Tryptophan Inhibits the [^{3}H]Glutamate Uptake into *Xenopus* Oocytes Injected with Rat Brain mRNA

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ABSTRACT — We characterized the glutamate (Glu) uptake in *Xenopus* oocytes injected with rat brain mRNA. The Glu uptake into oocytes was higher in mRNA-injected oocytes than in vehicle-injected ones. Na^+ omission or addition of tryptophan inhibited the uptake in mRNA-injected oocytes, although it did not affect that in vehicle-injected oocytes. These results suggest that Glu transporters with a tryptophan sensitivity different from that of Glu transporters in native oocytes are expressed after injection of rat brain mRNA.

Keywords: Glutamate uptake, Brain mRNA, *Xenopus* oocytes

There has been interest in neurotransmitter transporters as target sites of antidepressants (ex. imipramine, desipramine), psychotropic drugs (ex. amphetamine, cocaine) and neurotoxins (ex. 1-methyl-4-phenylpyridinum ion (MPP^+), 6-hydroxydopamine). Transporters for noradrenaline (NA) (1), γ-aminobutyric acid (GABA) (2), serotonin (5-HT) (3) and dopamine (DA) (4, 5) have been cloned. Glutamate (Glu), which plays important functional roles in the central nervous system such as learning and memory and neuronal damage, has been shown to reuptake into both neurons and glia, since the transporters exist in glia (6), synaptic vesicles (7) and synaptic membranes (8). In the present paper, although Glu transporters are also present in native *Xenopus* oocytes, the Glu transporters expressed in oocytes injected with rat brain mRNA possess different sensitivity to tryptophan from that in native oocytes.

Total RNA was extracted from the whole brains of male Wistar rats by the cesium chloride method (9). $Poly(A)^+$ mRNA was purified by oligo (dT) cellulose chromatography (10) and stored as a sterile aqueous solution (1 mg/ml) at −80°C until use. *Xenopus* laevis were purchased from Hamamatsu Seibutsu Kyozai (Shizuoka, Japan) and maintained on assorted food for rainbow trout at 23°C. From *Xenopus* laevis anesthetized by cooling on ice, small pieces of ovarian lobes were dissected and placed in sterile modified Barth's solution (MBS: 88 mM NaCl, 1 mM KCl, 0.4 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 7.5 mM Tris-HCl pH 7.6). Follicular cells surrounding the oocytes were removed by collagenase treatment (1 mg/ml in Ca^{2+}-free MBS at 23°C for 10 min). Denuded oocytes were allowed to stand overnight at 23°C in MBS, and any deteriorated cells were discarded. Each healthy oocyte was given 50 ng mRNA by pressure pulse of nitrogen gas and cultivated 1–2 days in MBS. Four mRNA-injected or vehicle-injected oocytes were transferred to 1 ml of MBS buffer containing 10 μM Glu/37 kBq [2,3-^{3}H]Glu and incubated at 23°C. After the incubation, the reaction medium was removed and washed 3 times with ice-cold MBS. The oocytes were then added into 500 μl of 0.1% deoxycholate and sonicated. The radioactivity of the 400 μl of supernatant was measured by a liquid scintillation spectrometer.

The uptake of [^{3}H]Glu was increased with increasing incubation time in oocytes injected with rat brain mRNA (Fig. 1). In the oocytes injected with the vehicle, the uptake activity was lower than that of mRNA-injected oocytes (Fig. 1). The Glu uptake into mRNA-injected oocytes was reduced by displacing 88 mM NaCl into 163 mM sucrose in the medium (Table 1). In contrast, the removal of NaCl did not affect the [^{3}H]Glu uptake in vehicle-injected oocytes (Table 1). Ouabain (1 mM) pretreatment for 1 hr inhibited [^{3}H]Glu uptake into mRNA-injected and vehicle-injected oocytes. Removal of Ca^{2+} but not Mg^{2+} inhibited the uptake (Table 1). The different characteristics of the [^{3}H]Glu

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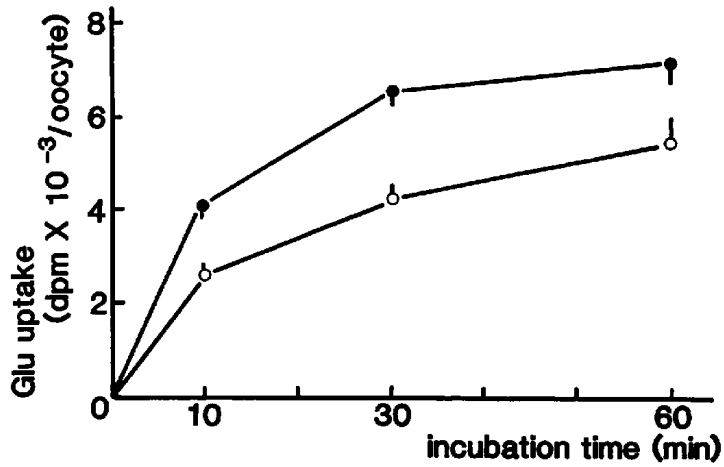

Fig. 1. Time course of the Glu uptake into native and mRNA-injected *Xenopus* oocytes. Oocytes were incubated with 10 μM Glu/37 kBq [^{3}H]Glu at 22°C. Each value shows the mean ± S.E. of the % of 4 experiments. ●: rat brain mRNA-injected oocytes, ○: vehicle-injected oocytes.

Table 1. Influence of extracellular ions and ouabain on glutamate uptake into native and brain mRNA-injected *Xenopus* oocytes

	Glu uptake (% of control)	
	vehicle-injected	mRNA-injected
Control	100.0 ± 6.8	226.9 ± 7.7 (100.0 ± 3.4)
Na^+-free	132.8 ± 11.2	114.3 ± 12.6 (50.4 ± 5.6)*
Ca^{2+}-free	16.3 ± 2.1*	63.2 ± 6.2 (27.9 ± 2.8)*
Mg^{2+}-free	109.0 ± 7.3	232.4 ± 17.0 (102.4 ± 7.5)
Control	100.0 ± 12.0	228.0 ± 25.1 (100.0 ± 11.0)
+ glutamate	29.3 ± 7.5*	29.2 ± 3.9 (12.8 ± 1.7)*
+ glutamine	54.6 ± 10.7*	93.4 ± 18.7 (41.0 ± 8.2)*
+ aspartate	44.2 ± 5.5*	49.7 ± 2.4 (21.8 ± 1.1)*
+ glycine	56.2 ± 3.8*	102.9 ± 11.1 (45.1 ± 4.9)*
+ tryptophan	93.9 ± 3.6	128.9 ± 17.9 (56.5 ± 7.9)*
+ taurine	69.3 ± 4.9	200.3 ± 35.2 (87.9 ± 15.4)
+ GABA	106.5 ± 13.0	236.4 ± 62.7 (103.7 ± 27.5)
1 mM ouabain	44.7 ± 3.2*	88.5 ± 4.7 (39.0 ± 2.1)*
at 0°C	21.5 ± 1.8	17.7 ± 2.1

Xenopus oocytes were incubated with 10 μM Glu/37 kBq [^{3}H]Glu at 22°C for 30 min. The Na^+-free medium was prepared exchanging 88 mM Na^+ to 163 mM sucrose. The Ca^{2+}-free medium also contained 2 mM EGTA. Each amino acid (at 100 μM) was simultaneously added with [^{3}H]Glu. Ouabain was treated 60 min before [^{3}H]Glu addition. Each value shows the mean ± S.E. of the % of the vehicle-injected control in 3 or 4 experiments. The values in parenthesis show the % of mRNA-injected control. Significance: *$P < 0.01$ vs. each control.

uptake between vehicle- and mRNA-injected oocytes were also shown in the influences of various amino acids at 100 μM on the uptake. The addition of Glu inhibited [^{3}H]Glu uptake, and the addition of glutamine, aspartate or glycine partially inhibited it in both mRNA- and vehicle-injected oocytes (Table 1). The addition of tryptophan inhibited the uptake into only mRNA-injected oocytes (Table 1). Taurine and GABA had no influence (Table 1).

Neurotransmitter transporters for NA (1), GABA

(2), 5-HT (3) and DA (4, 5) have recently been cloned. It has also been reported that brain Glu transporters are expressed in *Xenopus* oocytes injected with mRNA (11). The present findings regarding the properties of [^{3}H]Glu uptakes into mRNA- and vehicle-injected oocytes show that Glu transporters in the brain are different from those in oocytes with respect to two properties, Na^+-sensitivity and amino acid selectivity. The omission of Na^+ inhibited the reconstituted transporter activity, but it had no effects on native activity. The Glu uptake into synaptic vesicles (7) is Na^+-dependent. Thus it is suggested that the reconstituted Glu transporter is similar to the transporter of synaptic vesicles. Although the Glu uptake mechanisms are also reported in glial cells that are inhibited by 10 μM arachidonic acid (6), we found that both the mRNA- and the vehicle-injected oocytes were little affected by 10 μM arachidonic acid (data not shown), suggesting that the glial Glu transporters are not involved in the present results.

Tryptophan selectively inhibited the Glu uptake into mRNA-injected oocytes. The tryptophan uptake mechanism is present in the brain (12, 13) and the tryptophan is metabolized to kynurenic acid and quinolinic acid (13), which act at allosteric glycine sites in NMDA receptor/ion channel complexes as an endogenous agonist and antagonist, respectively (13, 14). Glycine also inhibited Glu uptake in both native and mRNA-injected oocytes. These results may possibly suggest that glycine regulates the Glu transport activity as well as the NMDA receptor/ion channel complex. It is interesting that tryptophan-induced selective inhibition of Glu uptake into brain mRNA-injected oocytes due to co-transport with Glu, to inhibition by tryptophan metabolites such as kynurenic acid and quinolinic acid, or to other reasons. Both native and reconstituted transporter activities were inhibited by pretreatment with ouabain, suggesting that both are dependent on ATP energy as previously reported (11). Furthermore, both activities were dependent on extracellular Ca^{2+}.

In conclusion, the present results suggest that although Glu transporter also exists on native oocytes, the characteristics of the transporters in the brain are different from those of the transporter in native oocytes in sensitivity to Na^+ and tryptophan.

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