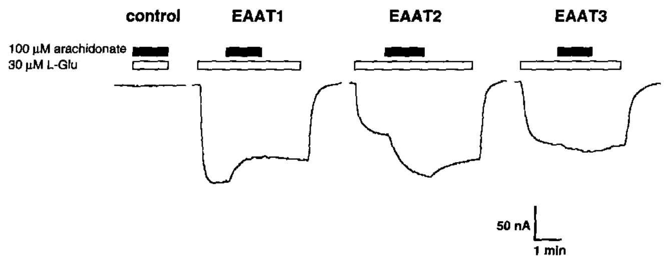

FIG. 1. **Differential effects of arachidonic acid on glutamate-induced currents recorded in voltage-clamped uninjected oocytes (control) and oocytes injected with RNA transcribed from human EAAT cDNAs.** Cells were clamped at −60 mV, and compounds were superfused for the times indicated by *open* (30 µM L-glutamate) and *closed* (100 µM arachidonic acid) *bars*. Identical results were seen in batches of oocytes from five frogs.

a rapid onset and reversed slowly upon washout of arachidonate (Fig. 1). These effects were due to specific interaction with the transporters, as application of arachidonate alone or with glutamate did not induce a current in uninjected oocytes (Fig. 1). In addition, arachidonate alone did not induce any current in oocytes expressing the transporters nor were the glutamate transport currents altered by co-application of vehicle (0.1% dimethyl sulfoxide) with glutamate (not shown). In order to determine whether the arachidonate-induced changes in transport currents mediated by EAAT1 and EAAT2 resulted from changes in glutamate uptake, measurements were made of radiolabeled L-glutamate transport mediated by these subtypes. Uptake of 10 µM L-[^{3}H]glutamate into oocytes expressing the transporters was linear for at least 20 min and was increased 10–100-fold over control (uninjected or water-injected) oocytes. In agreement with voltage clamp measurements, addition of arachidonic acid (100 µM) resulted in differential effects on the transporters (Fig. 2). Specific uptake in oocytes expressing EAAT1 was decreased from 275 ± 9 to 210 ± 10 fmol/oocyte/s (mean ± S.E. n = 6). In contrast, uptake in oocytes expressing EAAT2 was increased from 75 ± 6 to 143 ± 11 fmol/oocyte/s (n = 6; Fig. 2). Similar effects were seen in measurements of 10 µM L-[^{3}H]glutamate into the human embryonic kidney cell line HEK-293 stably transfected with EAAT1 or EAAT2. Co-application of 100 µM arachidonate reduced EAAT1-mediated uptake from 158 ± 8 to 123 ± 9 pmol/10^5 cells (n = 6), while uptake of L-[^{3}H]glutamate mediated by EAAT2 was increased from 120 ± 6 to 175 ± 6 pmol/10^5 cells (n = 6). The background in control (non-transfected) cells was not significantly changed by addition of arachidonate (10.9 ± 1.0 control *versus* 9.9 ± 1.0 pmol/10^5 cells; n = 6).

The effects of arachidonate on transport in oocytes expressing EAAT1 and EAAT2 were dose-dependent and saturable. The $K_{0.5}$ for the maximal inhibition of the EAAT1 current was 16 ± 6 µM, while the $K_{0.5}$ for maximal increase of the EAAT2 current was 59 ± 6 µM (n = 5; Fig. 3, *A* and *B*). The kinetic mechanism underlying the modulation of transport was investigated by analyzing the effect of arachidonic acid on the glutamate concentration response. Co-application of 100 µM arachidonic acid with varying doses of glutamate to oocytes expressing EAAT1 resulted in a 29 ± 1% decrease in the maximal current relative to control, while the apparent affinity for glutamate was not significantly altered (K_m = 42 ± 3 *versus* 37 ± 1 µM, n = 5; Fig. 4*A*). In contrast, the EAAT2 transport currents were enhanced via a decrease in the transport affinity constant for glutamate from 36 ± 7 to 14 ± 1 µM (n = 5; Fig. 4*B*).

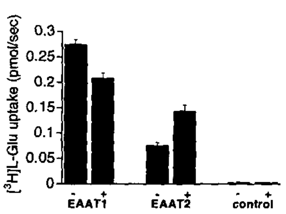

FIG. 2. **Arachidonic acid inhibits uptake of L-[^{3}H]glutamate into oocytes expressing EAAT1 and stimulates uptake mediated by EAAT2.** Uptake in control (uninjected) oocytes was unaffected by arachidonic acid.

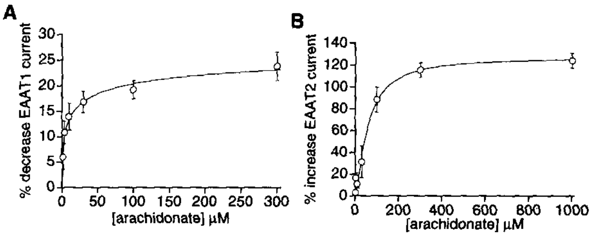

FIG. 3. **Concentration response for arachidonic acid effects on glutamate transport currents mediated by EAAT1 (*A*) and EAAT2 (*B*).** The percentage decrease or increase was calculated by comparing the current amplitudes induced by 30 µM L-glutamate co-applied with varying concentrations of arachidonic acid to the control transport current amplitudes in response to 30 µM L-glutamate in the same oocytes. Data points represent mean ± S.E. (n = 3–4).

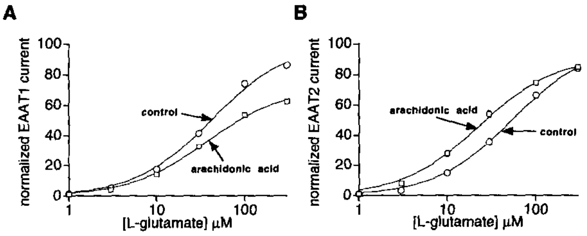

FIG. 4. **Differential effects of arachidonic acid on transport kinetic parameters for EAAT1 (*A*) and EAAT2 (*B*).** Data points representing normalized mean ± S.E. for 5 oocytes were fit to $I = I_{\max}([\mathrm{Glu}]/([\mathrm{Glu}] + K_m)$. *A*, co-application of 100 µM arachidonic acid reduced the maximal EAAT1 current 29 ± 1% without significantly changing the apparent affinity for glutamate. *B*, glutamate concentration dependence of EAAT2 transport currents was shifted by 100 µM arachidonic acid from 36 ± 7 to 14 ± 1 µM without significantly affecting the $I_{\max}$.

The pharmacological mechanism of action of arachidonic acid was investigated by testing structural analogues and inhibitors of its metabolism. The increase in EAAT2-mediated transport of 10 µM L-glutamate caused by 100 µM arachidonic acid was compared with equal concentrations of linolenic, linoleic, or arachidic acid. The order of efficacy relative to arachidonic acid was linolenic acid (91 ± 21%) > linoleic acid (49 ± 9%) ≫ arachidic acid (1 ± 5%; n = 3). The same rank order of efficacy relative to arachidonic acid was observed for inhibition of EAAT1: linolenic (109 ± 19%) > linoleic (43 ± 14%) ≫ arachidic (5 ± 5%; n = 3). Neither the cyclooxygenase inhibitor indomethacin (100 µM) nor the lipoxygenase inhibitor nordihydroguaritic acid (50 µM) affected arachidonate inhibition of EAAT1 (n = 3) or stimulation of EAAT2 (n = 6). These results, together with the rapid onset of modulation (Fig. 1), suggest that both effects on glutamate transport are mediated directly by arachidonic acid itself, rather than via a metabolite.

DISCUSSION

Arachidonic acid has been proposed to be a messenger molecule that influences synaptic transmission released by synaptic activation of ionotropic and metabotropic glutamate receptors (8, 9). Its release also occurs following ischemia (10, 11). Arachidonic acid-mediated inhibition, but not stimulation, of glutamate transport has been reported previously (13–16). Similar to the results in the present study, these inhibitory actions appear to be mediated by arachidonic acid itself, as are its potentiating actions on synaptic transmission in hippocampus (12). Although net L-[^{3}H]glutamate uptake into rat brain synaptosomes and slices is reduced by arachidonic acid (13–15), regional heterogeneity in transporter expression would not be resolved in such an assay (6, 7). EAAT1 is relatively abundant in many human brain regions, particularly in cerebellum, while EAAT2 is highly abundant in forebrain and midbrain regions including cortex and basal ganglia (6). EAAT3 is uniformly expressed in brain and periphery at lower levels (6). These distributions are consistent with the immunohistochemical localization in rat brain of the analogous glutamate transporters with which they share >90% sequence identities (7).

The glutamate receptor-mediated stimulation of arachidonate synthesis in glial cells (17) and neurons (8, 9, 18) suggests that arachidonic acid could modulate uptake in both cell types according to which transporter subtype is expressed. In one well defined system, the salamander retinal Mueller cell, arachidonic acid directly inhibits glutamate transport currents (16). In accord with this result, a transcript encoding a glutamate transporter homologous to EAAT1 is highly abundant in these cells, and expression of this salamander transporter in *Xenopus* oocytes confirms that it is negatively modulated by arachidonate.[2] While some studies on cultured mammalian glial cells have demonstrated arachidonic acid inhibition of glutamate uptake (15, 19), a recent study utilizing cultured astroglial cells demonstrated that inhibition of arachidonic acid synthesis during ischemia potentiates toxicity, suggesting a possible neuroprotective action of arachidonic acid (20).

The present results suggest the possibility that arachidonic acid could differentially influence the rate of clearance of synaptically released glutamate, which can in turn influence the kinetics of glutamatergic transmission at some synapses (21, 22). Moreover, modulation of interstitial glutamate levels could lead to changes in synaptic efficacy by tonic activation of receptors (23) or receptor desensitization (24). The actions of arachidonic acid on glutamate transport kinetics would allow for complex regulation of synaptic transmission as well as excitotoxicity via either positive or negative feedback according to which transporter subtypes are present in a particular pathway.

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