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The effect of glutamate uptake inhibitors on hippocampal evoked potentials *in vitro*

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Abstract. The influence of four inhibitors of the high-affinity glutamate uptake system (DL-aspartic acid β-hydroxymate, DL-AHM; L-aspartic acid β-hydroxymate, L-AHM; threo-β-methylaspartate, DLM; L-transpyrrolidine-2, 4-dicarboxylate, PDC) on potentials recorded from hippocampal slices was investigated. At low concentrations of DL-AHM, L-AHM and DLM (50 -150 μM) the population spike was permanently amplified. NMDA receptor antagonists blocked this facilitatory effect of L-AHM, DL-AHM and DLM. At higher concentrations (400-700 μM) DL-AHM and DLM abolished the population spike, while L-AHM did not eliminate the population spike at any concentration tested. None of these uptake inhibitors influenced an antidromic potential recorded in Ca^{2+} -free Ringer solution. PDC at lower concentrations (75 μM) did not affect the population spike and at higher concentrations (150 μM - 500 μM) induced only a transient elevation in population spike. Our data demonstrate that modification of glutamate uptake may be an important factor in the regulation of synaptic efficiency of glutamergic pathways.

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Key words: LTP, glutamate uptake, hippocampal slices, NMDA receptor

INTRODUCTION

The extracellular concentration of glutamate in the brain is normally maintained at a low level (1-3 μM) by a family of high-affinity glutamate transporters localized on neurons and glia cells (Danbolt 1994, Gegelashvili and Schousboe 1998). It is of critical importance that the extracellular glutamate concentration is kept low, as most neurons and even glia cells (Hosli and Hosli 1990) have glutamate receptors. Persistent and excessive activation of these receptors by prolonged increase in the extracellular glutamate concentration can lead to the cell death (Choi 1992). On the other hand an elevation in the extracellular glutamate level is suggested as one of the possible mechanisms responsible for a physiologically relevant, permanent increase in the synaptic efficiency called long-term potentiation (LTP) (Dolphin et al. 1982, Malinow and Tsien 1990, Bliss et al. 1991, Malgaroli and Tsien 1992).

LTP described for the first time by Bliss and Lomo (1973) represents an activity-dependent form of synaptic plasticity, which is currently thought to be a neurophysiological mechanism mediating learning and memory (Bliss and Collingridge 1993). Although LTP has been observed in several brain pathways, the most prominent LTP has been described so far in the hippocampus (Kuba and Kumamoto 1990). Triggering of LTP depends in most hippocampal pathways on activation of N-methyl--D-aspartate (NMDA) receptors (Collingridge et al. 1983). At present two very different types of hypothesis concerning the long-lasting changes underlying LTP exist (Malinow 1994). While the first hypothesis suggests an amplification of the sensitivity of the postsynaptic membrane (Lynch and Baudry 1984, Muller and Lynch 1988, Larson et al. 1991), the second hypothesis favors changes in the presynaptic part expressed as an increase in transmitter release (Malinow and Tsien 1990, Bliss et al. 1991, Ghijsen and Lope da Silva 1991). The presynaptic mechanism of LTP may, besides elevation of glutamate release, involve also modulation of the efficiency of high-affinity, Na^+-dependent glutamate uptake mechanism. Indeed, Wieraszko (1981) and others (Goh et al. 1986) demonstrated that while low-frequency, electrical stimulation of some of the hippocampal pathways amplifies glutamate uptake, triggering of LTP reduces the efficiency of the glutamate uptake system (Wieraszko 1981, Wieraszko 1983, Goh et al. 1986). In support of this data, an amplification of NMDA receptor-mediated response following inhibition of glial glutamate uptake system was recently reported (Mennerick and Zorumski 1994). The attenuation of glutamate uptake may result in an increase in the synaptic overflow of glutamate which can be falsely interpreted as LTP-induced increase in the glutamate release (Dolphin et al. 1982, Bliss et al. 1991, Ghijsen and Lopes da Silva 1991, Canevari et al. 1994).

In pathological conditions changes in glutamate uptake system can also occur (Cross et al. 1986, Cordero et al. 1994, Harris et al. 1996), although it is not known whether they are the cause or the consequence of the disease.

The aim of the present research was to evaluate the influence of elevated extracellular glutamate level achieved by attenuation of glutamate uptake system, on hippocampal evoked potentials.

METHODS

Seventy-eight slices from fifty-one C57BL/6J (B6) mice of both sexes were used for these experiments. The experiments were conducted in accordance of the guidelines of the CUNY Animal Care and Use Committee. The hippocampal slices were prepared as described previously (Wieraszko and Seyfried 1993). Briefly, following decapitation, the brain was removed and both hippocampi were dissected out and placed into ice-cold Ringer solution consisting of (in mM): NaCl 124, KCL 3.1, KH_2PO_4 1.3, $MgSO_4$ 1.3, $CaCl_2$ 3.1, $NaHCO_3$ 25.5, glucose 10.0. Both hippocampi were cut into slices (350 μ) with a manual tissue chopper and placed in an incubation chamber (33°C) constantly oxygenated with a CO_2/O_2 mixture. In some experiments $CaCL_2$ was omitted, as required by the experimental design. Following an hr preincubation period slices were transferred to the interface, recording chamber maintained at 33°C. A bipolar, stimulating electrode was placed on Schaffer collateral-commissural fibers (Sch. coll.) and recording electrodes were guided into pyramidal or radiatum layer of CA1 to record population spike or Excitatory Postsynaptic Potentials (EPSP), respectively. In order to observe antidromically-evoked potentials, the stimulating and recording electrodes were placed in the alveus and pyramidal cell layer, respectively. The magnitude of the population spike was measured as an average distance between the highest negativity and preceding and following positivities. The initial slope of the negative wave was taken as a measure of field EPSP. The strength of the stimulation in all tested slices was adjusted at the beginning of each

experiment to obtain a population spike in the range of 1-2 mV. The potential was monitored at this level for the next 15-20 min and experiments were performed only on slices demonstrating stable responses to low frequency (0.03 Hz) stimulation.

Stock solutions of glutamate uptake blockers: DL--threo-β-methylaspartate, Sigma (DLM), DL-aspartic acid β-hydroxyamate, Sigma, (DL-AHM), L-aspartic acid β-hydroxymate, Sigma, (L-AHM), L-transpyrrolidine-2, 4-dicarboxylate, Research Biochemicals (PDC) and antagonists of the NMDA receptor: LD-2-amino-5-phopshonovaleric acid, Sigma, (APV) and (+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10 imine malate, Research Biochemicals, (MK801) were prepared in Ringer's fresh every day. Each molecule was tested several times during different experimental days on slices prepared from different animals.

Ten or twenty µl of the stock solution was added directly to the recording chamber with Hamilton syringe to obtain the desired final concentration. The increase in the size of the potential and in the slope of EPSP was expressed as percentage of increase over the average control value observed before the treatment. The magnitude of amplification was routinely evaluated 30 min after the potential reached plateau, but in several experiments the elevated potential was followed for 2-3 h.

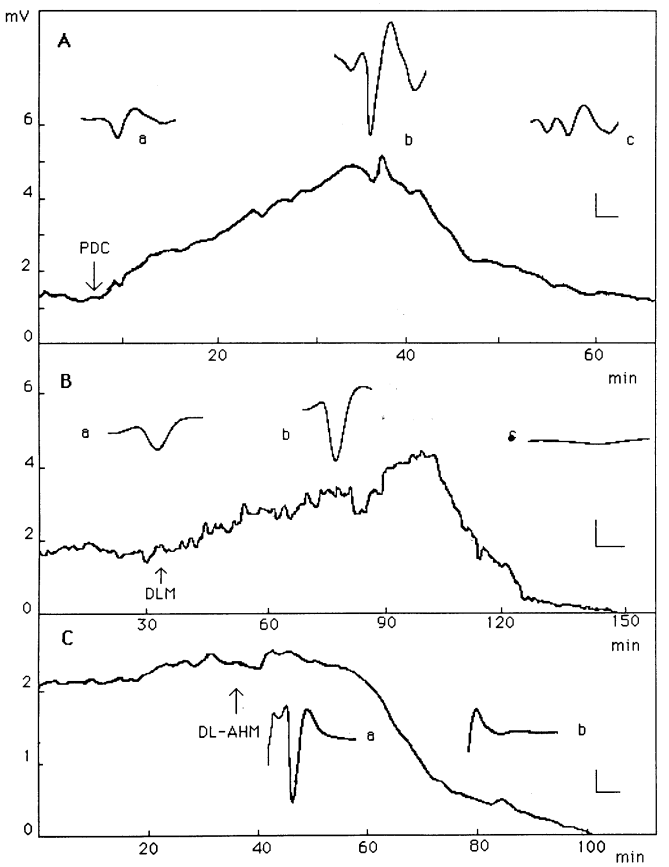

Fig. 1. The influence of 150 µM PDC (A), 500 µM DLM (B), and 550 µM DL-AHM (C) on the population spike. An arrow indicates addition of each of these molecules. Solid lines on each of the graph depict the change in the magnitude of the population spike during the whole experiment. In A and B letters a, b and c represent the potentials recorded 10 min before addition of the drug, at the maximum of its effect and at the end of experiment, respectively. In C, a and b represent the population spike 10 min before and at the end of experiment, respectively. Calibration: 2 mS (A, B, C), 1 mV (A, B), 0.4 mV (C).

RESULTS

L-transpyrrolidine-2, 4-dicarboxylate (PDC), a transportable (Danbolt 1994, Griffiths et al. 1994) antagonist of synaptosomal, high-affinity glutamate uptake (Bridges et al. 1991) at the concentration of 150.0 µM induced transient elevation of the amplitude of the population

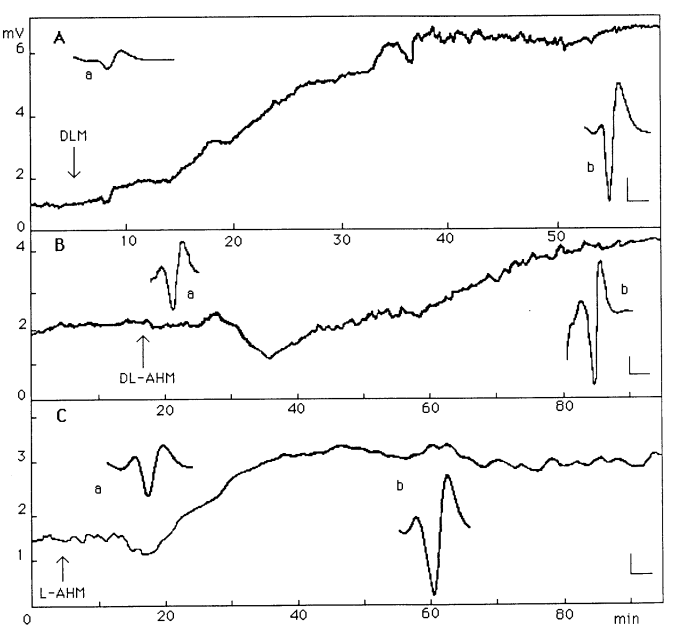

Fig. 2. Facilitation of the population spike by 150 µM DLM (A), 50 µM DL-AHM (B) and 75 µM L-AHM (C). In all figures a and b represent the examples of the population spike recorded 10 min before and at the end of the experiment, respectively. Note in B and C small depression of the potential before its amplification. Calibration: 2 mS (A,B,C), 1 mV (B,C), 0.4 mV (C).

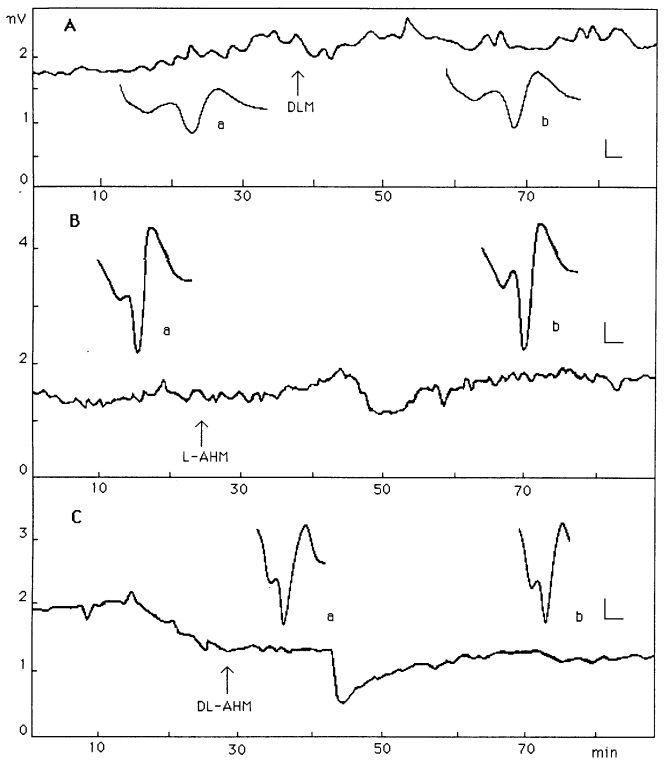

Fig. 3. The effect of 150 μM DLM (A), 75 μM L-AHM (B) and 50 μM DL-AHM (C) in the presence of 50 μM APV. APV was added to the incubation chamber 30 min before addition of the glutamate uptake inhibitor; a and b represent the population spike 10 min before, and at the end of the experiment, respectively. Calibration: 2 mS (A,B, C), 1.2 mV (A), 0.3 mV (B), 0.6 mV (C). See the legend to Fig. 2 for further details.

spike (212.6 ± 33 %, n = 6, Fig. 1A) followed by a decrease of the potentials to its control value. The changes in EPSP followed changes in the population spike (data not shown). At lower (75 μM and below) and at the highest concentrations (500 μM) PDC effect became erratic and nonreproducible. At none of the concentrations tested was there a permanent increase in the magnitude of the population spike.

DLM, which effectively blocks uptake of glutamate in the brain slices (Balcar and Johnston 1972) influenced the magnitude of the population spike in a concentration-dependent fashion. At higher concentrations (400-600 μM) DLM induced a short-lasting elevation of the population spike (172.0 ± 25%, n = 4), followed by fast, irreversible disappearance of the potential (Fig. 1B). At lower concentrations (75.0 μM) the effect of DLM was erratic, not reproducible and observed in 4 of 8 slices only (data not shown).The most reproducible effect was observed with 150.0 μM DLM, which evoked a permanent increase in the magnitude of the population spike (Figs. 2A and 4). The amplification of the population spike developed slowly with time, reached its maximum 30-45 min following DLM application and remained elevated for the duration of the experiment (Fig. 2A). DLM-induced amplification of the population spike was accompanied by an increase in the slope of EPSP (207 ± 32%, n = 5). The effects of DLM was markedly reduced by 60 μM APV (Figs. 3 and 4, 50.5 ± 36%, n = 6) and by 20 μM MK801 (15.9 ± 18%, n = 5). There was no change in the magnitude of antidromic potential recorded in Ca^{2+} -free medium following application of DLM (17.0 ± 11%, n = 4, Fig. 4).

Two other glutamate uptake inhibitors, DL-AHM and L-AHM also modified the population spike, although the mode of their action differed. DL-AHM at highest concentrations tested (400-700 μM) abolished the population spike (Fig. 1C), although in two of six experiments the potential recovered and even exceeded control value. The potentials have never been abolished by L-AHM. At lower concentrations (50-150 μM) DL-AHM and L-AHM amplified the population spike permanently (219 ± 38%, n = 4, Fig. 2B, and 168.1 ± 17.1%, n = 7, Fig. 2C, respectively). The amplification observed after addition of L-AHM and DL-AHM was often preceded by a 20-30% transient attenuation of the amplitude of the potential (Fig. 2B and C). This transient attenuation was more often observed following DL-AHM addition. As in the case of DLM, the effect of DL-AHM and L-AHM developed slowly with the time reaching its maximum 30-45 min following their application (Fig. 2B and C). While APV eliminated the facilitatory action of L-AHM (3.4±9.0%, n = 4, Fig 4) and markedly reduced the action of DL-AHM (50.4 ± 29.5%, n = 7, Fig 4), it did not influence transient attenuation of the potential (Fig. 3B and C). The antidromic potentials recorded in Ca^{2+} -free solution were not affected either by L-AHM or DL-AHM (Fig. 4).

DISCUSSION

The major goal of this research was to evaluate the effect of inhibitors of glutamate uptake system on hippocampal evoked potentials. We focused our attention on changes in the population spike to determine whether the number of neurons in the hippocampal slice, activated by a single electrical pulse can be modified by increased concentration of extracellular glutamate.

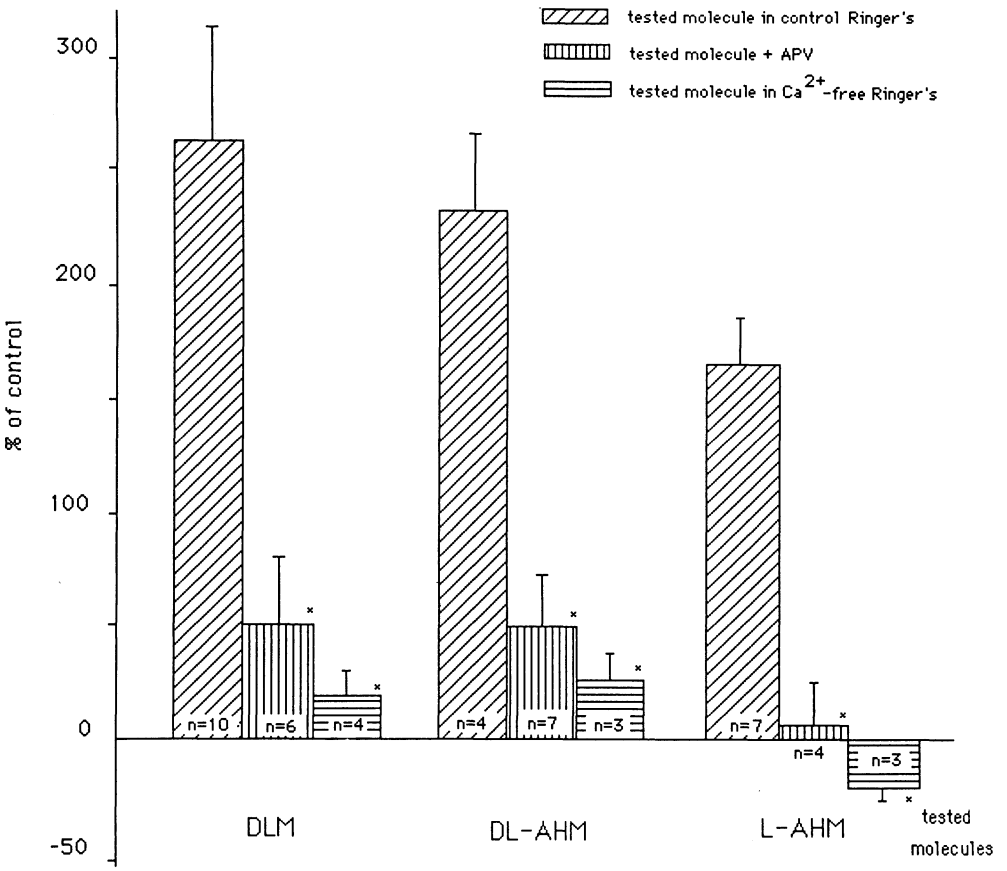

Fig 4. The amplification of the population spike induced by 150 μM DLM, 50 μM DL-AHM and 75 μM L-AHM in control and modified Ringer's solution. The results are expressed as percent of control value of the potential recorded before addition of the tested molecules. Each bar represents average results (±SEM) recorded from the number of slices indicated by "n". x denotes $P<0.05$, t-test, two-tailed, compared with an increase recorded in control Ringer's solution.

A number of studies have examined the effect of pharmacological inhibition of glutamate uptake, but unequivocal data interpretation was often difficult. Members of glutamate transporter family are expressed in glia cells and neurons (Obrenovitch and Urenjak 1997). Their relative contribution in the uptake of synaptically released glutamate remains unknown (Obrenovitch and Urenjak 1997), although recent reports suggest a significant contribution of glia to uptake of glutamate released by Sch. coll. synapses (Bergles and Jahr 1997, 1998). However, specific inhibitors of neuronal or glia cells glutamate transporters are still lacking.

We have selected for our research the most commonly used and most effective glutamate transport inhibitors (Porter et al. 1992, Kanai et al. 1994, Danbolt 1994, Griffiths et al. 1994, Obrenovitch and Urenjak 1997). Currently available glutamate analogs may, besides blocking glutamate uptake, interact with glutamate receptors, and serve as substrates for glutamate transporters replacing endogenous glutamate. Because of this potential diverse actions the concentrations of glutamate transporter inhibitors have been initially chosen by us based on two factors: (1) the concentrations were at least 10-15 times the IC_{50} for inhibition of glutamate uptake in neuronal preparations (Robinson et al. 1991, Robinson et al. 1993, Arriza et al. 1994), 2/ these concentrations have been used in previous investigations on variety of preparations (Danbolt 1994, Obrenovitch and Urenjak 1997). However, considering different properties (Robinson et al. 1991, Kanai et al. 1993, Gegelashvili and Schousboe 1998), diverse cellular localization (Garlin et al. 1995, Gegelashvili and Schousboe 1998) and the number of currently known types of the glutamate transporters (Danbolt 1994), in the course of our research we have actually used glutamate uptake inhibitors at much wider range of concentrations.

While three of glutamate uptake inhibitors tested by us (DLM, LD-AHM and L-AHM) exerted similar action, the effect of the fourth one, PDC was different and will be discussed separately (see below). At low concentrations DLM, L-AHM and DL-AHM induced a long--lasting, NMDA receptor-dependent amplification of the population spike. Our results are in line with previous data showing that application of exogenous glutamate can permanently enhance synaptic efficiency in hippocampal slices (Cormier et al. 1992, Kovalchuk et al.

1993, Collins and Davies 1994) and in cultured hippocampal neurons (Malgaroli and Tsien 1992) and blockers of glutamate transporters enhance EPSPs in cultured hippocampal neurons (Tong and Jahr 1994). In comparison to other neurotransmitter systems which showed a prolonged synaptic response in the presence of blockers of norepinephrine (Suprenant and Williams 1987), serotonin (Bobker and Williams 1991) and GABA (Thompson and Gahwiler 1992, Barbour et al. 1993) high affinity uptake systems, we concluded that an increase in hippocampal evoked potentials, induced by lower concentrations of DLM, L-AHM and DL-AHM was due to moderate elevation in the extracellular glutamate concentration. Glutamate uptake inhibitors at higher concentrations could further elevate extracellular glutamate concentration to a toxic level (Amin and Pearce 1997). This dual concentration-dependent mode of action of DLM and DL-AHM observed by us can be explained in a following way. Extreme elevation of extracellular glutamate concentration may desensitize (Zorumski et al. 1989), or overstimulate (Choi 1992) the glutamate receptors. In either case the population spike could be significantly reduced or eliminated. At lower concentrations an equilibrium may have been achieved, in which the concentration of glutamate is sufficient to stimulate NMDA receptors without their desensitization (Zorumski et al. 1989), but too low to exert neurotoxic effects (Choi 1992).

The effects observed in our experiments are NMDA receptor-dependent, and are probably mediated by modulation of the intracellular Ca^{2+} concentration. The changes in intracellular Ca^{2+} levels are crucial in the modulation of synaptic efficiency (Tsumoto 1992). Analogous to this data, our results which demonstrate a dual effect of glutamate uptake inhibitors (attenuation at higher concentrations and amplification at lower concentrations) may be also explained by fluctuations in Ca^{2+} level.

In comparison to the data reported by others (Hestrin et al. 1990, Isaacson and Nicoll 1993), we observed only a transient elevation of the population spike induced by PDC, regardless of its concentration. PDC was reported to inhibit the NMDA component of the synaptic response (Sarantis et al. 1993) and in our view this is the major reason for its inability to amplify the population spike permanently. Moreover, PDC, considered previously to be one of the most specific glutamate uptake inhibitors (Bridges et al. 1991, Arriza et al. 1994) appeared also to be a strong, competitive substrate for this system (Griffiths et al. 1994). Therefore, it does not prevent the molecular transport mechanism from working and may induce release of glutamate by heteroexchange, elevating its extracellular concentration to a toxic level (Amin and Pearce 1997). Transient, PDC-induced elevation of the population spike, prior to its decline, may be evoked by temporary stimulation of non-NMDA receptors (Porter et al. 1992). A failure to observe a permanent amplification of the population spike by PDC may be also related to the very short time of its application (Hestrin et al. 1990, Isaacson and Nicoll 1993) and subsequent, short-lasting elevation of the extracellular glutamate concentration. It has been shown that only a longer (more than 2-3 min) application of glutamate evoked a permanent amplification of the synaptic response (Kovalchuk et al. 1993). Although in our experiments the slices were incubated with PDC longer, its concentration could quickly diminish due to the action of the glutamate transporter (Griffiths et al. 1994).

Our data cannot conclusively rule out the possibility that the molecules used in our studies are acting directly on glutamergic receptors as agonists. This possibility, however seems to be very unlikely, as they are very weak agonists (Davies and Watkins 1973, Roberts and Watkins 1975, Porter et al. 1992).

We did not attempt to determine if the washing of glutamate uptake inhibitors away would reverse their action. We consider it irrelevant, since as long as glutamate uptake remains inhibited chemically (this study), by electrical stimulation (Wieraszko 1981, Goh et al. 1986) or by disease (Cross et al. 1986, Cordero et al. 1994, Harris et al. 1996) the cell's excitability is very likely to be modified due to the change in extracellular glutamate concentration following its exocitosis. This conclusion is further supported by recent results (Min et al. 1998) showing that retardation of glutamate diffusion by incubation of slices in dextran enhances postsynaptic responses.

Since the antidromic potential recorded in the presence of each of the glutamate uptake inhibitors in Ca^{2+}-free Ringer's remained unchanged, the amplification of the response due to elevation of the fiber potential seems to be very unlikely. These findings are consistent with the conclusion about synaptic localization of glutamate uptake inhibitors action and may imply that modulation of the glutamate uptake system may be involved in the mechanisms of LTP.

In comparison to the experiments employing exogenous glutamate (Cormier et al. 1993, Kovalchuk et al.

1993, Collins and Davies 1994), in our procedure neurons were exposed to elevated glutamate concentration only during short periods of their activity triggered every 30-s by an electrical stimulation. Therefore, the method employed in our experiments may be more relevant to physiological conditions, where neurons are exposed to a higher level of glutamate during synaptic excitation only. If the high-affinity glutamate transport remains compromised during this period, some glutamate released from excited terminals may "spillover" out of the synaptic cleft and activate adjacent synapses (Kullman and Asztely 1998). That increase in the number of recruited synapses and subsequent enhancement in the number of firing neurons would be expressed as amplification of the population spike recorded in our experiments.

An attenuation in the function of the glutamate uptake system may be correlated with profound changes in the function of the nervous system. Postmortem evaluation of brains from Huntington's disease subjects revealed over a 70% reduction in high affinity glutamate uptake sites (Cross et al. 1986). Amyloid β peptide, a main constituent of plaques characteristic of Alzheimer's disease significantly reduced glutamate uptake by astrocytes (Harris et al. 1996). Glutamate uptake is reduced following seizure episodes (Cordero et al. 1994) and is also attenuated by induction of LTP (Wieraszko 1981, Goh et al. 1986). Thus, it is reasonable to assume that the glutamate uptake system is involved in regulation of neuronal communication in pathological and physiological conditions.

ABBREVIATIONS

DL-AHM	-	DL-aspartic acid β-hydroxymate
L-AHM	-	L-aspartic acid β-hydroxymate
APV	-	LD-2-amino-5-phopshonovaleric acid
CNQX	-	6-cyano-7-nitroquinoxaline-2,3-dione
DLM	-	DL-threo-β-methylaspartate
LTP	-	long-term potentiation
MK801	-	(+)-5-methyl-10,11-dihydro-5H-dibenz o[a,d]cyclohepten-5,10imine malate
NMDA	-	N-methyl-D-aspartate
PDC	-	L-transpyrrolidine-2,4-dicarboxylate
Sch. coll.	-	Schaffer collateral-commissural fibers

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