Tryptophan Inhibits the $[^3]$HGlutamate Uptake into Xenopus Oocytes Injected with Rat Brain mRNA

Michihisa Tohda, Hisashi Urushihara and Yasuyuki Nomura*

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060, Japan

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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-phenylpyridinium ion (MPP$^+$), 6-hydroxydopamine). Transporters for noradrenaline (NA) (1), $\gamma$-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 $\mu$M Glu/37 kBq $[^3]$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 $\mu$l of 0.1% deoxycholate and sonicated. The radioactivity of the 400 $\mu$l 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...
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 [3H]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).

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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 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 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 Ca2+.

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.

REFERENCES

1. Pacholeczyk, T., Blakely, R.D. and Amara, S.G.: Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. Nature 350, 350 – 354 (1991)
2. Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedei, M.C., Davidson, N., Lester, H.A. and Kanner, B.I.: Cloning and expression of a rat brain GABA transporter. Science 249, 1303 – 1306 (1990)
3. Blakely, R.D., Berson, H.E., Fremeau, R.T., Jr., Caron, M.G., Peek, M.M., Prince, H.K. and Bradley, C.C.: Cloning and expression of a functional serotonin transporter from rat brain. Nature 354, 66 – 70 (1991)
4. Shimada, S., Kitayama, S., Lin, C.-L., Patel, A., Nanthakumar, E., Gregor, P., Kuhar, M. and Uhl, G.: Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science 254, 576 – 578 (1991)
5. Kilty, J.E., Lorang, D. and Amara, S.G.: Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254, 578 – 579 (1991)
6. Barbour, B., Szatkowski, M., Ingledew, N. and Attwell, D.: Arachidonic acid induces a prolonged inhibition of glutamate uptake into glial cells. Nature 342, 918 – 920 (1989)
7. Naito, S. and Ueda, T.: Characterization of glutamate uptake into synaptic vesicles. J. Neurochem. 44, 99 – 109 (1985)
8. Robinson, M.B., Hunter-Ensor, M. and Sinor, J.: Pharmacologically distinct sodium-dependent L-[3H]glutamate transport processes in rat brain. Brain Res. 544, 196 – 202 (1991)
9. Sambrook, J., Fritsch, E.F. and Maniatis, T.: Extraction of RNA with guanidium thiocyanate followed by centrifugation in cesium chloride solutions. In Molecular Cloning, p. 7.19 – 7.22, Cold Spring Harbor Lab. Press, New York (1989)
10. Sambrook, J., Fritsch, E.F. and Maniatis, T.: Selection of poly(A)+ RNA. In Molecular Cloning, p. 7.26 – 7.29, Cold Spring Harbor Lab. Press, New York (1989)
11. Blakely, R.D., Robinson, M.B. and Amara, S.G.: Expression of neurotransmitter transporter from rat brain mRNA in Xenopus laevis oocytes. Proc. Natl. Acad. Sci. U.S.A. 85, 9846 – 9850 (1988)
12. During, M.J., Freese, A., Heyes, M.P., Swartz, K.J., Markey, S.P., Roth, R.H. and Martin, J.B.: Neuroactive metabolites of 3-tryptophan, serotonin and quinolinic acid, in striatal extracellular fluid: effect of tryptophan loading. FEBS Lett. 247, 438 – 444 (1989)
13. Swartz, K.J., During, M.J., Freese, A. and Beal, M.F.: Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. J. Neurosci. 10, 2965 – 2973 (1990)
14. Kemp, J.A., Foster, A.C., Leeson, P.D., Priestley, T., Triggett, R., Iversen, L.L. and Woodrufl, G.N.: 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. Proc. Natl. Acad. Sci. U.S.A. 85, 6547 – 6550 (1988)