REVIEWS

Functional Interaction between Various Glutamate Receptors

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The interaction of glutamate with various receptors in glutamatergic neurons and functional interaction between glutamate receptors are reviewed. It is hypothesized that metabotropic receptors perform defensive functions in the brain by protecting neurons from neurotoxic effects caused by excessive glutamate release.

Key Words: *glutamate; ionotropic and metabotropic receptors; Na^+/K^+-ATPase; oxidative stress*

Excitatory effects of glutamic acid in neurons were demonstrated more than 40 years ago. Transmitter functions of glutamate in the central nervous system (CNS) of mammals were revealed only in the 1980s. Studies performed in the 1990s showed structural diversity of glutamate receptors (GR) and their important role in the CNS. Considerable progress in neurochemistry allowed researchers to reveal the interaction between GR subtypes. Biochemical mechanisms of this interaction are reviewed here.

Classification and role of GR

Depending on the result of activation, GR are classified into 2 subtypes: ionotropic (iGluR) and metabotropic (mGluR) receptors. iGluR are coupled to ion channels: their activation leads to opening of ion channels and generation of electric potentials. iGluR are rapid-response receptors activated within several milliseconds. mGluR do not mediate activation of ion channels. These receptors are coupled to G-proteins in the neuronal membrane. Binding of mGluR with the corresponding ligand modulates functioning of iGluR via changing cell metabolism. Functions of mGluR are realized over a longer period (from hundreds milliseconds to several minutes).

All GR (more than 10 classes) are activated by glutamate, but demonstrate various affinities for this compound. Apart from glutamate, GR selectively interact with synthetic compounds (steric analogues of glutamate), which underlies functional classification of these receptors (Table 1).

GR are heterooligomers containing binding sites for ligands and some modulators (e.g., glycine and Mg^{2+}). This modulation of GR is a very important property. Complete activation of NMDA receptors with glutamate is possible after removal of Mg^{2+} from the corresponding binding sites on these receptors. This process results from membrane depolarization and, therefore, NMDA receptors are involved in the formation of ion fluxes after activation of other iGluR.

Some synthetic regulators of natural GR are presented in Table 1. They selectively activate (or inactivate) different GR. Configuration of these ligands differs from that of glutamate. In this review, we do not consider structural and functional peculiarities of their effects. The names of ligands are given as in catalogues and manuals on neurochemistry.

Kainate and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors are similar by ions passing through their channels and inhibitors. In addition, kainate and AMPA activate both channels, which leads to partial summation of their effects (despite different receptor affinities for these agonists) [37].

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All iGluR are localized on the postsynaptic membrane of glutamatergic synapses, while mGluR are found on both pre- and postsynaptic membranes [4]. mGluR modulate activity of iGluR and coupled channels by changing neuron metabolism due to activation of G-proteins and generation of second messengers (Table 1). According to this property, mGluR are divided into 3 classes. Class I includes mGluR-1 and mGluR-5 responsible for activation of phospholipase C, hydrolysis of membrane phosphoinositides, formation of diacylglycerol and inositol phosphates, and Ca^{2+} accumulation in the cytoplasm. Class II comprises mGluR-2 and mGluR-3. Class III includes mGluR-4, mGluR-6, mGluR-7, and mGluR-8. Class II and III receptors have various structures and pharmacological properties, but both inhibit adenylate cyclase, decreases cAMP level, and increases cell cGMP concentration in the cell. Some receptors probably activate phospholipase D.

Functional activity of ionotropic receptors

Glutamate is a neurotransmitter causing various neuronal responses depending on the type of GR involved in the realization of the presynaptic signal. Various responses of the postsynaptic membrane to the same neurotransmitter are determined by different sensitivities of GR and the presence of various receptor subtypes interacting with each other. Activation of iGluR induces membrane depolarization, *i.e.* formation of the action potential), while activation of mGluR modulate its amplitude and length.

Activation of NMDA receptors on the postsynaptic membrane of glutamatergic synapses leads to the formation of an excitation potential due to opening of Na^+, Ca^{2+} (influx), and K^+ (efflux) channels. Activation of these receptors also stimulates intracellular production of reactive oxygen species (ROS, mainly superoxide anion and hydroxyl radical) [5,6] and leads to Ca^{2+}-dependent activation of nitric oxide (NO) synthase [17]. This enzyme catalyzes superoxide anion formation under conditions of arginine deficiency [14]. During excessive production of various radicals NO interacts with the superoxide radical with the formation of peroxynitrite, strong oxidant damaging cell structures. Excessive release of the excitatory neurotransmitter into the synaptic gap produces toxic effects. Therefore, glutamate is assigned to excitotoxic compounds [3]. NMDA produces no effects in a Ca^{2+}-free medium. Thus, the observed effects depend on extracellular, but not intracellular Ca^{2+} [11].

Activation of kainate/AMPA receptors also leads to generation of the excitation potential. These receptors are responsible for the formation of rapid excitation waves and perform the major neurotransmitter function in mammalian brain [22]. NMDA-activated structures also maintain electric activity of neurons and affect the state and activity of kainate receptors.

TABLE 1. Pharmacological Characteristics of GR [1-3]

Class	Agonists	Antagonists	Functions
Ionotropic receptors (iGluR)			
NMDA receptors	N-methyl-D-aspartate (NMDA)	MK-801, D-AP5, ketamine*, phencyclidine*	$Na^+/Ca^{2+}/K^+$ current across membrane
Kainate receptors	Kainic and domoic acids	NS 102	Na^+/K^+ current
AMPA receptors	α-Amino-3-hydroxy-5-methyliso-xazole-4-propionate (AMPA), 5-F-willardiine	NBQX, CNQX+, DNQX+	Ibid
Metabotropic receptors (mGluR)			
mGluR-1	DHPG, t-ACPD°	AIDA, CPCCO-Et, MCPG×	$G_{q/11}$ activation
mGluR-2	LY307452	PCCG-4, LY341495	$G_{i/o}$ activation
mGluR-3	Ibid	Ibid	Ibid
mGluR-4	L-AP4	MAP4	- " -
mGluR-5	DHPG	4CPG	$G_{q/11}$ activation
mGluR-6	homo-AMPA	MAP4	$G_{i/o}$ activation
mGluR-7	L-AP4	*Ibid*	Ibid
mGluR-8	Ibid	Unknown	- " -

Note. *Blocks opening of ion channels; +acts also as kainate receptor antagonist; °nonspecifically activates the majority of mGluR; ×nonspecifically inhibits the majority of mGluR.

TABLE 2. Effects of Rotenone (20 μM), Indomethacin (100 μM), 4-Methylpyrazole (4-MP, 50 μM), and Nialamide (100 μM) on ROS Formation in Cerebellar Granular Cells Induced by 30-min Incubation with Kainate, NMDA, or PMA

Conditions	Inhibition of ROS production, %
Kainate (0.25 mM)	—
+rotenone	0
+indomethacin	46.0±13.1
+4-MP	42.7±14.3
+nialamide	40.0±7.2
NMDA (0.25 mM)	—
+rotenone	0
+indomethacin	35.3±4.5
+4-MP	15.5±4.9
+nialamide	16.0±7.1
PMA (1 μM)	—
+rotenone	-7±5*
+indomethacin	-15±4*
+nialamide	-16±6*

Note. Negative inhibition indicates activation of ROS production. Respiratory chain, cyclooxygenase, cytochrome P-450, and monoamine oxidase are the targets for rotenone, indomethacin, 4-MP, and nialamide, respectively.

Ca^{2+} influx through ion channels of NMDA receptors leads to activation of kainate receptors due to Ca^{2+}-dependent phosphorylation of proteins by protein kinase.

It was assumed that activation of neurons via kainate or AMPA receptors would not cause ROS production, because these channels do not generate Ca^{2+} fluxes (Table 1). However, it was shown that kainate and AMPA increase intracellular ROS concentration, and this depends on extracellular Ca^{2+} [3,11,33]. All three ligands (NMDA, kainate, and AMPA) decrease mitochondrial membrane potential; the effect is inhibited by the corresponding antagonist [34]. These facts were difficult to explain until it was demonstrated that ion channels of non-NMDA receptors are also partially permeable for Ca^{2+} [29]. It is interesting that these channels are involved in the formation of epileptic activity (seizures) [30].

These data illustrate that the excitatory effect of ionotropic ligands can be easily transformed into excitotoxic effects. Even a minor rise of intracellular ROS can produce adverse effects, in particular, oxidative modification of Na^+/K^+-ATPase, the target enzyme in oxidative stress [4,12]. Damages to Na^+ pump alter the reverse glutamate transport. Furthermore, acidosis accompanying disturbances in oxygen metabolism stimulates dissociation of iron from transport protein (transferrin) complexes. This promotes oxidation of membrane lipids in nerve cells enriched with polyunsaturated and easily oxidized fatty acid chains. Disintegration of the neuronal membrane leads to massive Ca^{2+} influx, activation of enzymes (calpains, phospholipases, *etc.*), and can cause cell death.

The hazardous effects of ROS on brain neurons are often underestimated. It was believed that there is a multilevel antioxidant system in the brain (antioxidants inhibiting toxic effects of glutamate and preventing cell damage). This notion is now revised because of complex involvement of ROS in brain metabolism [2] and insufficiency of the antioxidant defense system in the brain [19].

Normal functioning of glutamatergic neurons would be impossible without a system counteracting damaging effects of ROS. We believe that mGluR attenuate toxic effects of various excitotoxic neurotransmitters, including glutamate.

Interaction between ionotropic and metabotropic receptors

Neuronal electric activity is provided by iGluR, while mGluR modulate this activity by regulating the duration and intensity of ion fluxes across the membrane and, therefore, contribute to long-term potentiation or inhibition of signals. During simultaneous activation of iGluR and mGluR by the corresponding ligands, mGluR prolong NMDA-induced fluxes in cerebellar neurons. This phenomenon can be considered as direct regulation of information processes by mGluR [31].

Unlike iGluR depending on extracellular Ca^{2+}, activation of mGluR stimulates mobilization of intracellular Ca^{2+}. This effect is realized via activation of phospholipase C and dissociation of inositol phosphate from membrane phosphoinositides, which initiates the release of Ca^{2+} into the cytoplasm [25,28]. Protein kinase C involved in this process probably affects NMDA and non-NMDA receptors [21]. Class I mGluR coupled to $G_{q/11}$ act via this mechanism. Although activation of class II and III mGluR also increases intracellular Ca^{2+} concentration, this process is mediated by other G-proteins (Table 1). They modulate protein kinases A and C, which elevates the cGMP/cAMP ratio in cells [8,24]. Since phosphorylation of NMDA receptors decreases their hyperactivity [18,24], this mechanism probably protects neuronal cells from neurotoxic effects of glutamate during activation of these receptors.

Normal functioning of iGluR is accompanied by intracellular accumulation of free radicals, which modulate the state and activity of receptor proteins. For example, NO forms complexes with thiols, which inhibit Na^+/K^+-ATPase in the presence of iron ions and aggravate toxic effects of glutamate by decreasing the uptake of neurotransmitters [29]. Other radicals (main-

ly $OH^{\bullet}$) inhibit, while antioxidants reactivate NMDA receptors [7].

Our studies showed that various metabolic processes are involved in ROS generation during activation of iGluR. Metabolic inhibitors reduce ROS signals measured on the model of single neuronal suspension [3] to a variable degree depending on the type of ligands inducing intracellular ROS generation (Table 2).

It was demonstrated that both NMDA and kainate generate rotenone-insensitive ROS signals. Nialamide inhibits kainate- and NMDA-induced ROS formation by 40 and 16%, respectively. The degree of inhibition of intracellular ROS formation by other compounds depends on the nature of activated receptors. These data indicate that activation of monoamine oxidases and cyclooxygenases contributes to ROS formation under the effects of kainate and NMDA, respectively. At the same time, phorbol myristate acetate (PMA) activating protein kinase C induces the formation of free radicals in reactions insensitive to these inhibitors.

According to a hypothesis of redox regulation of iGluR [32] the balance between pro- and antioxidants in the neuronal cell regulates neurocomputation and learning [32]. NMDA receptors are responsible for toxic effects of glutamate under conditions of cerebral ischemia accompanied by excessive production of NO or $O^{\bullet-}$ [6,20,23]. It was believed that antioxidants would protect neurons from toxic effects of excitotoxic neurotransmitters. Indeed, NMDA receptor antagonist orphenadrine partially protects neurons from neurotoxic effects of glutamate *in vitro* and *in vivo* [35]. At the same time, mGluR-activating compounds ACPD and L-AP4 and lazaroid U-83836E activating protein kinase C, but possessing no antioxidant properties, produce a more pronounced effect [36]. It was assumed that neuropeptide carnosine protects neurons from ROS also due to activation of protein kinase C [1,13].

We hypothesized that mGluR play a protective role in excitotoxic neurotransmitters. Various effects of glutamate on brain structures raise the question, which specific features of this simple molecule determine specific activation of more than 10 receptors in the same synapse. Studies of structural and functional interrelations by comparing structures of glutamate agonists and antagonists have failed. In many cases,

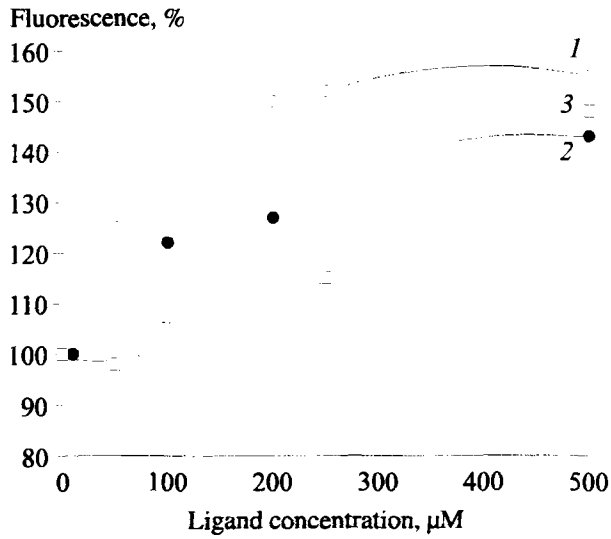

Fig. 1. Effects of various concentrations of ACPD (*1*), NMDA (*2*), and kainate (*3*) on reactive oxygen species signals measured in single-cell neuronal suspension using 2',7'-dichlorofluorescin diacetate.

these compounds have different structures, and even different localizations of receptor binding sites for natural and synthetic ligands can be suggested. This fact provides an excellent example of a variety of biological functions realized via combination of relatively simple biological elements.

Interaction of Na^+/K^+-ATPase with glutamate receptors

The interaction between structurally unrelated classes of GR determines synaptic plasticity. The mechanisms of this interaction include changes in the membrane potential modulating ligand binding, and the formation of second messengers induced by activation of specific enzymes (phospholipases, protein kinases, *etc.*). The interaction between iGluR and mGluR on the postsynaptic membrane regulates neuronal electric activity. However, the role of mGluR on the presynaptic membrane carrying no iGluR remains unclear.

Probably, these receptors and biochemical processes in the presynaptic membrane play a role in the regulation of neurotransmitter release and reuptake. Activation of mGluR induces the release of Ca^{2+} from

TABLE 3. Generation of ROS (% of Control) during Activation of Various GR in Rat Cerebellar Neurons [9] (*M±m*)

Conditions	Receptor type	ROS signal, %
NMDA	NMDA receptors	124±3
Kainate	Kainate receptors	109±2
ACPD	Metabotropic receptors	129±2
NMDA+ACPD	NMDA+metabotropic receptors	139±2
Kainate+ACPD	Kainate+metabotropic receptors	98±1

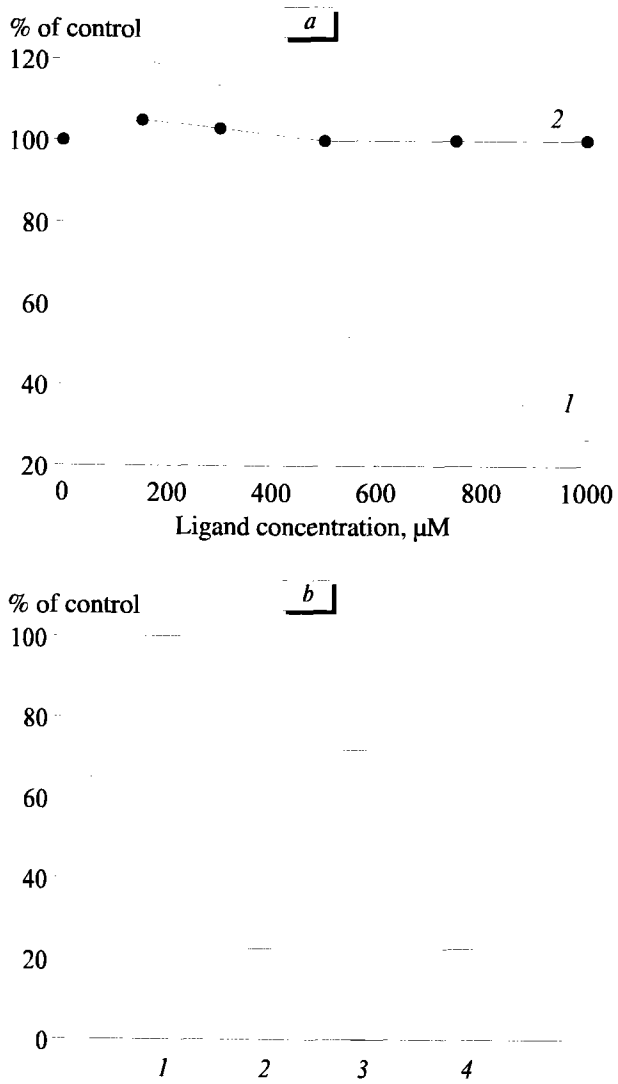

Fig. 2. Effects of glutamate and its analogues on Na^+/K^+-ATPase activity in rat cerebellar neurons: *a*) concentration-effect curve for glutamate (*1*) and kainate (*2*); *b*) enzyme activity in the control (*1*) and in the presence of 1 mM glutamate (*2*), 1 mM glutamate and 10 μM MK-801 (*3*), or 10 μM DNQX (*4*). Activity was measured after 30-min preincubation with the coresponding ligands.

intracellular stores, which participates in fusion of neurotransmitter vesicles with the presynaptic membrane. On the other hand, changes in the cAMP/cGMP ratio and modulation of protein kinase activities playing a role in the realization of metabotropic signals would affect Na^+/K^+-ATPase activity [5]. Since this enzyme provides asymmetric distribution of Na^+ and K^+ on the neuronal membrane, its activity is important for electric activity of cells and neurotransmitter reuptake. These facts prompt further studies on possible interrelations between Na^+/K^+-ATPase and GR.

Our recent studies showed that cerebral Na^+/K^+-ATPase is very sensitive to ROS: enzyme activity decreases after oxidation of its SH groups [4,10,12]. In addition, NO forms complexes with low-molecular-weight thiols containing iron ions and producing inhibitory or activating effects. Free radicals formed during cell metabolism can regulate active ion transport. The question arises of whether GR are involved in these regulatory mechanisms.

Surprisingly, measuring of free radical signals on single neuronal suspension revealed generation of ROS after activation of not only iGluR, but also mGluR (Table 3). The relative efficiency of signals induced by ACPD (mGluR activator, Table 1) was higher than that caused by kainate or NMDA (Fig. 1). Moreover, in combined application ACPD potentiated the effect of NMDA, but inhibited the effect of kainate (Table 3). Therefore, the interaction between these GR subtypes at the level of ROS generation differs from that realized via the electric activity, when mGluR stimulate kainate receptors and inhibit NMDA receptors.

To study the effects of ROS generated during GR activation, we measured Na^+/K^+-ATPase activity in neurons in the presence of various concentrations of glutamate (Fig. 2, *a*). The neurotransmitter produced a biphasic response: in high concentration it activated Na^+/K^+-ATPase, while in low concentration it inhibited the enzyme. Kainate in the same concentrations did not change enzyme activity. Taking into account different sensitivity of various GR to glutamate, it can be suggested that activation of the enzyme is mediated by mGluR, while its inhibition is realized via NMDA receptors. Inhibitory analysis (Fig. 2, *b*) showed that the effects of 1 mM glutamate were markedly attenuated by MK-801 (selective NMDA receptor antagonist), but not by DNOX (kainate/AMPA receptor antagonist). Moreover, NMDA also inhibited Na^+/K^+-ATPase [38].

It is unlikely that the opposite effects of iGluR (NMDA) and mGluR on Na^+/K^+-ATPase are due to generation of ROS. We hypothesized that activation of this enzyme by mGluR is mediated via protein kinase C. It was shown that Na^+/K^+-ATPase is inhibited during phosphorylation catalyzed by protein kinases [5, 15]. Hence, activation of phosphoprotein phosphatases by protein kinase C would activate Na^+/K^+-ATPase. At the same time, its inhibition by NMDA in high concentrations is probably related to neurotoxic effects of glutamate and ROS generation. Since toxic effects of glutamate increase the risk of cell death, protection of Na^+/K^+-ATPase from free radicals in neuronal cells seems to be very important.

Conclusion

Complex interaction between GR during functioning of glutamatergic structures attracts much attention, because these molecules play a role in cognitive functions of the brain and are most susceptible to neuro-

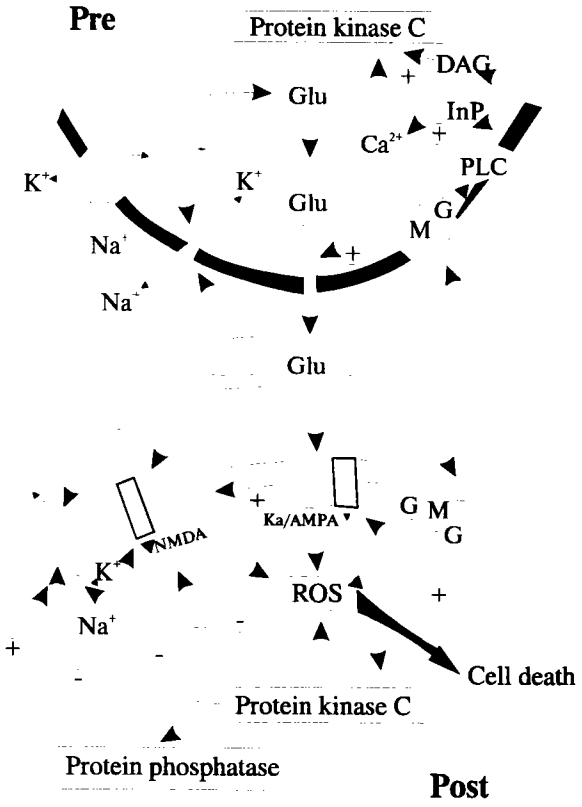

Fig. 3. Functional interaction between glutamate receptors and Na^+/K^+-ATPase in neuronal cells. Glutamate release from the presynaptic membrane (Pre) leads to activation of kainate/AMPA receptors (Ka/AMPA) on the postsynaptic membrane (Post), which are most sensitive to glutamate. Membrane depolarization caused by these receptors activates NMDA receptors. Glutamate produces no direct effects on Na^+/K^+-ATPase. Metabotropic receptors (M) coupled to G-proteins (G) trigger reactions (upper part of the scheme) leading to Ca^{2+} release in neurons and activation of protein kinase C. Kainate/AMPA receptors are activated, while NMDA receptors and Na^+/K^+-ATPase are inhibited under these conditions. Protein kinase C activates phosphoprotein phosphatase dephosphorylating and activating Na^+/K^+-ATPase. Hyperactivation of glutamate receptors leads to excessive production of intracellular reactive oxygen species (ROS) and causes cell death. DAG: diacylglycerol; InP: inositol phosphates; PLC: phospholipase C.

degenerative, age-related, and stress-induced damages. Studies of neurochemical mechanisms of their interaction will elucidate how the interaction between relatively simple molecules provides such a complex process as the higher nervous activity.

Figure 3 shows the key reactions triggered by glutamate-induced activation of various receptors. The effects of glutamate depend on the type of receptors. Complex regulation of the receptor sensitivity to ligands and modulation of key enzymes catalyzing the formation of second messengers in cells via membrane receptors contribute to the plasticity of the glutamatergic system.

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