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Nicergoline enhances glutamate re-uptake and protects against brain damage in rat global brain ischemia

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Abstract

Whereas a 2–3°C decrease in intraischemic brain temperature can be neuroprotective, mild brain hyperthermia significantly worsens outcome. Our previous study suggested that an ischemic injury mechanism which is sensitive to temperature may not actually increase the extracellular glutamate concentration ($[Glu]_e$) during the intraischemic period, but rather impairs the Glu re-uptake system, which has been suggested to be involved in the reversed uptake of Glu. We speculated that enhancing Glu re-uptake, pharmacologically or hypothermically, may shorten exposure to high $[Glu]_e$ in the postischemic period and thereby decrease its deleterious excitotoxic effect on neuronal cells. In the present study, rats treated with nicergoline (32 mg/kg, i.p.), an ergot alkaloid derivative, showed minimal inhibition of the $[Glu]_e$ elevation which characteristically occurs during the 10-min intraischemic period, while Glu re-uptake was dramatically improved in the postischemic period, when severe transient global ischemia was caused by mild hyperthermia. Moreover, the nicergoline (32 mg/kg, i.p.) treated rats showed reduced cell death morphologically and clearly had a far lower mortality. The present study suggests that the development of therapeutic strategies aimed at inhibition or prevention of the reversed uptake of glutamate release during ischemia, i.e., activation of the glutamate uptake mechanism, is a promising approach to reduce neural damage occurring in response to brain ischemia.

Keywords: Dialysis electrode; Real-time monitoring; Glutamate; Glutamate release; Glutamate re-uptake; Uptake, reversed; Ischemia; Hyperthermia, mild; Nicergoline; (In vivo); Electron-microscopy

1. Introduction

Brain temperature is reportedly related to the extracellular glutamate concentration ($[Glu]_e$), which is neurotoxic above a certain level in the brain, as demonstrated by brain microdialysis studies showing that intraischemic hypothermia is an extremely effective means of attenuating the acute $[Glu]_e$ rise which follows global ischemia (Busto et al., 1989; Mitani and Kataoka, 1991; Ginsberg and Busto, 1998). It is widely recognized that glutamic acid, which is an excitatory neurotransmitter, is released into the extracellular fluid in large amounts in the brain during ischemia and causes cell injury (Collingridge and Lester, 1989). The $[Glu]_e$ has been shown to increase during ischemia (Benveniste et al., 1984; Hagberg et al., 1985; Globus et al., 1988), hypoxia (Choi, 1990; Bickler and Hansen, 1996) and other central nervous system insults (Ginsberg et al., 1992; Bullock et al., 1995), and an increased $[Glu]_e$ is associated with neural cell death (Siesjö, 1981; Kirino, 1982). Although the mechanism by which this glutamate-mediated excitotoxic process is initiated is not known, glutamate accumulation in the extracellular space and overactivation of glutamate receptors are thought to play prominent roles (Choi and Rothman, 1990; Meldrum and Garthwaite, 1990).

We recently developed a system for oxygen-independent real-time measurement of $[Glu]_e$ (Kohno et al., 1998a,b), and used this system to the highly reproducible

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biphasic release of glutamate during ischemia (Asai et al., 1996). This system made separate evaluation of intraischemic biphasic glutamate release and postischemic glutamate re-uptake possible in ischemia-reperfusion models. Our previous study demonstrated that intraischemic brain temperature has a minimal influence on the initial glutamate release in the intraischemic period (Asai et al., 1998), but plays a major role in promoting glutamate re-uptake system during the postischemic period (Zhao et al., 1997). Furthermore, glutamate re-uptake was disrupted at a certain threshold of ischemic severity, with there being a synergistic action between intraischemic cerebral blood flow and brain temperature (Zhao et al., 1998). Although glutamate release induced by normothermia and mild hyperthermia significantly inhibits glutamate re-uptake after reperfusion, intraischemic hypothermia, which enhances re-uptake, should shorten the exposure to high $[Glu]_e$ in the postischemic period and thereby decrease its deleterious excitotoxic effect on neural cells. Thus, investigating the process of glutamate re-uptake disruption may reveal key steps in the process of cell damage. Furthermore, artificial activation of glutamate transport after ischemia is a potential approach to reducing the neural damage which occurs in response to brain ischemia.

Nicergoline is an ergot alkaloid derivative which reportedly blocks α1-adrenenoreceptors (Heitz et al., 1986), inhibits T- and L-type Ca^{2+} currents (Takahashi and Akaike, 1990), enhances protein synthesis in the rat brain, and affects cholinergic function in the aged rat brain (Ogawa et al., 1993). In in vivo studies, although nicergoline prevents antagonistic brain damage induced by transient bilateral carotid artery occlusion in mice and Mongolian gerbils (Shintomi et al., 1886), the mechanism of this protective effect is not known. In the present study, we investigated the effect of nicergoline, focusing on the relationship between $[Glu]_e$ dynamics and brain damage during acute severe global ischemia in the rat brain.

2. Materials and method

2.1. Reagents

Glutamate oxidase (EC 1.4.3.11) was purchased from Yamasa (Chiba, Japan). Ferrocene carboxylic acid was obtained from Tokyo Kasei Kogyo (Tokyo, Japan); bovine serum albumin (RIA grade) and *O*-phenylenediamine were from Sigma (St. Louis, MO); 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was from Pierce Chemical (Rockford, IL); 2-(*N*-morpholino)ethane sulfonic acid (MES) was from Dojindo Laboratories (Kumamoto, Japan); and Dulbecco's phosphate-buffered saline (PBS(+)) (product No. 21300) was from GIBCO/BRL (Grand Island, NY). All other chemicals used were of analytical grade.

2.2. Preparation of dialysis electrode for in vivo experiments

Glutamate oxidase was dissolved in PBS(−) and dialyzed against PBS(−) three times on ice. The solution of glutamate oxidase was stored at −80°C until use. The dialysis electrodes (Microdialysis Biosenser: type general, 10-2-2) used in this study were purchased from Sycopel International (Boldon, Tyne and Wear, U.K.). The principle of the dialysis electrode used for the measurement of glutamate was as previously described (Kohno et al., 1998a,b). Briefly, the dialysis electrode was filled with Dulbecco's phosphate-buffered saline (PBS(−) supplemented with 1.3 mM $CaCl_2$ (PBS(+)). The perfusate entered the fluid inlet tube at a rate of 0.5 μl/min via an EP70 perfusion pump (Eicom, Kyoto, Japan). The electrode was prepared by filling it with PBS(+) and immersing the membrane in a beaker of 5 mM *O*-phenylenediamine in PBS(+) bubbled with 100% N_2 for 15 min under constant stirring of the PBS(+). The dialysis electrode was connected to an EPS-800 potentiostat (Eicom, Kyoto, Japan), and a voltage clamp was switched on at +650 mV for 15 min to carry out electropolymerization with continuous bubbling and stirring. Upon completion of electropolymerization, the potentiostat was switched off. The dialysis electrode membrane was removed from the *O*-phenylenediamine solution, and the membrane portion was stored in PBS(+). After 20 min, the solution in the dialysis electrode was replaced with fresh PBS(+) by the perfusion pump, and the current, set at +650 mV, was allowed to stabilize. Finally, the mixed solution of ferrocene-conjugated bovine serum albumin and glutamate oxidase (1:1, v/v) was introduced into the dialysis electrode and perfused at a constant flow rate (0.5 μl/min) with a syringe pump. Calibration with ascorbate was then carried out while the solution was being stirred, and a small amount of concentrated ascorbate solution (up to 300 μM) was added. This procedure tested the efficacy of *O*-phenylenediamine coverage in preventing such compounds from being oxidized electrochemically by the working electrode.

2.2.1. Implantation in rat striatum

Adult male Wistar–Kyoto rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), weighing between 225 and 275 g, which had been allowed food and water ad libitum, were used in all experiments. The rats were anesthetized with an intraperitoneal injection of urethane (1.25 g/kg). After tracheotomy, the rats were orotracheally intubated and artificially ventilated by means of a small-animal ventilator (Rodent Ventilator, Ugo Basile, Italy) and an appropriate stroke volume to maintain normocapnia. Arterial pCO_2 was kept between 30 and 35 mmHg, and was monitored with an i-STAT 200A (i-STAT, NJ). The head was fixed in a stereotactic frame, and rectal temperature was kept at 36.5°C–37°C by means of a

heating pad. The skull was exposed, 2-mm holes were drilled through the skull bilaterally, and the dura was carefully pierced. An electroencephalogram electrode attachment was placed on the brain surface. The laser Doppler blood flow monitor FLO-1 (Omegawave, Tokyo, Japan) includes a helium–neon laser, an electronic processor and a contact-type of fiber-optic probe (0.5 mm ad). The tip of the temperature sensor probe (Physitemp Instrument, Clifton, NJ) (0.5 mm od) was in contact with the outer tip of the laser probe, 1 mm above the lowest portion. The unit with the laser Doppler probe and the temperature probe were carefully implanted stereotactically, separately, in the other side of the striatum to the insertion of the electrode. The exposed brain surface was covered with aluminum foil to prevent the Doppler laser from responding to the warm light. All signals were monitored continuously using Mac Lab systems (AD instruments, Pty, Castle Hill, Australia). The brain temperature was maintained manually throughout the experiment with red light irradiation for heating and a cold blower. Both common carotid arteries were isolated. Nicergoline or saline was administered intraperitoneally (i.p.) 8 mg/kg ($n = 7$), 16 mg/kg ($n = 9$) or 32 mg/kg ($n = 8$), 60 min before ischemia induction. Acute brain ischemia was induced by a combination of bilateral carotid artery clamping and rapidly reducing mean arterial blood pressure to approximately 30 mmHg by blood withdrawal. This is known as the Smith model for investigating ischemia-reperfusion of the brain. The brain temperature dropped spontaneously from 37°C to 32°C (Saline, $n = 6$) or was maintained at a constant 39°C (Saline, $n = 6$) during the ischemic period while body temperature was kept constant at around 37°C throughout the experimental period. At the end of the 10-min occlusion period, the occlusion was released to allow reperfusion via a combination of resumed blood flow and reinfusion of the withdrawn blood. After each in vivo monitoring procedure, the dialysis electrode was removed from the brain and immediately recalibrated in vitro to simulate the in vivo condition. The calibration was carried out approximately 15 min after ischemia induction. The value of 100% cerebral blood flow was calculated from the difference between the baseline normal condition and the minimum value of the postmortem condition. The time needed for recovery to baseline after reperfusion was plotted and showed an approximately 90% decrease in $[Glu]_e$ as compared with the $[Glu]_e$ value at the start of reperfusion. The cut-off time for $[Glu]_e$ recovery was 40 min after starting reperfusion.

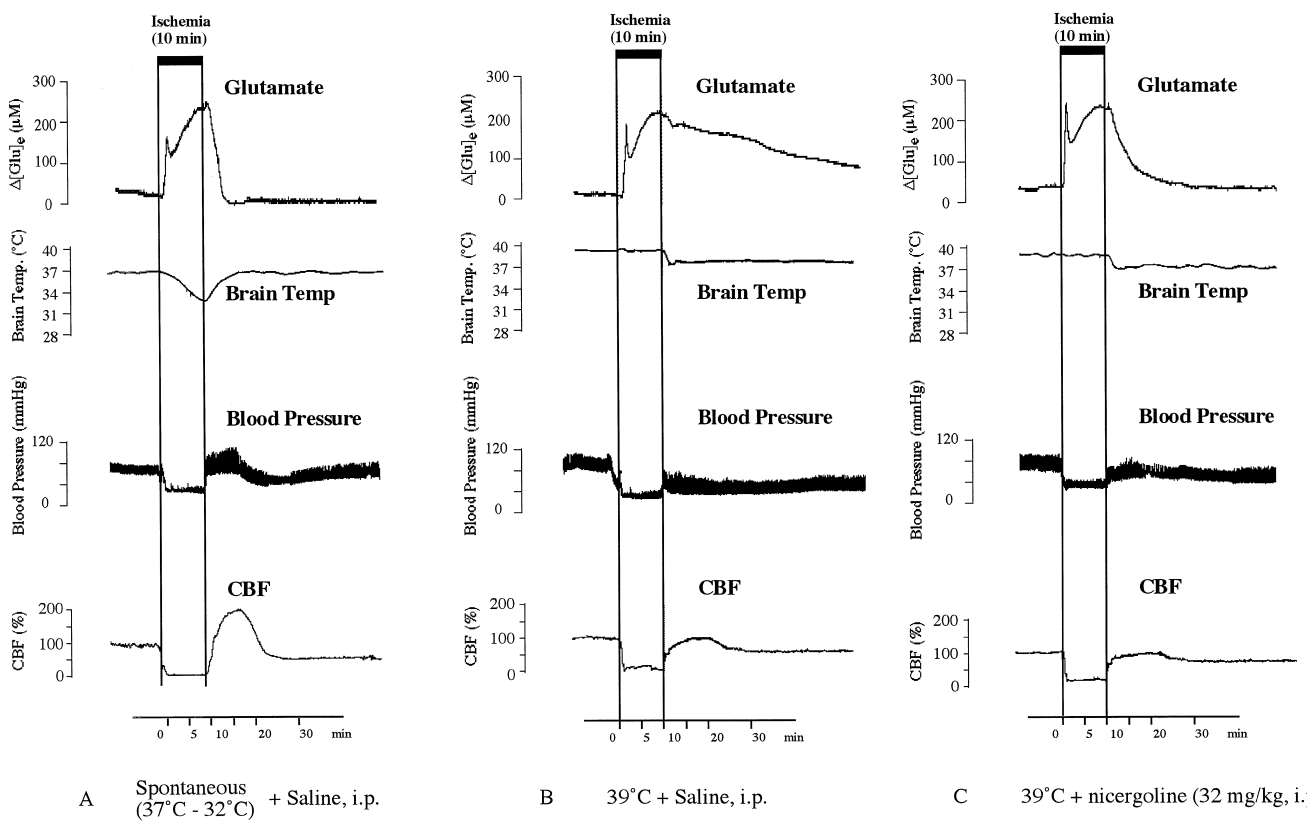

Fig. 1. Effects of nicergoline on extracellular glutamate concentration ($[Glu]_e$) response, blood pressure, cerebral blood flow and brain temperature (Brain Temp). Real-time $[Glu]_e$ monitoring revealed the events occurring when the brain temperature dropped spontaneously from 37°C to 32°C (A) and was then kept constant at 39°C followed by injection of saline alone (B) or nicergoline (C) during the ischemic period. Body temperature was kept at a nearly constant 37°C. Saline or nicergoline was administered intraperitoneally 60 min before the induction of ischemia. Results are from a representative experiment performed three times with similar results.

2.2.2. Electron-microscopic observation

The rats were killed 2 h after completion of reperfusion. After decapitation under deep anesthesia, treated animals were immediately perfused through the common carotid artery with cold PBS, then with cold 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After perfusion, the brain was quickly removed and cut into thin blocks which were postfixed in 2% glutaraldehyde and 4% paraformaldehyde 0.1 M phosphate buffer, pH 7.4 for 3 h at 4°C. The blocks were then immersed in 2% OsO_4 at 4°C for 1 h, dehydrated in Ethanol and embedded in Epon resin (Sigma). Ultra-thin sections (silver-gold) were cut on an Ultramicrotome (Leica Ultracut UTC, Wein, Austria) and mounted on a copper mesh (100 grid). The sections were stained with uranyl acetate and lead citrate for observation under an electron microscope (JEM-1200 EX, JEOL, Tokyo, Japan). To avoid double-counting of cells, we scanned the entire surface of one section from a certain block. Fifty cells were counted in the striatum from every animal treated with saline ($n = 3$) or nicergoline, 32 mg/kg, i.p. ($n = 3$), while the brain temperature was kept at 39°C during the intraischemic period.

2.2.3. Statistical analysis

Data are presented as means ± S.E.M. Statistical comparisons between groups of samples were made by analysis of variance (ANOVA) with the Fischer least significant difference test.

3. Results

Real-time $[Glu]_e$ monitoring revealed the events occurring when brain temperature dropped spontaneously from 37°C to 32°C (Fig. 1A; $n = 3$) versus being maintained at a constant of 39°C (Fig. 1B; $n = 3$ and 1C; $n = 3$) during the ischemic period while body temperature was kept constant at around 37°C, and the events occurring when bilateral carotid artery clamping and immediate reduction of mean arterial blood pressure were used in combination under the latter conditions. Most rats with a decrease in blood pressure of up to 30 mmHg during the intraischemic period had a very low cerebral blood flow, i.e., below 5%, which had no relation to the changing intraischemic brain temperature (data not shown). A sharp and rapid elevation in glutamate release took place (first phase) and then decreased slightly before continuing to rise throughout the 10-min ischemic period (second phase). As shown in Fig. 1A, following reperfusion, the brain temperature recovered to 37°C and 90% of the $[Glu]_e$ increase produced by the ischemic insult was cleared, and cerebral blood flow and blood pressure immediately returned to the baseline. This compensatory upregulation briefly exceeded, and then returned to the baseline levels. As shown in Fig. 1B, real-time $[Glu]_e$ monitoring revealed the events occurring when the brain temperature was maintained at a constant 39°C. Although the first phase of $[Glu]_e$ elevation began approximately half a minute earlier than that of the brain temperature conditions, the biphasic $[Glu]_e$ elevation curve was similar to that shown in Fig. 1B. However, the Glu re-uptake phase during reperfusion was markedly inhibited, and the increased $[Glu]_e$ remained at a high level during the postischemic period. Following reperfusion, cerebral blood flow and blood pressure did not fully return to the baseline value recorded during the experimental period and no compensatory upregulation was seen. Brain temperature dropped spontaneously from 37°C to 32°C, but most animals showed a recovery of cerebral blood flow to more

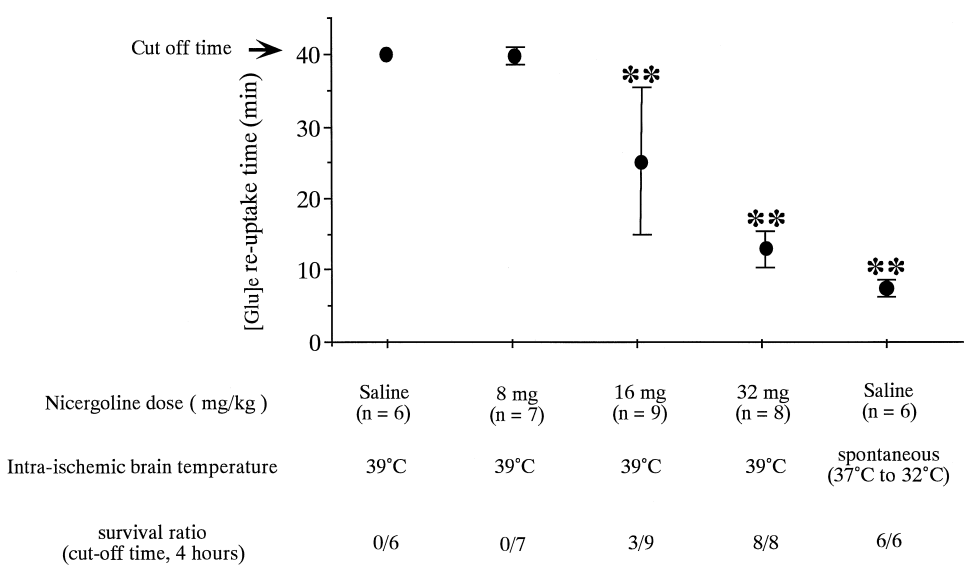

Fig. 2. The glutamate re-uptake activity in the postischemic period was measured as the time required for 90% clearance of the increased $[Glu]_e$. Saline or nicergoline was administered intraperitoneally 60 min before the induction of ischemia. The cut-off time for Glu re-uptake was set at 40 min and that for calculating the survival ratio in the postischemic period at 4 h. The data are presented as means ± S.E.M. $^{**}P < 0.01$ vs. saline; intraischemic brain temperature at 39°C (ANOVA with Fischer LSD).

than 150% above baseline during the postischemic period (Fig. 1A). When brain temperature was maintained at a constant 39°C, cerebral blood flow did not completely return to baseline and there was no compensatory upregulation as seen when the temperature decreased from 37°C to 32°C. There was, however, recovery to nearly the baseline cerebral blood flow in most cases during the postischemic period (Fig. 1B). As shown in Fig. 1C, when nicergoline was administered (32 mg/kg i.v.) 60 min before the induction of global ischemia, the changes in cerebral blood flow dynamics and blood pressure were similar to those seen without drug treatment at 39°C (see

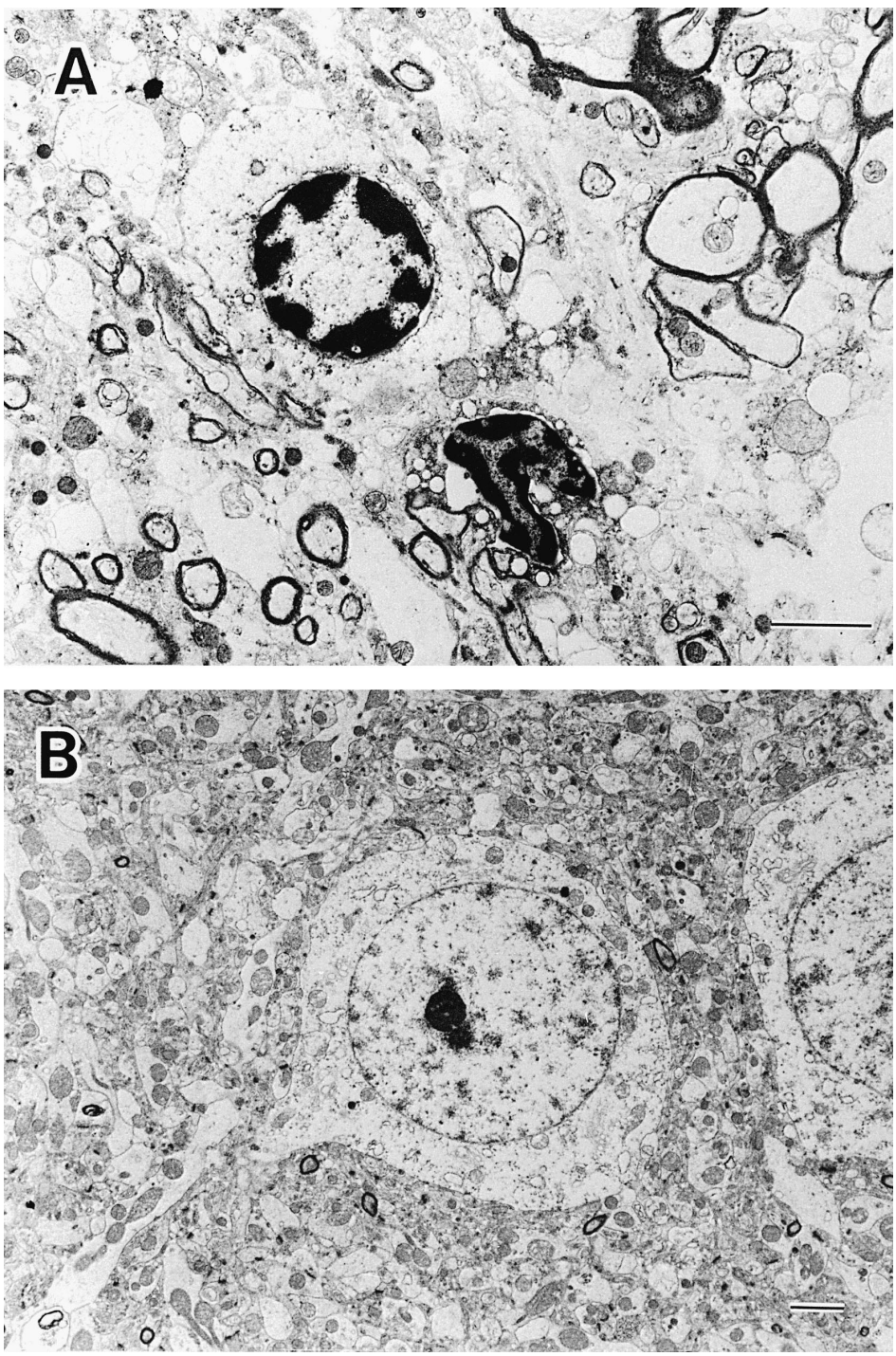

Fig. 3. Electron-micrographs of neuronal cells in the striata of ischemic animals that had been treated without (A) and with (B) nicergoline. Tissue preparations were processed as described in Materials and methods. (A) Saline (i.p.); the nuclei of some neuronal cells show chromatin condensation, indicating neuronal degeneration (ratio of abnormal cell, 42%/150 neurons, $n = 3$). (B) Nicergoline (32 mg/kg, i.p.); nearly all neuronal cells show normal morphology (ratio of abnormal cells, 4%/150 neurons, $n = 3$). Bars indicate 2 μm.

Fig. 1B). In the postischemic period, however, the Glu re-uptake phase was markedly decreased following reperfusion, with 90% of the $[Glu]_e$ increase produced by the ischemic insult being cleared within 20 min during the postischemic period.

As shown in Fig. 2, the time required for 90% clearance of the increased $[Glu]_e$ was measured as the activity of glutamate re-uptake in the postischemic period. Mild intraischemic hyperthermia prolonged the $[Glu]_e$ elevation more than 40 min during the postischemic period, although with a spontaneous intraischemic decrease in brain temperature, only about 10 min was required for clearance of the elevated $[Glu]_e$. The $[Glu]_e$ clearance time with nicergoline administration (saline, 8, 16 or 32 mg/kg, i.p.) was shortened in a dose-dependent manner. Survival 4 h after ischemia was dose-dependently reduced by nicergoline treatment. The cerebral blood flow changes in the postischemic reperfusion period with nicergoline administration (saline, 8, 16 or 32 mg/kg, i.p.) at 39°C were 92.5 ± 5.6, 92.3 ± 6.8, 94.3 ± 5.8 and 93.5 ± 6.9% at 5 min post reperfusion and 87.5 ± 7.8, 84.8 ± 9.3, 83.3 ± 8.8 and 85.5 ± 9.2% at 30 min post reperfusion, respectively. The cerebral blood flow changes under mild hyperthermia (39°C) at 5 min and 30 min post reperfusion were not significantly different between saline and nicergoline treatment groups.

Fig. 3 is an electron micrograph showing the striatal brain damage in rats receiving an injection of saline (A) or nicergoline, 32 mg/kg, i.p., (B), when the temperature was kept at 39°C during the intraischemic period. The nuclei of some neuronal cells in ischemic rats that were not treated showed chromatin condensation, indicating that neuronal degeneration had occurred in these ischemic animals. Forty-two percent (63/150) of the cells had abnormal nuclei in the striata of ischemic animals that had been treated with saline ($n = 3$) (A). In contrast, neuronal cells in the striata of ischemic rats that had been treated with nicergoline were morphologically normal. Only 4% (6/150) of the cells had abnormal nuclei in the striata of ischemic animals that had been treated with nicergoline, 32 mg/kg, i.p. ($n = 3$) (B). Similarly, when the brain temperature dropped spontaneously from 37°C to 32°C, 6% (9/150) of striatal neuronal cells were abnormal ($n = 3$; data not shown).

4. Discussion

The importance of variations in brain temperature in pathological and functional outcomes (Ginsberg and Busto, 1998) has been demonstrated in various models of brain injury (Ginsberg et al., 1992). Mildly increased temperature has been shown to significantly increase $[Glu]_e$ (Busto et al., 1989; Mitani and Kataoka, 1991), and to exacerbate brain damage, which is intimately related to the pathogenesis of neuronal cell toxicity (Butcher et al., 1990; Bullock et al., 1991). Our previous study suggested that variation in brain temperature is not important in the initial biphasic increase in $[Glu]_e$ (Asai et al., 1998), but intraischemic normothermia is crucial to glutamate re-uptake during the reperfusion phase after an ischemic insult. (Zhao et al., 1997). Although intraischemic mild hyperthermia during severe ischemia has a more pronounced disruptive effect on glutamate re-uptake and on brain damage in the postischemic period, in the present study, nicergoline had little effect on $[Glu]_e$ during the intraischemic period, but dramatically enhanced Glu re-uptake in the postischemic period (Fig. 1). It is well known that neuronal death occurs subsequent to excessive glutamate-mediated excitation, suggesting that the enhancement of Glu re-uptake shortens the period of exposure to $[Glu]_e$ over a certain level of excitotoxicity. Nicergoline treatment reduced cell death (Fig. 3) and improved the survival rate (Fig. 2), indicating that the duration of exposure to a high $[Glu]_e$ level during the postischemic period correlates closely with the extent of damage during ischemia. Since cerebral blood flow and blood pressure during the postischemic period did not show good recovery with either nicergoline or saline treatment when the brain temperature was maintained at 39°C, ATP production may not have differed markedly between treatment and non-treatment groups (Katsura et al., 1992). These data suggest that the mechanism underlying the action of nicergoline might have little effect on cerebral blood flow, and is instead more closely related to the mechanism of Glu re-uptake during the postischemic period.

When the temperature was kept at 39°C during the intraischemic period, most of the rats died of cardiac arrest within 4 h during the postischemic period, even when continuously artificially ventilated (Fig. 2). Since neuronal alterations caused by mildly hyperthermic forebrain ischemia reportedly occur a few hours after recirculation, as determined in light and electron microscopic analyses (Dietrich et al., 1991; Kawai et al. 1992), the nicergoline effect in the mildly hyperthermic brain was evaluated at 2 h after reperfusion of the ischemic brain. As shown in Fig. 3, electron microscopic analysis revealed the striatal brain damage to be ameliorated in rats receiving a 32 mg/kg, i.p. injection of nicergoline. It is well known that ischemic neuronal injury is linked to the excessive activation of glutamate receptors (Choi and Rothman, 1990), which induces lethal Ca^{2+} entry and/or intracellular Ca^{2+} overload (Miljanich and Ramachandran, 1995; Siesjö et al., 1995). Excessive influx of Ca^{2+} into the cell under ischemic conditions, which has been suggested to be involved in *N*-methyl-D-aspartate (NMDA)-linked Ca^{2+} channel function (Morley et al., 1994; Bickler and Hansen, 1996), and/or the release of Ca^{2+} from intracellular stores (Alps, 1992; Morley et al., 1994; Siesjö et al., 1995) is due to intracellular calcium overload and is regarded as a major mechanism of cell injury and death (Ginsberg et al., 1991; Siesjö et al., 1995). According to Takahashi and

Akaike (1990), nicergoline blocks not only the L-type Ca^{2+} current but also the T-type Ca^{2+} current, suggesting that one of the neuroprotective effects of nicergoline, the prevention of ischemic brain damage, may be attributable to its inhibitory actions on T- and L-type Ca^{2+} channels in brain cells. Nicergoline reportedly protects against ischemic brain damage induced by bilateral carotid occlusion in the ICR-strain of mice and in Mongolian gerbils (Shintomi et al., 1886). In the present study, nicergoline-treated rats showed a dose-dependent reduction not only in the acute mortality rate, but also in neuronal damage, as demonstrated by electron microscopy.

A number of studies have indicated that a difference in the amount of glutamate released during ischemia affects the subsequent brain damage. Moreover, owing to the breakdown of ion gradients across neuronal and glial membranes during ischemia, glutamate uptake mechanisms are inhibited by the clearance of glutamate from the extracellular space and reversed operation of glutamate transporters, which constitute the so-called ''reversed uptake'' phenomenon (Attwell et al., 1993; Szatkowski and Attwell, 1994; Kanai, 1997). The ''reversed uptake'' phenomenon modulates the operation of glutamate transporters from the intracellular spaces and is probably related to altered glutamate release in the postischemic phase. The mechanisms underlying the neural protective effect and enhancement of Glu re-uptake by nicergoline remain obscure. Activation of NMDA receptors allows calcium ions to move passively into neurons and prompts the hydrolysis of arachidonic acid from membrane phospholipids (Sanfeliu et al., 1990; Miller et al., 1992). Increasing the concentration of exogenous arachidonic acid reduces the electrogenic uptake of glutamate into astrocytes (Barbour et al., 1989). An increased $[Glu]_e$ has been shown to induce rapid hydrolysis of arachidonic acid from membrane phospholipids and to synergistically activate protein kinase C together with diacylglycerol (Nishizuka, 1992; Berridge, 1993). Furthermore, the protein kinase C-dependent phosphorylation site in glutamate transporters is located in the intracellular loop between transmembrane domains (Casado et al., 1993), and a decrease in protein kinase C activity in both the cytosol and the membrane fraction has been demonstrated in a global brain ischemia-reperfusion model (Busto et al., 1994). TPA (12-*O*-tetradecanoylphorbol 13-acetate) activation of high-affinity sodium-dependent glutamate uptake in glial cells was shown to be mediated by the activation of protein kinase C, with an elevation in the intracellular free Ca^{2+} concentration, presumably involving phosphatidylinositol turnover (Casado et al., 1991). Recently, Carfagna et al. (1996) suggested that the enhanced response of phosphatidylinositol turnover to stimuli in the striata of nicergoline-treated rats reflected either an increase in receptor density or increased coupling between the receptor and phospholipase C (Carfagna et al., 1996). These authors also demonstrated an increase in the responsiveness of the phosphatidylinositol system when stimulated with an antagonist. These data suggested that intracellular calcium influx and its main pathway, as described above, might play an important role in the glutamate re-uptake mechanism, which operates mainly via glutamate transporters (Attwell et al., 1993; Kanai, 1997), although the regulation of glutamate transporters during ischemia has not yet been clarified.

Our previous observation of enhanced re-uptake suggests that there is a briefer exposure to high $[Glu]_e$ in the postischemic period and thereby, possibly, a decrease in its deleterious excitotoxic effect on neural cells (Zhao et al., 1997). Our present results raise the possibility of novel therapeutic strategies, because nicergoline treated rats showed minimal inhibition of the increase in $[Glu]_e$ elevation which characteristically occurs during the intraischemic period, while Glu re-uptake was dramatically improved in the postischemic period. Moreover, the treated rats clearly had a far lower mortality rate in association with severe transient global ischemia. Niecergoline was shown to exert a protective effect via a novel mechanism. The development of therapeutic strategies aimed at inhibition or prevention of the reversed uptake of glutamate release during ischemia, i.e., activation of the glutamate uptake mechanism, which may be intimately associated with glutamate transporters during ischemia, is a promising approach for reducing the neural damage occurring in response to brain ischemia.

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