon after the AMPA-receptor subunits had been cloned (18 – 20), expression studies showed that AMPA receptors are permeable to calcium except if they contain subunit glutamate receptor (GluR)-2 in its edited form at the Q/R site (21 – 23). The earliest expression studies stressed the fact that GluR-2 is almost always edited (at least in the adult) and that its presence dominates the channel properties concerning ion permeability even in heteromeric receptors (23).

Since GluR-2 mRNA or protein is detectable in most neurons, AMPA receptors permeable to calcium were initially expected to be present in only a small minority of cells. However, in addition to the pioneering electrophysiological studies published in the late eighties and the early nineties, cobalt-loading studies (see below) have provided ample evidence that numerous cells bearing AMPA receptors permeable to calcium are present in many regions of the nervous system. The apparent discrepancy can probably be explained by the following facts: a) Even if the presence of a single edited GluR-2 subunit substantially reduces permeability to calcium in a given receptor, it may not completely abolish calcium influx, yielding intermediate permeability (17, 24 – 26); b) Even if a given cell carries receptors that are more or less impermeable to calcium, it may express in addition other receptors that display higher permeability (27 – 29).

If there is spatial segregation between the two types of receptors, for instance one being targeted to the soma or proximal dendritic segments, the other to more distal parts (30, 31), then the frequency of a given type may be underestimated if the method of detection is biased. Whereas it is considerably more difficult to obtain patch-clamp recordings from fine terminal dendritic branches than from the soma, the cobalt-loading method published in 1991 by Pruss and co-workers (32; see also the methods section of the present paper) does not have this problem. In addition, this method can be used for rapid screening of cell cultures or whole brain regions, which reduces the bias due to selection as a function of the experimenter’s expectations. Cobalt-positive neurons have been found in (or, in the case of cell cultures, derived from) many regions of the developing or adult nervous system, including neocortex (33 – 38), hippocampus (39, 40), basal ganglia (41, 42), retina (43 – 45), diencephalon (46), mesencephalon (46), cerebellum (46, 47), brainstem (48 – 50) and spinal cord (28, 51 – 57).

All these studies concern animal models (from lower vertebrates to mammals). There is in addition one study demonstrating the existence of functional AMPA/kainate receptors permeable to calcium in human embryonic and fetal central nervous system. Strong increase in cobalt staining after co-application of cyclothiazide, but also remarkably high numbers of cells stained after stimulation by AMPA alone, have been reported (58).

In summary, the number of neurons bearing AMPA/kainate receptors permeable to calcium is far above the initial estimations. It is also clear today that, while such receptors are frequently expressed by interneurons (55, 59 – 64), they are not at all restricted to this cell type. The functions of these receptors may also be more important than hitherto believed.

In addition to neurons, there are also non-neuronal cells, including floorplate (53) and glial cells, that have such receptors. Although most of these receptors are probably non-synaptic, being involved in paracrine signaling, some may be associated with synapses or synapse-like contacts (65, 66).

Whereas AMPA receptors permeable to calcium have been reported in astrocytes, oligodendrocytes, Bergmann and Müller cells (24, 25, 67 – 76), microglial cells seem to express receptors displaying relatively low permeability to calcium (77).

Several studies have reported declining expression of permeable receptors during differentiation of macroglial cells (25, 78). However, consistent with the continuous proliferation of glial cells, “immature” cells having permeable receptors are probably present in the adult nervous system, and even “mature” astrocytes displaying such receptors have been reported (79).

AMPA receptors permeable to calcium have also been found in several cell lines (80 – 84).

Although most AMPA receptors are presumably involved in synaptic transmission, some receptors may support non-synaptic, paracrine, signaling. This is manifestly the case at very early stages of development, well before the onset of synaptogenesis. mRNAs for AMPA receptor subunits have been detected in the rat neural tube by embryonic
Fluorescence resonance energy transfer from the same cells. Optical responses can be recorded during several hours. In addition, cell damage with this method is reduced, and for bias mentioned for patch-clamp recordings (89, 90). Imaging with voltage-sensitive dyes is underrepresented or even neglected. This method allows the simultaneous analysis of membrane potential in large groups of cells, without the risks for bias mentioned for patch-clamp recordings (89, 90). In addition, cell damage with this method is reduced, and optical responses can be recorded during several hours from the same cells.

Imaging with fluorescent calcium chelators
This family of methods is based upon incorporation of a fluorescent marker into the cells under study, either by micro-injection (which may lesion the cell and which is difficult to do for a large number of cells) or by using a lipophilic complex that permeates the cytoplasmic membrane and is then hydrolyzed by the cell’s endogenous enzymes. Of course, the marker will react with any free calcium ion. Therefore, a fluorescent chelator can identify AMPA receptors permeable to calcium without ambiguity only if other potential sources such as NMDA receptors, voltage-gated channels or release of intracellular calcium are under control by pharmacological tools or voltage-clamping (92, 93). Once calcium has entered the cell, it may be spatially restricted by calcium-binding proteins such as calretinin (94).

2. Methods for the detection and analysis of AMPA receptors permeable to calcium

Electrophysiology
Using the patch-clamping technique, spontaneous as well as evoked current modifications can be recorded, either in whole-cell configuration to evaluate global activity or in more localized membrane patches. It is also possible to probe the receptor properties not only with molecules applied to the extracellular side of the membrane, but also to its intracellular side (88). The principal advantage of these methods is that the ionic nature of the outward or inward currents can be determined directly with a time resolution below a millisecond. This is even possible in very restricted areas of the cytoplasmic membrane, so that single receptor channel activity can be recorded. This possibility is invaluable for studies correlating, for instance, subunit composition with physiological channel properties.

As with every method, this one has some drawbacks. First, only a small number of cells or cell regions can be recorded simultaneously. It is therefore difficult to detect rare responses in a heterogeneous cell population. Second, while it is relatively easy to record electrical activity from the cell body or thick proximal dendrites, this is not the case for more distant (and often very thin) neurites. This may lead to biased analyses where distally located receptors are underrepresented or even neglected.

Imaging with voltage-sensitive dyes
This method allows the simultaneous analysis of membrane potential in large groups of cells, without the risks for bias mentioned for patch-clamp recordings (89, 90). In addition, cell damage with this method is reduced, and optical responses can be recorded during several hours from the same cells.

Fluorescence resonance energy transfer
This method requires specialized equipment and at least two fluorescent probes. The excitation wavelength of one probe must overlap with the emission wavelength of the other. Under these conditions, ion movements (for instance across an ion channel) can be measured with high accuracy at the molecular level (91).
Detection of AMPA receptors permeable to calcium using imaging methods
Fig. 1. Dissociated brainstem cells taken from rat embryos at day 13 of gestation, cultured for 3 days. The cells have been loaded with the fluorescent calcium-chelator Fluo-3 (for methodological details, see ref. 173). The three images represent a time-series (interval is 2 min) taken with an ACAS laser microscope (Meridian, Okemos, USA). Each peak represents an individual cell. The concentration of free cytosolic calcium is coded by the height and the pseudo-color of the peak (violet represents very low, red very high concentrations). Top: before stimulation. Center: after stimulation by the agonist AMPA (50 μM) alone. Bottom: after co-application of cyclothiazide (50 μM), which blocks desensitization of AMPA receptors. Several tens or even hundreds of cells can be analyzed simultaneously using this method, and those responding to AMPA alone, or to AMPA in the presence of cyclothiazide, can be detected easily. In contrast with cobalt-loading (see Figs. 4 and 5), calcium imaging takes into account not only calcium influx through AMPA receptor channels, but also cytosolic calcium from all other sources.

Fig. 2. The fluorescence intensities of individual cells can be plotted against time to get insight into the kinetics of the calcium responses to pharmacological agents applied sequentially or simultaneously. This three-dimensional graph represents the relative calcium kinetics of two groups of four cells (dissociated from embryonic day 14 rat neocortex and grown in vitro for 2 days), each cell being identified by a number representing its location in the analyzed microscope field. During the first minute recorded (first segment of the individually colored bands), the cells were exposed to plain buffer. During the second minute, they were exposed to kainate (400 μM) alone; then, cyclothiazide (100 μM) was added. The two groups of cells were chosen to show extreme examples of calcium kinetics after stimulation by kainate. The first group (at left) displayed the usual strong steady-state response to kainate, which was not significantly modified by the co-application of cyclothiazide. In contrast, the cells of the second group (at right) showed a low-amplitude steady-state response to kainate alone, which was dramatically enhanced by cyclothiazide.

Fig. 3. Calcium kinetics of two groups of four cells in a sister culture. In this experiment, cyclothiazide was added only during the 6th minute, and cobalt (5 mM) was added 1 min later. In spite of the high dose of kainate (400 μM), the calcium concentration in the cells of the right group remained fairly low until cyclothiazide was added. When cobalt was added, the competition of this ion with calcium led to a decrease in fluorescence intensity. The two cells corresponding to the red and the green bands, respectively, are shown in Fig. 4.

Fig. 4. Digital image with electronic contrast enhancement of the culture used for the calcium analysis shown in Fig. 3. After cobalt-loading during 10 min, cobalt had been precipitated by ammonium sulfide, which led to reddish-gray staining of the cells bearing AMPA receptors permeable to divalent cations (for methodological details, see refs. 37, 38, 58). The red and the green arrows indicate the cells corresponding to the red and the green bands, respectively, in Fig. 3. In cases where further characterization of the cells is required, cobalt labeling can be combined with other methods; for instance, immuno-cytotoxic staining to show the expression of proteins of interest. Cobalt labeling can also be enhanced by silver precipitation to yield high-contrast staining as shown in Fig. 5.

Fig. 5. Dissociated brainstem cells taken from rat embryos at day 14 of gestation, cultured for 5 days. The cells have been exposed to kainate (200 μM) and cyclothiazide (50 μM) in the presence of cobalt (5 mM). After cobalt precipitation by ammonium sulfide, the cells have been fixed by paraformaldehyde, and the cobalt labeling has been enhanced by silver precipitation. The resulting staining shows clearly not only the cell bodies, but also the processes. Some of these processes are even more intensely stained than the cell bodies, suggesting that they possess numerous AMPA receptors permeable to divalent cations and that cobalt, once it has entered the cell, does not freely diffuse within it.

wavelength probes is lower than with single-wavelength probes since an image-pair (instead of a single image) has to be recorded at every time point.

An important feature of fluorescent calcium chelators is their affinity to calcium. Whereas low affinity markers may be inappropriate for the detection of minute calcium fluctuations, high affinity markers may a) severely disturb the intracellular cascades of events triggered by calcium elevation and b) lead to underestimation of high calcium peaks (95). Since living cells are sensitive to light, every effort should be made to reduce both the time and intensity of fluorescence excitation. A particularly interesting method for high resolution imaging with moderate light impact is two-photon imaging (96, 97).

Radioactive calcium

Unlike imaging with a fluorescent calcium chelator, incubation with radioactive calcium (45Ca2+) allows one to distinguish between calcium influx on the one hand and intracellular calcium release on the other. This, together with the possibility to quantify not only influx but also efflux (98) easily, has made the method quite popular (99–102). However, it still does not distinguish between influx through AMPA receptors as opposed to other channels. Furthermore, unlike imaging with a fluorescent calcium chelator, this method cannot provide fast calcium kinetics corresponding to a single stimulation or to a whole sequence of pharmacological agents.

Cobalt-loading

In contrast with the above-mentioned methods, exposure to cobalt allows direct and specific identification of AMPA receptors permeable to calcium. Indeed, the only entry route for cobalt hitherto rigorously documented is through the ion channels of such receptors: neither NMDA receptors, nor voltage-gated calcium channels, seem to be permeable for this ion, which is so closely related to calcium (32).

The natural intracellular cobalt concentration is below the threshold of detection with the method used; therefore, cobalt staining unequivocally results from influx. In healthy cells, almost all, if not all the influx seems to go through
AMPA receptors, since co-application of an antagonist usually prevents any staining. However, when the integrity of the cytoplasmic membrane is lost, there may be staining even in the presence of an antagonist.

In order to become visually detectable, cobalt has to be precipitated with ammonium sulfide. When used with tissue slices containing numerous receptor-bearing cells, staining of whole cell groups is visible even at low magnification (58).

In individual cells, for instance in monolayer cell cultures, cobalt staining is more difficult to detect due to the low contrast, and digital imaging with contrast enhancement (as in Fig. 3) is useful to show cobalt-positive cells. Alternatively, cobalt staining can be enhanced by silver precipitation, which yields high-contrast Golgi-like staining (as shown in Fig. 4). Interestingly, cobalt does not seem to diffuse freely in the cytosol, which allows to some extent the localization of membrane regions with high receptor density (Fig. 5).

Although cobalt alone (prior to precipitation with ammonium sulfide) is not directly visible, its competition with calcium can be seen as a decrease in Fluo-3 fluorescence (Fig. 3).

At least some AMPA receptors permeable to calcium and cobalt seem to be permeable also to zinc (103). However, unlike cobalt, zinc cannot be used to identify AMPA receptors permeable to calcium unequivocally, since, even if it has been reported to enter the cell preferentially through AMPA receptors (104), there are other routes of entry into the cells, including NMDA-receptor channels (105).

3. Molecular and physiological properties of AMPA receptors permeable to calcium

Subunit composition and edition of subunit GluR-2

At the receptor level, permeability to calcium is determined by the presence and the proportion of edited GluR-2 (21 – 23, 106, 107). RNA editing at the Q/R site of GluR-2, which leads to substitution of a single glutamine residue with neutral charge in the M2 (channel forming) transmembrane segment by a positively charged arginine residue, is due to a highly specific enzyme requiring a double-stranded RNA structure formed by exonic and intronic sequences (108, 109). Homologous enzymes seem to exist in *Drosophila* (110) and goldfish (111).

In rodents, RNA editing of GluR-2 at the Q/R site is developmentally regulated; however, the percentage of edited RNA rapidly reaches very high values already at embryonic stages of development (23). In contrast, studies in humans have reported substantial amounts of unedited GluR-2 in normal brains (112), and even very high percentages in the spinal ventral gray of patients with amyotrophic lateral sclerosis (113).

In order to help understand apparent contradictions in the literature, it may be useful to emphasize that the presence of edited GluR-2 generally reduces permeability to calcium at the receptor level, but not necessarily at the cellular level, since a given cell may express both permeable and more or less impermeable receptors, either mixed together or targetted to different sites (45, 114, 115). This is notably the case for hippocampal pyramidal neurons (30) and spinal motor neurons (28, 29).

Specific channel properties

When comparing AMPA receptors permeable to calcium with NMDA receptors, important differences have been found in spite of the fact that these receptors share calcium permeability. For instance, the pore of NMDA receptor channels has multiple sites for Ca$^{2+}$, whereas that of AMPA receptor channels has only one (116), which implies different mechanisms of Ca$^{2+}$ transport in the two types of receptors. Concerning biophysical differences between the ion channels of calcium-permeable vs impermeable AMPA receptors, their ion selectivity might in principle be determined either by the size or form of the pore or by charge density of the channel. The latter parameter seems to be more probable (117).

Inward rectification

One of the electrophysiological characteristics of calcium-permeable receptors is inward rectification (118 – 125). Polyamines seem to be responsible for this behavior, and the loss of rectification described in some studies may be due to washout of intracellular polyamines (120). In addition to the largely studied intracellular site for polyamines, there seem to exist also other sites, one being extracellular (88).

Closely related to the fact that receptors permeable to calcium generally display inward rectification is their affinity to spider toxins, which offers an interesting possibility to selectively block such receptors (see the following section concerning agonists and antagonists).

4. Pharmacological properties of AMPA receptors permeable to calcium

Agonists and antagonists

The agonists most commonly used to activate AMPA receptors (glutamate, AMPA or kainate) cannot discriminate between the permeable and the impermeable forms. Agonists displaying marked subunit selectivity have been developed recently (126). However, they discriminate better within the permeable and the permeable forms than between them. As to the antagonists, Evans blue may be useful, but its selectivity varies in a complex manner as a function of the concentration used (127). In addition, it
has been reported to antagonize not only AMPA but also kainate receptors (128).

Conversely, spider toxins such as Joro toxin seem to have little effect on kainate receptors (129), and they have been widely used to block specifically AMPA receptors permeable to calcium (130 – 135). However, dissociation between the Joro spider toxin sensitivity of recombinant AMPA receptors and their ability to increase intracellular calcium has been reported (26).

The anti-epileptic drug Topiramate seems to have interesting properties: it has been reported to antagonize cobalt uptake in immature, but not mature cultured cerebellar granule cells (47).

Modulation of desensitization

When stimulated by AMPA or glutamate, AMPA receptors usually display fast and strong desensitization. Therefore, time-resolution is an important feature for methods devoted to the detection of calcium responses to AMPA. Any method whose resolution does not approach the millisecond range will probably yield weak, if any, signals unless desensitization is reduced or blocked by pharmacological tools. Nootropic drugs such as aniracetam have been used (136 – 138); however, these drugs have two drawbacks: a) they need to be used at high (millimolar) concentrations; and b) they reduce desensitization not only at AMPA, but also at kainate receptors.

Cyclothiazide, which has been used as a diuretic, significantly reduces desensitization at micromolar concentrations and seems to be relatively specific for AMPA receptors (100, 139, 140). This drug is therefore useful not only for unmasking AMPA receptor responses (see Fig. 1), but also for distinguishing between AMPA and kainate receptors. Other desensitization blockers have been developed recently, and comparing their effect with that of cyclothiazide may help detect AMPA receptor heterogeneity (141).

Kainate is sometimes described as a “non-desensitizing” agonist at AMPA receptors. However, experiments using fast perfusion have shown that there is substantial desensitization that can be reduced by cyclothiazide (139). Subsets of glial (142) as well as neuronal (143) cell populations have been reported to express AMPA receptors that were strongly desensitized by kainate. When analyzing very immature dissociated cells in culture, we even found cells that showed hardly detectable calcium responses (or cobalt labeling) after exposure to high doses of kainate alone, whereas co-application of cyclothiazide induced very strong calcium or cobalt influx (37, 53).

As shown in Figs. 2 and 3, the additional increase in calcium when cyclothiazide was co-applied with kainate varied widely within one and the same culture. This heterogeneity was region- and development-specific: strong increase was more frequent in neocortical cells than in brainstem cells and more frequent at early than at later stages of in vitro development.

5. Factors that can modify the functional properties of AMPA receptors permeable to calcium

Activity / inactivity

Depolarization or synaptic activity can induce significant changes, including switches in receptor subtype (144 – 146).

Growth factors

Basic fibroblast growth factor and platelet-derived growth factor were found to modify subunit expression, either directly or when combined (147, 148).

Protein kinases

Calcium- and calmodulin-dependent phosphorylation and action of cAMP dependent protein kinase has been reported (149 – 155).

Calpains and caspases

AMPA receptor specific action of calpains has been described (156 – 159), and an interesting mechanism (involving caspase-mediated degradation of AMPA receptor subunits) for preventing excitotoxic necrosis and ensuring apoptosis has been proposed (160).

Ethanol and other drugs

Effects of exposure to ethanol (161), dopamine or psychostimulants (162) have been reported.

Ischemia

Global ischemia has been claimed to induce downregulation of GluR-2 mRNA and to increase AMPA receptor-mediated Ca\(^{2+}\) influx in hippocampal CA1 neurons of gerbils (163). Several groups did not find a comparable downregulation in various models for ischemia (164 – 166). On the other hand, differential effects on GluR-5 and GluR-6 have been reported (165). In a study of ischemic tolerance, no change of GluR-2 RNA editing, but a transient decrease in R/G editing was found (167).

Epilepsy

Mice having an editing-deficient GluR-2 allele (168) or different levels of the Q/R site-unedited AMPAR subunit GluR-B (169) display epileptic seizures. On the other hand, kindling or epileptic activity seem to modify the properties of AMPA receptors. Amygdaloid kindling down-regulates GluR-2 (170, 171) and the kindled seizures can be blocked by 1-naphthylacetyl spermine, an analogue of Joro spider toxin (133).
Hypothermia

Hypothermia has been known for a longtime as one of the best protections against glutamate neurotoxicity, and AMPA/kainate receptors seem to be particularly sensitive (172).

We have found a narrow temperature range (between 24°C and 27°C) in which repeated stimulation by AMPA switched from inducing reversible to irreversible effects on cytosolic calcium levels (173).

In a study analyzing the effects of three different temperatures, 30°C and 20°C were found to be protective, whereas cooling to 12°C was toxic (174).

6. Effects and possible roles of AMPA receptors permeable to calcium

Cell proliferation

There are several reports indicating that AMPA receptors are involved in the control of cell proliferation (175 – 177). However, it remains to be clarified to which extent receptors permeable to calcium play a specific role in this domain.

Migration

Calcium-permeable receptors are expressed on tangentially (non-radially) migrating cells in the embryonic as well as the adult brain (36, 38, 86). AMPA-receptor activation leads to neurite retraction, which suggests that one of the possible roles of these receptors is to mediate a stop signal.

Neurite growth and circuit making

Effects of glutamate on dendritic growth in embryonic rat motoneurons have been demonstrated (178), and a role for AMPA receptors in the establishment of inner retinal circuits has been suggested (179).

Regulation of gene expression / transcription factors

Regulation of gene expression has been reported in astrocytes (180) and glial progenitors (181, 182). AMPA receptor-mediated, calcium-dependent CREB phosphorylation has been shown in a subpopulation of auditory neurons surviving activity deprivation (183).

Differentiation and trophic signaling

AMPA receptors seem to be involved in very early differentiation steps during terminal cell division and the earliest commitment to neuronal cell lineage of rat neuroepithelial cells (184). Early expression of AMPA receptors, soon after neurite initiation, has also been detected in differentiating Xenopus spinal neurons. Interestingly, AMPA receptors were found before NMDA receptors (184 – 186), in contrast with the widely held opinion that NMDA receptors come first. The latter may be true for synaptic receptors developing during the postnatal period (187), but not for receptors on embryonic cells. Another example showing the implication of AMPA receptors in early developmental steps is their expression during the differentiation of NTERA2 human embryonal carcinoma cells into neurons (188).

Concerning glial cells, the best documented example is the involvement of AMPA receptors in the differentiation of oligodendrocyte progenitors (189 – 192).

In contrast with the well known “excitotoxic” effects of AMPA or kainate on differentiated neurons (193), activation of calcium-permeable AMPA receptors in developing cells has been reported to be trophic (194, 195), and exposure to antagonists at early stages of in vitro development has been shown to lead to massive cell death (37, 196).

Plasticity of the cytoskeleton

AMPA receptors regulate neuritic plasticity in the developing as well as the adult nervous system (44, 197, 198). However, the underlying mechanisms, and the interaction with other receptor types, may be quite different. For instance, a two-step process in which spines initially formed in response to NMDA receptor activation are subsequently stabilized by AMPA receptors has been proposed (199).

On the other hand, we have found neurite retraction in tangentially migrating neurons in the intermediate zone of the embryonic rat neocortex. These cells express calcium-permeable AMPA receptors, but no NMDA receptors, and the effects are presumably due to direct calcium influx via the AMPA receptor channels (38).

Synaptic plasticity / interactions with NMDA receptors

AMPA receptors permeable to calcium are involved in synaptic strengthening (200), long-term potentiation (201 – 206), short-term potentiation (207) and long-term depression (208 – 210), often in interaction with NMDA receptors (211, 212). Interestingly, AMPA seems to induce nitric oxide (NO) production through a NO synthase (NOS)-independent pathway, whereas NMDA receptor-mediated NO production is dependent on NOS activity (202).

Modulation of neurotransmitter release

AMPA receptors are involved in glycine release from hippocampal slices in developing and aging mice (213). Prostaglandins stimulate calcium-dependent glutamate release in astrocytes (214). AMPA can induce release of [3H]-noradrenaline from hippocampal slices (215), or ATP from rat cortical astroglial cells (216). It also can modulate the glycine response in rat spinal neurons (217).
Modulation of neurosecretion

AMPA receptors stimulate oxytocin release in the lactating rat (218). They can modulate cytosolic calcium in isolated rat melanotropes (219) and in isolated rat supraoptic neurons (220).

Presynaptic and autoreceptors

Presynaptic AMPA receptors (presumably autoreceptors) have been reported to enhance the synaptic release of excitatory amino acids in the mammalian forebrain (221) and the presence of such receptors on taste receptor cells has been suggested (222).

7. Cell death

Are cells bearing AMPA receptors permeable to calcium particularly “vulnerable” (45, 223 – 225)?

Although particularly “vulnerable” cells presumably do exist, it is not likely that all cells displaying AMPA receptors permeable to calcium are vulnerable, if one considers their number and variety. In other words, there are probably additional features that, taken together, put a given cell into the “low risk” or the “high risk” category. The quality and quantity of calcium-binding proteins is probably one of these features, and it happens that the cells bearing permeable receptors have usually a rather high amount of these proteins (226). However, it has been shown that the presence of calcium-binding proteins alone is not sufficient to protect neurons from a toxic calcium overload (227).

The proximity of glial cells may be another determinant of vulnerability. Again, the relationship with cell death is complex since both positive and negative effects have been reported (75, 228).

Is there a direct correlation between cytosolic calcium concentration and cell death?

The answer in the literature is generally yes (34, 41, 52, 54, 224, 229 – 231). However, careful analysis of the fate of individual neocortical neurons in culture has shown that the correlation may be rather complex (232).

Intracellular cascades leading to the formation of reactive oxygen species

Once calcium has entered the cell, it may can trigger numerous cascades of events that change the cell’s fate reversibly or irreversibly. One of these cascades leads to mitochondrial stress and the formation of reactive oxygen species (233). Zinc may enter the cells through the same channels and generate toxic free radicals (234 – 237).

Normal and pathological aging

Calcium homeostasis is one of the most important aspects of cell maintenance. Calcium-conducting AMPA receptor channels have therefore been privileged targets for studies of animal as well as human brain. The principal regions known to be involved in normal or pathological age-related changes have been investigated: the hippocampus (238), the entorhinal cortex of patients with Alzheimer’s disease (239) and the nucleus basalis of Meynert (240).

AMPA-receptor channels highly permeable to calcium have been described, but also negative feedback systems that might protect the cells from excessive calcium influx (241). Studies comparing primate and rodent brain have highlighted species differences concerning the distribution of GluR-1 in the entorhinal cortex (242). It is useful to know such differences before drawing conclusions based upon work with rodents.

8. Conclusions and perspectives

After having been considered for a longtime to be either non-existent or as “atypical”, AMPA receptors permeable to calcium are now becoming almost “ordinary” receptors, although the whole range of their functions is far from being fully understood. Their progress in notoriety is due in part to the invaluable cobalt-loading method published by Pruss and co-workers (32). This method is on the one hand a powerful research tool complementary to other approaches such as the patch-clamp techniques. On the other, it can be used as a routine method for easy and fast screening of drug effects. For instance, competition assays can be easily performed. No method is perfect for every purpose; thus, the other approaches mentioned in this paper may be more appropriate for specific experiments.

A completely different approach for studying such receptors is to have a look at appropriate mutants. Precious results have already been obtained with this method (243), although their interpretation is not easy.

Many AMPA receptors are presumably involved directly or indirectly in synaptic transmission, according to the conventional concept of receptors for a “neurotransmitter”. Others seem to function in a much less well-known way: paracrine signaling between neurons or even glial cells (65, 216, 244).

This type of communication starts at the very earliest stages of differentiation of glial as well as neuronal cells (245). The early expression of functional receptors capable to modulate cytosolic calcium, which may have profound and long-lasting effects, should be taken into account for food consumption and medical prescriptions during pregnancy: drugs that are able to cross the placental barrier and to act on the early expressed receptors may constitute a risk for normal embryonic development.

In contrast with earlier reports, AMPA receptors do not appear after NMDA receptors, but before them (184 – 186). This emphasizes their role in the construction of the brain.
and shows that the sequence NMDA / AMPA may apply to
the formation of synapses (187), but not to more “pri-
tive” fundamental functions.

Many interesting results during the next years will pre-
sumably come from the exploration of adult neurogenesis.
Glutamate receptors are expressed on cultured neural stem
cells derived from adult rat hippocampus (246), and cells
of the rostral migratory stream express functional AMPA
receptors (86). Newborn neurons in the adult brain presum-
ably have some features reminiscent of embryonic cells,
and some of the mechanisms controlling embryonic tan-
gential migration (38) may also apply to them.

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