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Specificity of Glutamate Receptors in P_2 Synaptosomal Fraction from Rat Brain Cortex

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Specificity of glutamate receptors in the P_2 synaptosomal fraction from the cerebral cortex of newborn rats was studied by measuring $^{45}Ca^{2+}$ uptake by synaptosomes in the presence of agonists of ionotropic and metabotropic glutamate receptors. It was shown that P_2 synaptosomal fraction from rat cortex contains NMDA receptors, kainate receptors, and group 1 metabotropic receptors.

Key Words: *ionotropic receptors; metabotropic receptors; synaptosomes; $^{45}Ca^{2+}$ uptake*

Glutamic acid, the major excitatory neurotransmitter in the central nervous system, activates ionotropic and metabotropic glutamate receptors (GR) [8,9]. Ionotropic GR include cation-specific ion channels and are divided into NMDA receptors and AMPA/kainate receptors [7]. As differentiated from NMDA receptors and AMPA/kainate receptors, metabotropic GR are not directly coupled to ion channels, but participate in the regulation of intracellular Ca^{2+} concentration through G proteins and secondary messengers. Metabotropic GR are divided into 3 groups. Group 1 receptors (mGluR 1 and 5) stimulate inositol-1,4,5-triphosphate synthesis, receptors of groups 2 (mGluR 2 and 3) and 3 (mGluR 4, 6, 7, and 8) inhibit adenylate cyclase and mediate the decrease in cAMP concentration [10].

Among various endogenous compounds, glutamate probably activates all subtypes of GR. The use of agonists specific for a certain subtype of receptors allows dividing ionotropic and metabotropic GR. Since activation of the receptor recognition site is directly or indirectly associated with the function of ion channels, opening of channels, and increase in current through the neuronal membrane, activity of GR can be estimated biochemically by recording changes in Ca^{2+} current through the membrane [13].

Here we studied GR subtypes in the P_2 synaptosomal fraction from the cerebral cortex of newborn rat pups. This *in vitro* model is suitable for evaluation of the effect of compounds on Ca^{2+} uptake in nerve endings.

MATERIALS AND METHODS

Synaptosomes were isolated from rat brain cortex by the standard method [6]. Experiments were performed on newborn Wistar rats (days 9-10 of life). For accumulation of the radioactive label, the P_2 synaptosomal fraction was suspended in incubation buffer A containing 132 mM NaCl, 5 mM KCl, and 5 mM HEPES (pH 7.4, final protein concentration 1.5-2.0 mg/ml). Ca^{2+} concentration in the final solution was 1.25 mM (1.4 μCi/ml). Glutamate [1] and agonists of ionotropic and metabotropic GR were used to stimulate $^{45}Ca^{2+}$ uptake by synaptosomes. After 3-min incubation with agonists of ionotropic

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and metabotropic GR at 37°C, $^{45}Ca^{2+}$ uptake was stopped by filtering the mixture through GF/B fiberglass filters (Whatman). The samples were washed 3 times with cold buffer solution B containing 145 mM HEPES, 10 mM Tris, and 54 mM Trilon B (pH 7.4) and radioactivity was measured in a liquid scintillation β-counter. The amount of $^{45}Ca^{2+}$ accumulated in synaptosomes was calculated as the difference between the concentrations of the radioactive label in the presence and absence of agonists and expressed in percents of the control (100%). The measurements were performed in 4-5 parallel samples (3-4 independent experiments).

The results were analyzed by Student's *t* test.

RESULTS

Glutamic acid in various concentrations activated all subtypes of GR in the P_2 synaptosomal fraction from rat brain cortex. $^{45}Ca^{2+}$ uptake by synaptosomes was maximum under the influence of glutamic acid in a concentration of 200 μM (Fig. 1).

The following agonists of GR were used to evaluate the subtype of ionotropic GR involved in $^{45}Ca^{2+}$ uptake: NMDA (5 μM glycine), kainic acid, and AMPA. $^{45}Ca^{2+}$ uptake by synaptosomes was maximum in the presence of 100-200 μM NMDA (Fig. 1). NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes decreased after addition of NMDA receptor antagonists MK-801 (IC_{50} ~1 μM), CPP (IC_{50} ~100 μM), memantine (IC_{50} ~0.4 μM), and Mg^{2+} (IC_{50} ~100 μM, Table 1). The estimated values of IC_{50} for CPP, Mg^{2+}, MK-801, and memantine are consistent with published data [3,14]. Our results indicate that the P_2 synaptosomal fraction from the cerebral cortex includes NMDA receptors.

Functional division of AMPA receptors and kainate receptors is difficult due to the absence of selective agonists and antagonists. Kainic acid produces non-desensitizing and rapid desensitizing effects on AMPA receptors and kainate receptors, respectively. By contrast, AMPA causes rapid desensitization of AMPA receptors and activates kainate receptors (non-desensitizing response). Kainic acid slightly increased $^{45}Ca^{2+}$ uptake by synaptosomes. This effect of kainic acid did not depend on its concentration (Fig. 1). $^{45}Ca^{2+}$ uptake did not decrease after addition of kainate receptor antagonist kynurenic acid (200 μM) and AMPA/kainate receptor inhibitor DNQX (1 μM). The stimulatory effect of AMPA on $^{45}Ca^{2+}$ uptake by synaptosomes depended on the concentration of this agent. $^{45}Ca^{2+}$ uptake was maximum in the presence of 0.5-1.0 μM AMPA (Fig. 1). DNQX in a concentration of 1 μM inhibited AMPA-induced $^{45}Ca^{2+}$ uptake. Cyclothiazide and concanavalin A (ConA) were used to divide AMPA receptors and kainate receptors. Desensitization of AMPA receptors and kainate receptors can be selectively modified with cyclothiazide and ConA, respectively. Cyclothiazide and ConA block desensitization of AMPA receptors and kainate receptors, respectively [11].

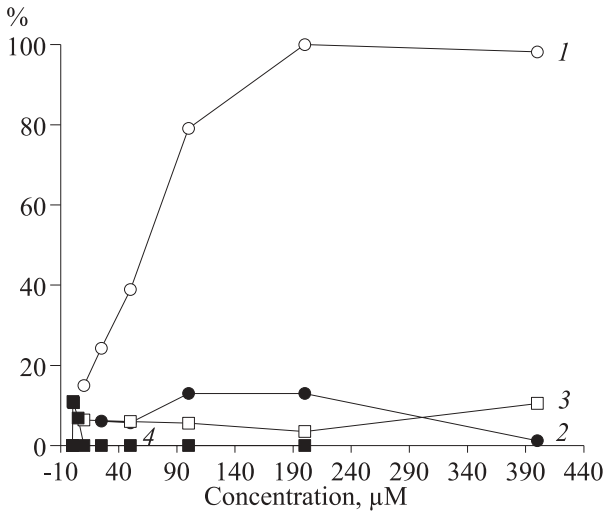

Fig. 1. Dependence of $^{45}Ca^{2+}$ uptake by synaptosomes of rat brain cortex on the concentration of glutamic acid (*1*), NMDA (*2*), kainic acid (*3*), and AMPA (*4*).

We attempted to divide AMPA receptors and kainate receptors and evaluate the subtype of GR in the P_2 synaptosomal fraction. Cyclothiazide did not potentiate the effects of AMPA and kainic acid.

TABLE 1. Effect of NMDA Receptor Antagonists on $^{45}Ca^{2+}$ Uptake by Synaptosomes of Rat Brain Cortex after Stimulation with NMDA (200 μM NMDA and 5 μM glycine, *M*±*m*)

Antagonist	Concentration, μM	Ca^{2+}, % of the control	IC_{50}, μM
MK-801	0.25	75.0±2.4	
	1.0	49.6±0.2	
	2.5	35.0±3.1	~1.0
	10	9.6±1.8	
	50	0	
Memantine	0.1	69.2±1.9	
	0.5	45.0±1.1	
	1.0	35.8±3.9	~0.4
	10	9.0±2.3	
	50	0	
CPP	50	26.1±0.9	~100
	100	53.3±1.4	
$MgCl_2$	100	54.9±0.7	~100
	1000	0	

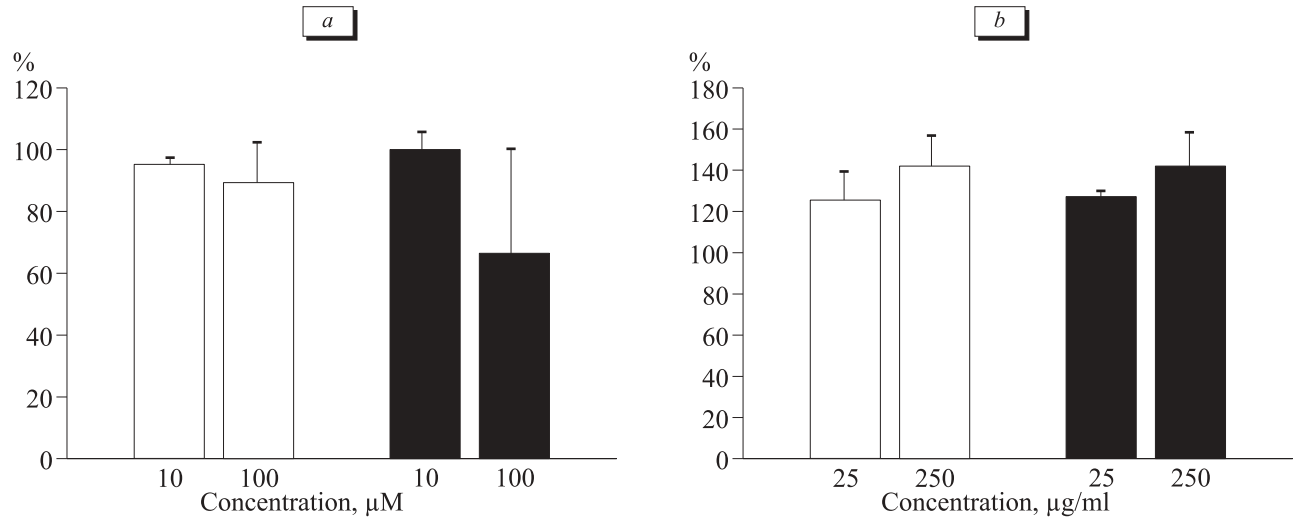

Fig. 2. Effects of cyclothiazide (*a*) and ConA (*b*) on $^{45}Ca^{2+}$ uptake by synaptosomes of rat brain cortex induced by 1 μM AMPA (light bars) or 100 μM kainic acid (dark bars).

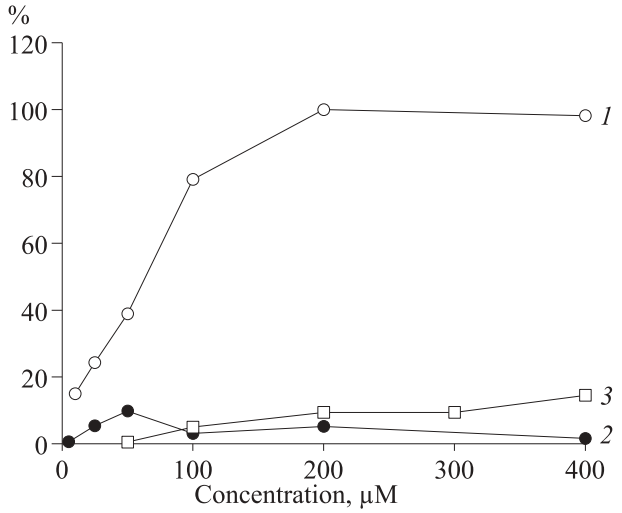

Fig. 3. Dependence of $^{45}Ca^{2+}$ uptake by synaptosomes of rat brain cortex on the concentration of glutamic acid (*1*), quisqualic acid (*2*), and ACPD (*3*).

Incubation of synaptosomes with ConA was accompanied by an increase in $^{45}Ca^{2+}$ uptake by synaptosomes upon stimulation with AMPA and kainic acid (Fig. 2). Therefore, the P_2 synaptosomal fraction from rat brain cortex includes kainate receptors.

$^{45}Ca^{2+}$ uptake by synaptosomes increased in the presence of metabotropic GR agonist quisqualic acid (maximum uptake was observed at 50 μM quisqualic acid). This effect remained unchanged after addition of DNQX and, therefore, was not mediated by ionotropic receptors. Quisqualate metabotropic GR were previously identified on synaptoneurosomes of 12-16-day-old rat pups [12].

Specific metabotropic GR agonist ACPD induced a dose-dependent increase in $^{45}Ca^{2+}$ uptake by synaptosomes (Fig. 3). $^{45}Ca^{2+}$ uptake was maximum in the presence of 200 μM ACPD. Metabotropic receptor antagonists L-AP3 and L-AP4 in a concentration of 10 μM completely inhibited ACPD-induced $^{45}Ca^{2+}$ uptake by synaptosomes.

L-AP3 and L-AP4 in high concentrations act as metabotropic receptor agonists [2]. We showed that L-AP3 and L-AP4 in high concentrations (200-800 and 200-400 μM, respectively) did not increase $^{45}Ca^{2+}$ uptake.

$^{45}Ca^{2+}$ uptake by synaptosomes in the presence of glutamate was higher compared to that observed after combined treatment with specific agonists (Figs. 1 and 3). This phenomenon requires further investigations. As differentiated from exogenous agonists of various subtypes of GR, glutamate is probably accumulated in synaptosomes and modulates Ca^{2+} release from intracellular stores (*e.g.*, mitochondria and endoplasmic reticulum) [4,5].

We conclude that the P_2 synaptosomal fraction from the cerebral cortex of newborn rat pups contains NMDA receptors, kainate receptors, and group 1 metabotropic receptors. These receptors are associated with phospholipase C and inositol-3-phosphate formation.

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