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Research Paper

Amitriptyline inhibits the activity of the rat glutamate transporter EAAT3 expressed in *Xenopus* oocytes

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Abstract

Objectives Evidence suggests that glutamatergic systems may be involved in the pathophysiology of major depression and the mechanism of action of antidepressants. We have investigated the effects of amitriptyline, a tricyclic antidepressant, on the activity of the excitatory amino acid transporter type 3 (EAAT3), a protein that can regulate extracellular glutamate concentrations in the brain.

Methods EAAT3 was expressed in *Xenopus* oocytes. Using a two-electrode voltage clamp, membrane currents were recorded after application of 30 μM L-glutamate in the presence or absence of various concentrations of amitriptyline or after application of various concentrations of L-glutamate in the presence or absence of 0.64 μM amitriptyline.

Key findings Amitriptyline concentration-dependently reduced EAAT3 activity. This inhibition reached statistical significance at 0.38–1.27 μM amitriptyline. Amitriptyline 0.64 μM reduced the pharmacokinetic parameter V_{max}, but did not affect the pharmacokinetic parameter K_m, of EAAT3 for L-glutamate. The amitriptyline inhibition disappeared after a 4-min washout. Phorbol-12-myristate-13-acetate, a protein kinase C activator, increased EAAT3 activity. However, 0.64 μM amitriptyline induced a similar degree of decrease in EAAT3 activity in the presence or absence of phorbol-12-myristate-13-acetate.

Conclusions Our results suggested that amitriptyline at clinically relevant concentrations reversibly reduced EAAT3 activity via decreasing its maximal velocity of glutamate transporting function. The effects of amitriptyline on EAAT3 activity may have represented a novel site of action for amitriptyline to increase glutamatergic neurotransmission. Protein kinase C may not have been involved in the effects of amitriptyline on EAAT3.

Keywords amitriptyline; glutamate; glutamate transporters; protein kinase C

Introduction

Glutamate is the principal excitatory amino acid neurotransmitter in the central nervous system.[1] Glutamate transporters (also called excitatory amino acid transporters, EAATs) regulate glutamate concentrations in the synaptic cleft by transporting glutamate from extracellular space to intracellular compartments under physiological conditions, which can prevent extracellular glutamate accumulation and regulate glutamatergic neurotransmission.[2–4] Alterations in EAAT functions have been suggested to play a role in several acute and chronic nervous system diseases, such as psychosis, epilepsy and amyotrophic lateral sclerosis.[5,6] Five EAATs have been identified: EAAT 1–5.[4] The transporting functions of all five EAATs are Na^+-dependent. They use the transmembrane gradient of Na^+, K^+ and H^+ as the driving force for glutamate uptake.[4,7]

A growing body of evidence from preclinical and clinical research suggests that brain glutamatergic systems may be involved in the pathophysiology of major depression and the mechanism of action of antidepressants.[8–12] Tricyclic antidepressants are commonly used drugs for major depressive disorders and are also widely used in chronic pain states, such as neuropathic and inflammatory pain.[13–15] The effects of tricyclic antidepressants have been thought to be mediated mainly by inhibition of monoamine reuptake. However, recent studies have suggested the involvement of the glutamatergic system in the action of these

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drugs.[8,16,17] Amitriptyline, a commonly used tricyclic antidepressant, has been shown to regulate the mRNA expression of EAAT3, the major neuronal EAAT, in rat brain.[17] However, it is not known whether tricyclic antidepressants could affect EAAT activity. Thus, we have designed this study to determine the effects of amitriptyline on EAAT3 activity by using the *Xenopus* oocyte expression system.

Materials and Methods

Animal protocols

The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Virginia (Charlottesville, VA, USA). The recommendations from the Declaration of Helsinki and the internationally accepted principles in the care and use of experimental animals were adhered to during the study. Mature female *Xenopus laevis* frogs were purchased from Xenopus I (Ann Arbor, MI, USA). All reagents, unless specified below, were obtained from Sigma (St Louis, MO, USA).

Oocyte preparation and injection

As described by Do *et al.*[18], frogs were anaesthetized in 500 ml 0.2% 3-aminobenzoic acid ethyl ester in water until unresponsive to painful stimuli (toe pinching) and underwent surgery on ice. Oocytes were surgically retrieved and placed immediately in modified Barth's solution. The Barth's solution comprised (in mM): 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.41 $CaCl_2$, 0.82 $MgSO_4$, 0.3 $Ca(NO_3)_2$, 0.1 gentamicin and 15 HEPES, with pH adjusted to 7.5. The oocytes were defolliculated with gentle shaking for approximately 2 h in calcium-free OR-2 solution. The OR-2 solution comprised (in mM): 82.5 NaCl, 2 KCl, 1 $MgCl_2$, 4 HEPES, and 0.1% collagenase type Ia with pH adjusted to 7.5. The oocytes were then incubated in modified Barth's solution that did not contain collagenase at 16°C for one day before the injection of EAAT3 mRNA.

The rat EAAT3 complementary DNA (cDNA) construct was provided by Dr M. A. Hediger (Brigham and Women's Hospital, Harvard Institutes of Medicine, Boston, MA, USA). The cDNA was subcloned in a commercial vector (BluescriptS Km). The plasmid DNA was linearized with a restriction enzyme (NotI) and mRNA was synthesized *in vitro* with a commercially available kit (Ambion, Austin, TX, USA). The resulting mRNA was quantified spectrophotometrically and diluted in sterile water. This mRNA was used for the cytoplasmic injection of oocytes in a concentration of 40 ng/40 nl by using an automated microinjector (Nanoject; Drummond Scientific Co., Broomall, PA, USA). Oocytes were then incubated at 16°C in modified Barth's solution for three days before voltage-clamping experiments.

Electrophysiological recordings

Experiments were performed at room temperature (approximately 21–23°C). A single oocyte was placed in a recording chamber that was <1 ml in volume and was perfused with 5 ml Tyrode's solution/min. The Tyrode's solution comprised (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 dextrose and 10 HEPES, with pH adjusted to 7.5. Clamping microelectrodes were pulled from capillary glass (10 μl Drummond Microdispenser, Drummond Scientific Co.) on a micropipette puller (model 700C; David Kopf Instruments, Tujunga, CA, USA). Tips were broken at a diameter of approximately 10 μm and filled with 3 M KCl obtaining resistance of 1–3 MΩ. Oocytes were voltage-clamped using a two-electrode voltage clamp amplifier (OC725-A; Warner Corporation, New Haven, CT, USA) that was connected to a data acquisition and analysis system running on a personal computer. The acquisition system consisted of a DAS-8A/D conversion board (Keithley-Metrabyte, Taunton, MA, USA). Analyses were performed with pCLAMP7 software (Axon Instruments, Foster City, CA, USA). All measurements were performed at a holding potential of −70 mV. Oocytes that did not show a stable holding current less than 1 μA were excluded from analysis. L-Glutamate was diluted in Tyrode's solution and superfused over the oocyte for 25 s (5 ml/min). L-Glutamate-induced inward currents were sampled at 125 Hz for 1 min: 5 s of baseline, 25 s of L-glutamate application and 30 s of washing with Tyrode's solution. The glutamate-induced peak currents were calculated to reflect the amount of glutamate transported. We used 30 μM L-glutamate, unless indicated otherwise, in this study because the value of the pharmacokinetic parameter K_m of EAAT3 for L-glutamate was shown to be 27–30 μM in previous studies.[18,19]

Administration of experimental chemicals

Amitriptyline was dissolved in methanol (Fisher Scientific, Fair Lawn, NJ, USA) and then diluted by Tyrode's solution to the appropriate final concentration (10, 60, 120, 200, 280 or 400 ng/ml that corresponded to 0.032, 0.19, 0.38, 0.64, 0.89 or 1.27 μM, respectively). In the control experiments, oocytes were perfused with Tyrode's solution for 4 min before the application of Tyrode's solution containing L-glutamate for the electrophysiological recording. In the amitriptyline-treated group, oocytes were perfused with Tyrode's solution for the first minute for stabilization followed by Tyrode's solution containing amitriptyline for 3 min before the application of Tyrode's solution containing L-glutamate for the electrophysiological recording. To determine the reversibility of the effects of amitriptyline, the responses to L-glutamate were assayed, oocytes were then treated with 0.64 μM amitriptyline and the responses to L-glutamate were recorded again. Subsequently, oocytes were perfused with Tyrode's solution for 4 min and the responses to L-glutamate were measured for the third time. To study the effects of protein kinase C (PKC) activation on amitriptyline-induced change of EAAT3 activity, oocytes were pre-incubated with 100 nM phorbol 12-myristate-13-acetate (PMA), a PKC activator, for 10 min just before the application of 0.64 μM amitriptyline.

Data analysis

Responses are reported as mean ± SD. Each experimental condition was performed with oocytes from at least three different frogs. Since the expression level of transporter proteins in oocytes of different batches may vary, variability in response among batches of oocytes is common. Thus,

responses were normalized to the mean values of the same-day controls for each batch. Similarly, in the reversibility experiments, responses were normalized to the responses of the same oocytes to 1 mM glutamate under control conditions (before the amitriptyline treatment). This concentration of glutamate was the highest concentration used to induce EAAT3 activity and this normalization allowed us to pool together data from different batches of oocytes for analysis. Statistical analysis was performed by one-way repeated measures analysis of variance (for the reversibility study) or one-way analysis of variance (for all other experiments) followed by the Tukey multiple comparison test. A $P < 0.05$ was accepted as significant.

Results

L-Glutamate (30 μM) induced no current in the oocytes without injection of EAAT3 mRNA (data not shown) and an inward current in oocytes injected with EAAT3 mRNA (Figure 1). Our previous studies showed that this current was mediated via EAAT3.[18,19] The glutamate-induced current in oocytes expressing EAAT3 was not affected by 0.04% (v/v) methanol, the solvent for amitriptyline (0.92 ± 0.48-fold compared with control, $n = 11$, $P > 0.05$). This concentration of methanol was the highest concentration expected in the Tyrode's solution containing amitriptyline. While amitriptyline alone did not induce any current in oocytes injected with or without EAAT3 mRNA (data not shown), amitriptyline concentration-dependently reduced EAAT3 responses with an IC50 value (concentration required for 50% inhibition) of 0.11 μM (Figure 1). Since maximal inhibition was reached at concentrations higher than 0.38 μM, we used 0.64 μM for further experiments. The EAAT3 response to L-glutamate in the presence of 0.64 μM amitriptyline was reduced by ~20% (Figure 1).

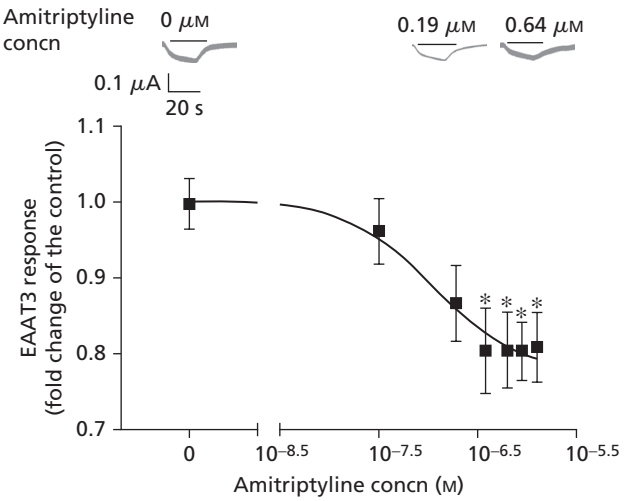

Figure 1 Dose–response of amitriptyline inhibition of excitatory amino acid transporter 3 responses to L-glutamate. The IC50 value for this inhibition was 0.11 μM. Three typical current traces are shown at the top of the figure. The short line above each current trace represents the time of L-glutamate (30 μM) application. EAAT3, excitatory amino acid transporter 3. Data are mean ± SD, $n = 27$–48 in each data point. *$P < 0.05$ compared with control.

Table 1 Pharmacokinetic parameters of the excitatory amino acid transporter 3 response to L-glutamate

Pharmacokinetic parameter	Control	Amitriptyline	Tyrode's washout
V_{max}	1.20 ± 0.10	0.94 ± 0.10*	1.08 ± 0.10
K_m (μM)	35 ± 16	42 ± 15	37 ± 14

The Michaelis–Menten elimination constants V_{max} and K_m were measured. The excitatory amino acid transporter 3 (EAAT3) responses to various L-glutamate concentrations were measured either before (control) or immediately after amitriptyline treatment (amitriptyline), and after a 4-min Tyrode's perfusion (Tyrode's washout). Data are mean ± SD, $n = 6$. *$P < 0.05$ compared with the corresponding values in the control group.

Consistent with our previous studies, the value of K_m of EAAT3 for L-glutamate was 35 ± 16 μM (Table 1).[18,19] This K_m value was not significantly affected by 0.64 μM amitriptyline. However, amitriptyline significantly decreased the value for V_{max} of EAAT3 for L-glutamate (from 1.20 ± 0.10 of control group to 0.94 ± 0.10 of amitriptyline group, $P < 0.05$). This amitriptyline effect disappeared in oocytes perfused with Tyrode's solution for 4 min after amitriptyline treatment (Figure 2).

Pre-incubation of the oocytes with 100 nM PMA, a PKC activator, increased EAAT3 activity significantly compared with the control (Figure 3). However, 0.64 μM amitriptyline caused a similar degree of decrease in EAAT3 response no matter whether PMA was present (EAAT3 responses in the amitriptyline groups were 0.84 ± 0.32 and 0.78 ± 0.34-fold of the corresponding controls respectively in the presence or absence of PMA, $n = 24$–45, $P > 0.05$).

Discussion

Antidepressants have been used for decades and their therapeutic effects have been thought to be mediated by inhibition of monoamine uptake. However, antidepressant-like activity can be produced not only by drugs modulating the glutamatergic synapses, but also by agents that affect subcellular signalling systems linked to excitatory amino acid receptors.[20] Since EAATs can regulate extracellular glutamate concentrations and glutamatergic neurotransmission, EAATs may be a target for antidepressants.[4] Consistent with this idea, it has been shown that amitriptyline regulates EAAT3 mRNA expression in rat brain.[17] Amitriptyline also blocks morphine-induced redistribution of EAAT1 and EAAT2, but not EAAT3, from the plasma membrane to intracellular space in rat spinal cord and such effects have been considered to play a role in the reduction of morphine tolerance by amitriptyline.[21]

Our results showed that amitriptyline concentration-dependently reduced EAAT3 activity with an IC50 value of 0.11 μM. This inhibition was statistically significant at concentrations higher than 0.38 μM. The exact amitriptyline concentrations around EAAT3 in the brains of patients on therapeutic doses of amitriptyline are not known. However,

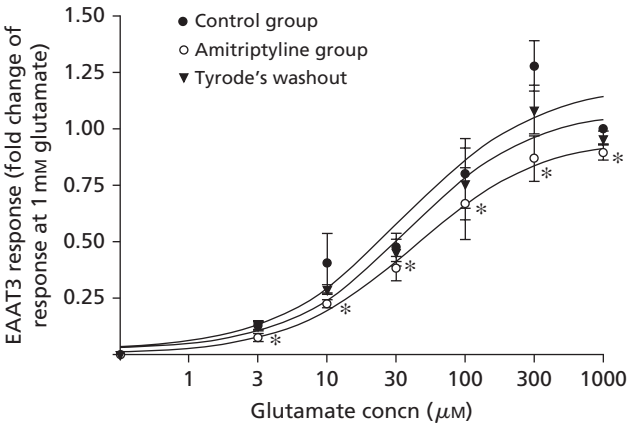

Figure 2 Effects of amitriptyline on excitatory amino acid transporter 3 activity. The excitatory amino acid transporter 3 (EAAT3) responses to various L-glutamate concentrations were measured either before or immediately after amitriptyline treatment, and after a 4-min Tyrode's perfusion. See Table 1 for the V_{max} and K_m values of the EAAT3 response to L-glutamate. Data are means ± SD, $n = 6$ in each data point. *$P < 0.05$ compared with the corresponding values in the control group.

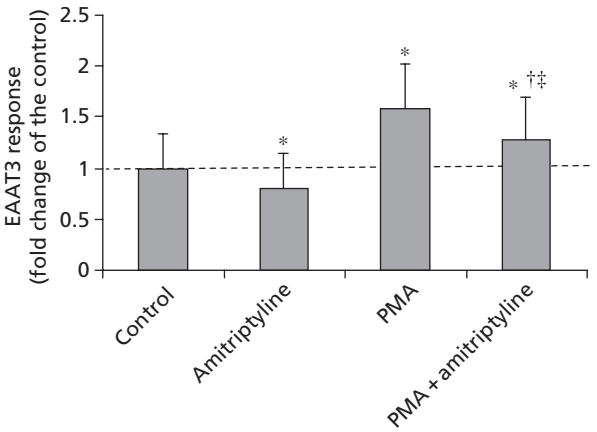

Figure 3 Effects of protein kinase C activation on excitatory amino acid transporter 3 activity. Excitatory amino acid transporter 3 (EAAT3) activity was observed in the presence or absence of 0.64 μM amitriptyline. Oocytes were either exposed to 100 nM phorbol-12-myristate-13-acetate (PMA, a protein kinase C activator) for 10 min or not before they were stimulated by 30 μM L-glutamate in the presence or absence of amitriptyline. The dotted line represents the mean value of the control group. Data are means ± SD, $n = 24$–45 in each data point. *$P < 0.05$ compared with control; †$P < 0.05$ compared with PMA alone; ‡$P < 0.05$ compared with amitriptyline alone.

the therapeutic range of plasma concentrations of amitriptyline for depression is 0.3–0.8 μM.[22] In humans the concentration ratio of amitriptyline in the cerebral spinal fluid vs blood is ~0.3.[23] Thus, the therapeutic range of amitriptyline concentrations in the cerebral spinal fluid may fall into the effective concentrations that could inhibit EAAT3 activity. In addition, it has been estimated that the amitriptyline concentrations in the brains of patients on therapeutic doses is approximately 1–10 μM, concentrations that are high enough to maximize the inhibitory effects of amitriptyline on EAAT3 activity as shown in our study.[24] Thus, our results suggested that amitriptyline at clinically relevant concentrations could inhibit EAAT3 activity.

Our results showed also that amitriptyline reduced the V_{max} but did not affect the K_m of EAAT3 for L-glutamate. This suggested that amitriptyline did not affect the affinity of EAAT3 for L-glutamate but decreased the total EAAT3 available for transporting glutamate. Interestingly, the amitriptyline-decreased EAAT3 activity recovered after a short washout, indicating that the amitriptyline effect was reversible. It has been proposed that amitriptyline, by inhibiting the uptake of monoamines, could increase serotonin in the synaptic cleft, which could then activate the glutamatergic synapses and increase the amount of glutamate in the synaptic cleft.[25] Our results suggested that direct inhibition of EAAT activity may have been another mechanism for amitriptyline to increase glutamate concentrations in the synapses. Since reduced glutamate levels are seen in anterior cingulated cortex of patients with depression, the inhibition of EAAT activity by amitriptyline may have contributed to its antidepressant effects.[10]

It has been shown that the loss of EAAT3 produced mild neurotoxicity and induced epilepsy.[26] Therefore, inhibition of EAAT3 activity by amitriptyline may have caused neurotoxicity. However, this effect may not have occurred for the following reasons. Firstly, EAATs other than EAAT3 may have partially compensated the function of EAAT3. Secondly, the maximal inhibition of EAAT3 activity by amitriptyline was ~20% and this ceiling effect may have safeguarded the degree of glutamate increase in the synaptic cleft. Finally, amitriptyline has been shown to block N-methyl D-aspartate (NMDA)-induced toxicity, although the ED50 (effective dose, 50%) for this protection was ~7 μM, higher than the concentrations needed to inhibit EAAT3 activity in this study.[27]

Multiple studies have shown that PKC activation could increase EAAT3 activity and amitriptyline could inhibit PKC.[18,21,28,29] Thus, it was possible that the amitriptyline effects on EAAT3 activity may have been mediated by PKC. Our results showed that the degree of inhibition of EAAT3 activity caused by amitriptyline at 0.64 μM, a concentration that had maximized its effects on EAAT3 activity, was not affected by PMA. These results suggested that the amitriptyline-induced reduction of EAAT3 activity was not mediated by PKC. Consistent with this idea, our previous studies suggested that PKC may not have played an important role in regulating the basal EAAT3 activity because PKC inhibition did not affect the basal EAAT3 activity.[18,28]

EAATs use the transmembrane gradient of Na^+, K^+ and H^+ as the driving force for glutamate uptake.[4,7] Drugs may have affected the gradients of these ions to change EAAT activity. It is known that amitriptyline can block sodium channels.[30] This effect would hyperpolarize the cell membrane and could increase the transmembrane gradients of sodium. Consequently, the EAAT3 activity would be expected to increase. This expectation was in contrast to our results. Thus, the effects of amitriptyline on sodium channels may not have contributed to the inhibition of EAAT3 activity caused by amitriptyline.

Conclusions

Amitriptyline, a tricyclic antidepressant, dose-dependently inhibited EAAT3 activity via reducing the V_{max} of EAAT3 response to L-glutamate. This effect represented a novel site of action for amitriptyline to increase the glutamatergic neurotransmission. PKC may not have been involved in this effect of amitriptyline.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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Dr H.-J. Baik and Dr S.-A. Lee contributed equally to this work.

References

1. Fonnum F. Glutamate: a neurotransmitter in mammalian brain. *J Neurochem* 1984; 42: 1–11.
2. Kanai Y, Hediger MA. Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature* 1992; 360: 467–471.
3. Kanai Y *et al*. A new family of neurotransmitter transporters: the high-affinity glutamate transporters. *Faseb J* 1993; 7: 1450–1459.
4. Danbolt NC. Glutamate uptake. *Prog Neurobiol* 2001; 65: 1–105.
5. Rothstein JD *et al*. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis [see comments]. *N Engl J Med* 1992; 326: 1464–1468.
6. Torp R *et al*. Differential distribution of the glutamate transporters GLT1 and rEAAC1 in rat cerebral cortex and thalamus: an in situ hybridization analysis. *Anat Embryol (Berl)* 1997; 195: 317–326.
7. Billups B *et al*. Physiological and pathological operation of glutamate transporters. *Prog Brain Res* 1998; 116: 45–57.
8. Cai Z, McCaslin PP. Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainate- and N-methyl-D-aspartate-induced intracellular Ca2+ levels in neuronal culture. *Eur J Pharmacol* 1992; 219: 53–57.
9. Duman RS *et al*. Neural plasticity to stress and antidepressant treatment. *Biol Psychiatry* 1999; 46: 1181–1191.
10. Auer DP *et al*. Reduced glutamate in the anterior cingulate cortex in depression: an *in vivo* proton magnetic resonance spectroscopy study. *Biol Psychiatry* 2000; 47: 305–313.
11. Palucha A, Pilc A. The involvement of glutamate in the pathophysiology of depression. *Drug News Perspect* 2005; 18: 262–268.
12. Pittenger C *et al*. The NMDA receptor as a therapeutic target in major depressive disorder. *CNS Neurol Disord Drug Targets* 2007; 6: 101–115.
13. Eisenach JC, Gebhart GF. Intrathecal amitriptyline acts as an N-methyl-D-aspartate receptor antagonist in the presence of inflammatory hyperalgesia in rats. *Anesthesiology* 1995; 83: 1046–1054.
14. McQuay HJ *et al*. A systematic review of antidepressants in neuropathic pain. *Pain* 1996; 68: 217–227.
15. Abdel-Salam OM *et al*. Evaluation of the anti-inflammatory and anti-nociceptive effects of different antidepressants in the rat. *Pharmacol Res* 2003; 48: 157–165.
16. Golembiowska K, Dziubina A. Involvement of adenosine in the effect of antidepressants on glutamate and aspartate release in the rat prefrontal cortex. *Naunyn Schmiedebergs Arch Pharmacol* 2001; 363: 663–670.
17. Andin J *et al*. Modulation of neuronal glutamate transporter rEAAC1 mRNA expression in rat brain by amitriptyline. *Brain Res Mol Brain Res* 2004; 126: 74–77.
18. Do SH *et al*. Effects of volatile anesthetics on glutamate transporter, excitatory amino acid transporter type 3. *Anesthesiology* 2002; 96: 1492–1497.
19. Kim JH *et al*. Effects of ethanol on the rat glutamate excitatory amino acid transporter type 3 expressed in *Xenopus* oocytes: role of protein kinase C and phosphatidylinositol 3-kinase. *Alcohol Clin Exp Res* 2003; 27: 1548–1553.
20. Paul IA, Skolnick P. Glutamate and depression: clinical and preclinical studies. *Ann N Y Acad Sci* 2003; 1003: 250–272.
21. Tai YH *et al*. Amitriptyline preserves morphine's antinociceptive effect by regulating the glutamate transporter GLAST and GLT-1 trafficking and excitatory amino acids concentration in morphine-tolerant rats. *Pain* 2007; 129: 343–354.
22. Baldessarini RJ. Drugs and the treatment of psychiatric disorders: depression and mania. In: Hardman JG *et al.* eds. *Pharmacological Basis of Therapeutics*. New York: McGraw Hill, 1996: 431–459.
23. Engelhart DA, Jenkins AJ. Comparison of drug concentrations in postmortem cerebrospinal fluid and blood specimens. *J Anal Toxicol* 2007; 31: 581–587.
24. Glotzbach RK, Preskorn SH. Brain concentrations of tricyclic antidepressants: single-dose kinetics and relationship to plasma concentrations in chronically dosed rats. *Psychopharmacology (Berl)* 1982; 78: 25–27.
25. Hasuo H *et al*. Activation of presynaptic 5-hydroxytryptamine 2A receptors facilitates excitatory synaptic transmission via protein kinase C in the dorsolateral septal nucleus. *J Neurosci* 2002; 22: 7509–7517.
26. Rothstein JD *et al*. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 1996; 16: 675–686.
27. McCaslin PP *et al*. Amitriptyline prevents N-methyl-D-aspartate (NMDA)-induced toxicity, does not prevent NMDA-induced elevations of extracellular glutamate, but augments kainate-induced elevations of glutamate. *J Neurochem* 1992; 59: 401–405.
28. Huang Y, Zuo Z. Isoflurane induces a protein kinase C alpha-dependent increase in cell surface protein level and activity of glutamate transporter type 3. *Mol Pharmacol* 2005; 67: 1522–1533.
29. Huang Y *et al*. Critical role of serine 465 in isoflurane-induced increase of cell-surface redistribution and activity of glutamate transporter type 3. *J Biol Chem* 2006; 281: 38133–38138.
30. Dick IE *et al*. Sodium channel blockade may contribute to the analgesic efficacy of antidepressants. *J Pain* 2007; 8: 315–324.