# Effect of Uridine of Presynaptic NMDA and Kainate Receptor of Rat Brain Cortex

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It was demonstrated that uridine affects presynaptic NMDA and kainite receptors of rat brain cortex. Uridine considerably inhibited $^{45}Ca^{2+}$ uptake into synaptoneurosomes ($IC_{50}$=7.1×10$^{-12}$ M) under conditions NMDA stimulation and increased it under conditions AMPA stimulation (157.8%).

**Key Words:** *uridine; presynaptic NMDA and kainite receptors; synaptosomes; $^{45}Ca^{2+}$ uptake*

The important role of uridine in the CNS was demonstrated in experiments *in vivo* (by uridine release) and *in vitro* (by uridine binding and uptake). Uridine completely inhibits activity of isolated single neuronal cells of the hypothalamus, modulates the rate of neuronal transmission *in vivo* [2,3], and induces transmembrane $^{45}Ca^{2+}$ current *in vitro*. It can be hypothesized that uridine is recognized by specific excitation receptors of the brain. Two types of uridine-binding centers were identified on membrane fragments and nerve terminals of rat cortex, which confirms the role of uridine in nerve transmission [6]. The existence of uridine-specific receptor in the brain is widely discussed [2,3,5-7,10]. Uridine can act not only directly through adenosine receptor [11]. It was shown that GTP-binding site of AMPA/kainite receptors (KR) also demonstrates affinity for uridine [4]. Experiments on hippocampal slices employing a fluorescent label showed that uridine application led to an increase in $[Ca]_{in}$, which attests to the involvement of uridine into the regulation of intracellular calcium via G protein [10]. It should be noted that [S]-willardiine, an uridine derivative, acts as agonist of AMPA receptors [14]. It is known that uridine potentiates GABA receptors, which leads to relaxation and behavioral sleep [9].

Presynaptic glutamate receptors can be possible targets for uridine. Thus, uridine acting on presynaptic NMDA receptors (NMDA-R) and KR can modulate synaptic transmission in CNS. Since activation of the recognition site of the receptor is directly or indirectly related to ionic channels, their opening, and activation of ionic current through the neuronal membrane, glutamate receptors can be studied using biochemical methods by measuring changes in $Ca^{2+}$ flux through the membrane [14].

Here we studied the effect of uridine on presynaptic NMDA-R and KR by evaluating $^{45}Ca^{2+}$ uptake into synaptosomes of rat brain cortex.

## MATERIALS AND METHODS

Synaptosomes were isolated routinely from the cortex of newborn (9-day-old) Wistar rats. The brain was homogenized in 10 volumes of cold 0.32 M sucrose (900 rpm). The homogenate was centrifuged at 1500*g* for 10 min, the resultant supernatant was centrifuged at 10,000*g* for 20 min. For accumulation of radioactive label, $P_2$ fraction of synaptosomes was suspended in incubation buffer A (132 mM NaCl, 5 mM KCl, 5 mM HEPES, pH 7.4, protein concentration 1.5-2.0 mg/ml). The con-

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centration of $Ca^{2+}$ in the final volume was 1.25 mM (1.4 μCi/ml). $^{45}Ca^{2+}$ uptake into synaptosomes was stimulated with NMDA (200 μM+5 μM glycine) and AMPA (1 μM). After 3-min incubation with NMDA or AMPA at 37°C, the uptake was stopped by filtering through GF/B fiberglass filters (Whatman) followed by 3-fold washout with cold buffer B (145 mM HEPES, 10 mM Tris, and 5.4 mM trilon B, pH 7.4). All experiments were performed in 3-4 parallels in 2-4 independent experiments. The radioactivity was measured using a liquid scintillation β-counter. $^{45}Ca^{2+}$ uptake into synaptosomes was determined by the difference in sample radioactivity after stimulation with the agonists and without stimulation and expressed in percents of the control (100%).

Specific $^{45}Ca^{2+}$ uptake was calculated by the formula:

$$K_{(42/21)}=[(Ca_4-Ca_3)/(Ca_2-Ca_1)]\times 100\%,$$

where $Ca_1$ is $^{45}Ca^{2+}$ uptake in the control (without agonists and test compounds); $Ca_2$ is $^{45}Ca^{2+}$ uptake after stimulation with an agonist (NMDA or AMPA); $Ca_3$ is $^{45}Ca^{2+}$ uptake in the presence of uridine (without agonist); $Ca_4$ is $^{45}Ca^{2+}$ uptake in the presence of agonist and uridine.

The data were processed statistically using Student *t* test.

## RESULTS

Uridine in concentrations of $10^{-12}$-$10^{-7}$ M inhibited the NMDA-induced $^{45}Ca^{2+}$ uptake into synaptosomes in a concentration-dependent manner (Fig. 1, *a*). The maximum inhibition of $^{45}Ca^{2+}$ uptake into synaptosomes was observed at uridine concentration of $10^{-8}$-$10^{-9}$ M ($IC_{50}$=7.1×$10^{-12}$ M). $^{45}Ca^{2+}$ uptake into synaptosomes upon NMDA stimulation is associated with activation of glutamate NMDA-R. The NMDA-induced $^{45}Ca^{2+}$ uptake into synaptosomes decreased after application of NMDA-R inhibitors MK-801 ($IC_{50}$ ~1 μM), CPP ($IC_{50}$ ~100 μM), memantin ($IC_{50}$ ~0.4 μM), $Mg^{2+}$ ($IC_{50}$ ~100 μM). These findings confirm that NMDA activates ionotropic NMDA-R of $P_2$ synaptosomal fraction from rat brain cortex [1]. Thus, our results suggest that uridine affects presynaptic NMDA-R of $P_2$ synaptosomal fraction from rat brain cortex.

Functional separation of AMPA-receptors (AMPA-R) and KR is difficult because of the absence of selective agonists and antagonists of these two types of ionotropic glutamate receptors. Kainic acid acts on AMPA-R as a non-desensitizing agent and on KR as a rapid desensitizing agent. AMPA induces rapid desensitization of AMPA-R and activates KR, demonstrating a non-desensitizing response. Desensitization of AMPA-R and KR can be selectively modulated by cyclothiazide and concanavalin A, respectively. Desensitization of AMPA-R and KR can be blocked by cyclothiazide and KR concanavalin A, respectively [12]. We showed that cyclothiazide does not increase the response induced by AMPA and kainic acid. AMPA- and kainic acid-stimulated $^{45}Ca^{2+}$ uptake into synaptosomes increased upon incubation of synaptosomes with concanavalin A. Thus, these results attest to the existence of KR in $P_2$ synaptosomal fraction from rat brain cortex. Stimulation of $^{45}Ca^{2+}$ uptake into synaptosomes from rat brain cortex with kainic acid yielded insignificant response, because of rapid desensitization of KR. AMPA significantly activates

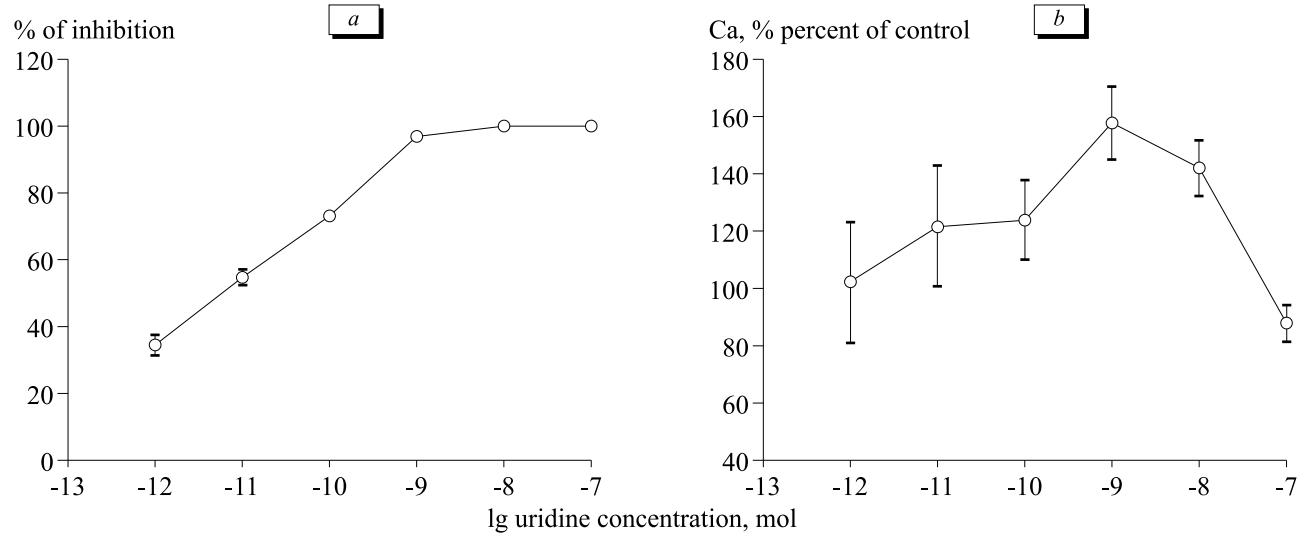


**Fig. 1.** Effect of uridine on $^{45}Ca^{2+}$ uptake into synaptosomes of rat cortex under conditions of NMDA (*a*) and AMPA (*b*) stimulation.

KR and not induces their desensitization. Maximum $^{45}Ca^{2+}$ uptake was induced by 0.5-1.0 μM AMPA [1]. Therefore, for activation of KR we used AMPA in a concentration of 1 μM. Uridine increased $^{45}Ca^{2+}$ uptake into synaptosomes under conditions of AMPA stimulation in the studied concentration range ($10^{-12}$-$10^{-7}$ M). The maximum increase in $^{45}Ca^{2+}$ uptake into rat cortex synaptosomes was observed at uridine concentration of $10^{-9}$ M (157.8% from the control). The dependence of $^{45}Ca^{2+}$ uptake into rat cortex synaptosomes on uridine concentration is described by a bell-shaped curve (Fig. 1, *b*).

These results suggest that uridine modulates presynaptic NMDA-R and KR, which can be a mechanism of uridine modulation of synaptic transmission in the CNS.

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