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Original Article

Size-controllable striatal lesion model for evaluation of neuroprotective agents in rats

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ABSTRACT

Objectives: Various focal brain lesions are primarily caused by excessive glutamate release. An easy and convenient *in vivo* striatal lesion model was developed in which reliable and controllable lesion sizes were created by glutamate for evaluation of neuroprotective agents.

Materials and Methods: Dose-dependent striatal lesions were created by intrastriatal infusion of sodium glutamate in anesthetized male Sprague-Dawley rats (250–350 g). The lesion sizes were estimated with a 2% triphenyltetrazolium chloride stain. Motor disturbances were evaluated by the rotarod test and rotational behavior test. This experimental model was attested with neuroprotective effects of granulocyte colony-stimulating factor (G-CSF, 200 μg/kg), a cytokine growth factor, and estradiol (2 mg/kg), a female sex hormone.

Results: Intrastriatal infusion of 2–6 μmol of 1 M (i.e., 2 Osm) sodium glutamate produced dose-dependent (or controllable) increases in sizes and volumes of striatal lesion in which unpredictable data were also included in analyses. The infusion rate appeared not to affect the lesion size or volume. Although the 2 Osm (1 M) glutamate solution had a much higher osmolarity than brain extracellular fluid (0.3 Osm), control infusions with the 2 Osm of glucose or sodium chloride (NaCl) produced only negligible lesions. The striatal lesion or volume induced by glutamate (4 μmol, 1 M) was reduced by pretreatment with the neuroprotective agents G-CSF and estradiol. The motor disturbance caused by glutamate infusion was also improved by G-CSF administration.

Conclusion: Local intrastriatal infusions with controlled doses of glutamate can create a reliable, controllable striatal lesion size. This model is easy and convenient for evaluation of neuroprotective agents.

1. Introduction

Glutamate is a major neurotransmitter at most excitatory synapses in the mammalian central nervous system. It activates two kinds of receptors, the ionotropic and metabotropic glutamate receptors [1,2]. The ionotropic action of glutamate acts on three distinct receptor systems, the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainate receptors [3], to mediate most of the excitatory synaptic transmissions in the brain. Excitotoxicity of over-released glutamate is a key factor in central neuronal loss consequent to hypoxic–ischemic insult [4].

The extracellular concentration of glutamate is normally maintained at ~1 μM by sodium (Na^+) flux-coupled transport [5]. Excessive releases and/or accumulations of glutamate in the extracellular fluid commonly occur in brain injuries, including acute insults (such as cerebral ischemia, cerebral hemorrhage, brain infection, traumatic brain injury) and chronic neurodegenerative diseases (such as Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis) [6,7]. Brain injuries cause falls in Na^+ gradients and cellular energy reserves, leading to an increased release and impaired uptake of glutamate in which toxic extracellular glutamate is built up. This toxic glutamate induces an

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over-stimulation of glutamate receptors and consequently neuronal cell damage and death. Because the mechanisms of the neuronal damage or death caused by glutamate are not yet well clarified, therapeutic and preventive approaches for brain injuries remain unsatisfactory.

The striatum, a main input nucleus of the basal ganglia, receives a great number of glutamatergic afferents from the cortex and thalamus [8]. Therefore, glutamate excitotoxicity in striatum disorders has attracted considerable attention [6,9]. Focal striatal lesions (injuries) have been produced by local administration of quinolinate (an NMDA agonist of glutamate) or/and kainate (a non-NMDA agonist of glutamate) into the striatum for evaluation of various neuroprotective agents [10–12]. Administration of quinolinate or kainate into the striatum causes excitotoxicities by over-stimulation of the glutamatergic receptors. It is still important to ask whether a neuroprotective agent effective in striatal damage induced by kainate or quinolinate is also effective in that induced by directly increasing the extracellular glutamate concentration by direct injection of glutamate in the striatum. As far as we know, the evaluation of neuroprotective agents in striatal lesions induced by directly increasing the extracellular glutamate concentration in the striatum has seldom been addressed.

The mechanisms of neuronal damage or death caused by glutamate are not yet well clarified. Therefore, developing a good animal model, in which the degree of neuronal damage or death and the size of the damaged brain area (lesion) produced by glutamate are reliable and controllable, has become very important. In this experiment, lesions were produced in the striatum, because the approach to the nucleus is easy with insertion of a tube for glutamate microinfusion. In addition, evaluation of motor disturbance is possible because striatal lesions disturb motor function. This is important because evaluation of therapeutic effects should be based on functional recovery as well as biochemical, morphological, and histological recovery.

This study reports a novel focal striatal lesion model in which lesion sizes were reliable and controllable, and lesion-induced motor disturbance could be evaluated. This model has proven to be valid and convenient for evaluation of neuroprotective agents.

2. Materials and methods

2.1. Chemicals

Chloral hydrate, urethane, sodium glutamate, triphenyltetrazolium chloride (TTC), and estradiol were purchased from Sigma-Aldrich (St Louis, MO, USA). Granulocyte colony-stimulating factor (G-CSF, 300 μg in 0.7 mL) was purchased from Kirin Brewery Company (Gunma, Japan). Chloral hydrate (0.04 g/mL) or urethane (0.5 g/mL) was dissolved in normal saline and stored at room temperature for further use. Sodium glutamate (1 M) and TTC (0.02 g/mL) were dissolved in normal saline and stored at 4°C. G-CSF (300 μg in 0.7 mL) was stored at 4°C. G-CSF (200 μg/kg) and estradiol (2 mg/kg) were freshly dissolved in 0.5 mL normal saline and 1.0 mL 1% dimethyl sulfoxide, respectively.

2.2. Surgical procedures

The experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and with the Guidelines of the Tzu Chi University Ethical Committee for Animal Research. The Committee has approved this investigation.

Male Sprague-Dawley rats (250–350 g) were anesthetized with urethane 1.0 g/kg or chloral hydrate 0.04 g/kg. All surgical procedures were done under aseptic conditions. The femoral vein was cannulated with a PE-50 polyethylene tube for fluid supplements and drug administration, and the femoral artery for monitoring of arterial blood pressure. The arterial blood pressure and heart rate were recorded and computed with a MP35 amplifier (BIOPAC Inc, Goleta, CA, USA) and stored in a computer. The body temperature (rectal temperature) was automatically maintained at 37.5 ± 0.5°C by a rectal temperature sensor and a heating pad (CMA-150; CMA Microdialysis AB, Stockholm, Sweden). The head of the rat was fixed on a Stoelting stereotaxic instrument (Stoelting Co, Wood Dale, IL, USA). A midline incision on the scalp exposed the skull for insertion of a microinfusion tube (28-gauge, Small Parts, Inc., Miami, FL, USA). To facilitate insertion of the infusion tube into the striatum, a hole about 1.0–1.2 mm was created in the skull with a dental drill at 0.5 mm rostral to the bregma, 3.0 mm lateral to the midline, and 6.0 mm ventral to the surface of the cortex. Animals were stabilized for 20 minutes before experiments.

2.3. Creation of striatal lesions

To create striatal lesions, sodium glutamate was microinjected through the infusion tube implanted in the striatum as described above. The lesion was routinely created unilaterally on the right striatum. One end of the infusion tube was connected to PE-50 polyethylene tubing and the other end to the needle of a Hamilton syringe (Hamilton Company, Reno, NV, USA). The microinfusion was accomplished by pushing the Hamilton syringe with a CMA-200 microinfusion pump (CMA Microdialysis).

Estradiol or G-CSF was used for pretreatment 30 minutes before the glutamate infusion. For the rotational behavior test, G-CSF (200 μg/kg, subcutaneously) was given immediately after and every 24 hours for 1 week after glutamate administration.

2.4. Measurement of striatal lesions

To evaluate lesion volume, animals were sacrificed 18 hours after the infusion of glutamate and the brains were removed. The brains were fixed with rat brain stainless steel coronal slices matrices RBS-SS1-C (ASI Instruments, Inc, Warren, MI, USA) in 9% normal saline at 4°C. Coronal sections (1 mm thickness) were made from the olfactory bulb to the cerebellum. The sections were taken at 2 mm, 1 mm, 0 mm, −1 mm, −2 mm, and −3 mm from the injected site (0 mm) to contain the entire lesion. They were placed in 2% TTC in normal saline and stained for 20 minutes at 37°C in the dark, then washed twice with saline, and fixed with 4% paraformaldehyde for 30 minutes at room temperature.

TTC is a reagent for oxidative enzymes first introduced by Jestaedt and Sandritter [13] as a stain to detect ischemic injury of the myocardium. TTC is widely applied in localization of various brain injuries. This salt is reduced by accepting a proton from succinate dehydrogenase in the inner membrane of the mitochondria to form red insoluble formazan. Therefore, in an area with inactive enzymes, the lesion area is not stained and appears pale. The pale area (not stained by TTC) was defined as the core area, a slightly reddened area (partially stained by TTC) was defined as the penumbra area, and a dark red area (completely stained by TTC) was defined as the undamaged area.

Anterior face images of collected TTC sections were photographed and the images stored as JPG computer files. Image J software (NIH, Bethesda, MD, USA) was used to measure and analyze the lesion size (area) and volume [14].

2.5. Motor behavior test (rotational behavior test)

Rotational behavior was measured 7 days after the administration of glutamate (4 μmol, 1 M, 0.05 μL/min, intrastriatally).

Amphetamine (2.0 mg/kg, intraperitoneally) was administered to observe rotational behavior. Complete rotation (360°) was recorded every 5 minutes in both clockwise (ipsiversive to the infused hemisphere) and counterclockwise (contraversive to the infused hemisphere) directions for 60 minutes according to the method of Altar et al [15] with modifications. Numbers of complete ipsiversive and contraversive rotations were recorded every 5 minutes for 60 minutes. I/(I + C) indicates ipsiversive rotations/(ipsiversive rotations and controversive rotations), reflecting changes in rotational behavior. Rats were sacrificed immediately after the measurement.

2.6. Rotarod test

Motor impairment was assessed by a TSE RotaRod System equipment (Technical & Scientific Equipment GmbH, Bad Homburg, Germany). Briefly, the animal was place on a rod, a 5 cm diameter steel beam covered with high-profile rubber to ensure firm gripping. This rod turned on its longitudinal axis at an initial rate of 4 rpm and accelerated to 40 rpm in 300 seconds. Side panels were inserted at each end of the rod to prevent escape from the apparatus. The training room for the rotarod test was maintained at a temperature 22–24°C, and noise was kept to a minimum.

Before the rotarod test, the rats had daily training sessions, in which they were gently moved to the training room and gently placed on the rod with the orientation opposite to that of the already rotating rod so that they could acquire the necessary skilled behavior to prevent a fall. Rats were allowed into the experiment when they could stay on the rod for at least 150 seconds. The rotarod test was done 10 times 1 hour before the glutamate infusion and at 17 hours, 3 days, 5 days, and 7 days after the glutamate infusion. The test scored based on the rats' baseline ability. Rats that did not fall off the rod within 10 minutes were given a maximum score of 600 seconds [16].

2.7. Statistical analysis

All data are presented as mean ± standard error of the mean. Differences between groups with identical treatments were determined with unpaired Student *t* test. In all cases, differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Cardiovascular parameters

In urethane-anesthetized rats, the basal systemic arterial pressure and heart rate were 101.6 ± 3.0 mmHg and 429.0 ± 19.1 beats/min, respectively. They were not altered by microinfusion of sodium glutamate and normal saline (vehicle) into the striatum. The glutamate infusion into the striatum, however, induced a striatal blood flow increase (124.6 ± 2.6% of basal level) in 5 minutes and a maximum increase (153.3 ± 19.36% of basal level) in 60 minutes. The flow gradually returned to the basal level in 120–150 minutes (Fig. 1). The striatal blood flow was not affected by microinfusion of vehicle.

In chloral hydrate-anesthetized rats, the basal systemic arterial pressure and heart rate were 99 ± 5.0 mmHg and 400.0 ± 21.3 beats/min, respectively, essentially similar to those in urethane-anesthetized rats. These parameters, however, started to fluctuate and increase at 60 minutes after anesthesia, because chloral hydrate is a short acting anesthetic agent. A supplemental dose of 0.1 g/kg was subcutaneously administered to maintain stable blood pressure. The glutamate infusion into the striatum essentially induced an increase in striatal blood flow similar to that in the urethane-anesthetized rats.

Because rats anesthetized with urethane were unable to wake up in 24 hours, they were used for long-term monitoring of cardiovascular parameters (Fig. 1). However, they tended to suffocate under 24-hour anesthesia conditions. By contrast, those anesthetized with chloral hydrate woke up in 3–4 hours, and hence suffocation was generally avoided. In the following experiments (Figs. 2–7), therefore, all rats were anesthetized with chloral hydrate.

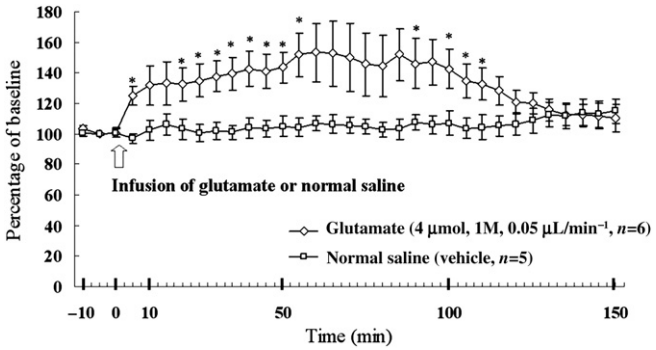

Fig. 1. Effects of continuous 80-minute infusion of sodium glutamate into the right striatum on ipsilateral striatal blood flow as measured with a laser Doppler flowmeter probe. * $p < 0.05$ versus normal saline group (unpaired *t* test).

3.2. Dose-dependent effects on striatal lesions

Fig. 2 shows that unilateral intrastriatal glutamate infusions induced similar dose-dependent increases in striatal lesion sizes

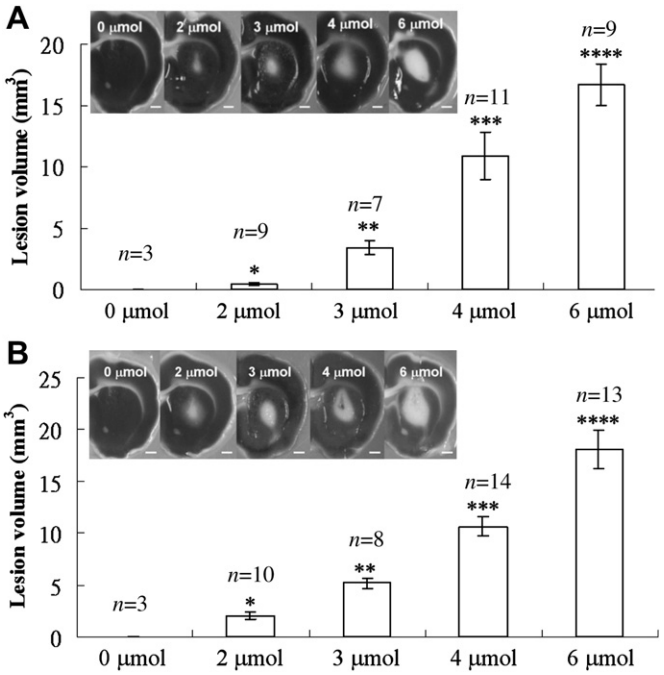

Fig. 2. Effects of striatal lesion sizes and volumes upon infusion of normal saline (sodium glutamate 0 μmol), and glutamate 2 μmol, 3 μmol, 4 μmol, and 6 μmol in rats anesthetized with (A) urethane (1.5 g/kg) and (B) chloral hydrate (0.4 g/kg). Normal saline and sodium glutamate (1 M or 2 Osm) were infused at 0.05 μL/min for 120 min, 40 min, 60 min, 80 min, or 120 min. The rats were sacrificed 18 h after infusion. Representative brain slides (A) were stained with triphenyltetrazolium chloride (TTC). Scale bar, 1 mm. The histogram summarizes the results. For A, *, $p < 0.01$ versus 0 μmol group (vehicle with normal saline for 120 min); **, $p < 0.01$ versus 2 μmol group; ***, $p < 0.01$ versus 3 μmol group; ****, $p < 0.01$ versus 4 μmol group. For B, *, $p < 0.001$ versus 0 μmol group; **, $p < 0.001$ versus 2 μmol group; ***, $p < 0.001$ versus 3 μmol group; ****, $p < 0.01$ versus 4 μmol group (unpaired *t* test).

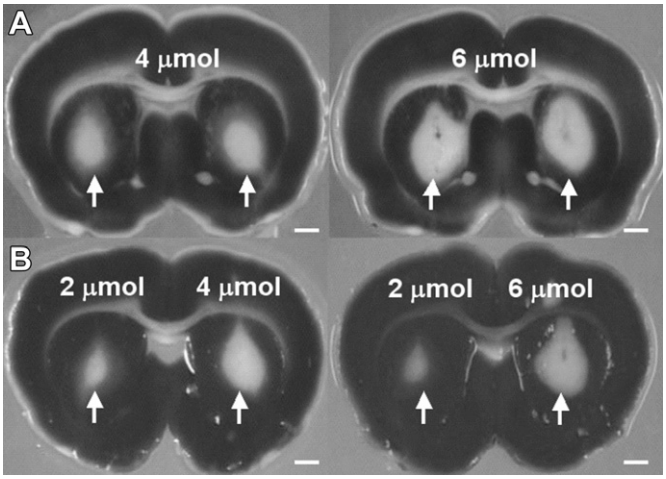

Fig. 3. Triphenyltetrazolium chloride (TTC)-stained brain slices indicate striatal lesion sizes produced by intrastriatal infusion of (A) the same doses and (B) different doses of glutamate (1 M, 0.05 μL/min) on both sides of a rat brain. The doses were adjusted by injection time as in Fig. 2. The rats were sacrificed 18 h after infusion for TTC staining of brain slides. Note the similarity or reliability (A) and the dose-dependency (B) of the lesion sizes on both sides of the brain. Scale bar, 1 mm.

and volumes in rats anesthetized with either anesthetic, indicating the lesion sizes and volumes are controllable. The average lesion volumes with 2 μmol, 3 μmol, 4 μmol, and 6 μmol of sodium glutamate, were 0.5 ± 0.1 mm^3 ($n = 10$), 3.2 ± 0.5 mm^3 ($n = 8$), 10.5 ± 1.9 mm^3 ($n = 14$), and 16.7 ± 1.7 mm^3 ($n = 13$), respectively, in rats anesthetized with urethane (1.5 g/kg; Fig. 2A). The lesion volumes with 2 μmol, 3 μmol, 4 μmol, and 6 μmol of sodium glutamate, were 2.0 ± 0.4 mm^3 ($n = 10$), 5.2 ± 0.5 mm^3 ($n = 8$), 10.7 ± 0.9 mm^3 ($n = 14$), and 18.1 ± 1.9 mm^3 ($n = 13$), respectively, in rats anesthetized with chloral hydrate (0.4 g/kg; Fig. 2B). Highly reliable (less varied) lesion sizes could be created on both sides of the striatum of the same rat with the same dose of glutamate (Fig. 3A). Furthermore, markedly dose-dependent lesion sizes were produced by different doses of glutamate on both sides of a rat brain (Fig. 3B). Because 4 μmol produced a moderate lesion volume (Figs. 2, 3), this dose was used for the experiments in Figs. 5–7.

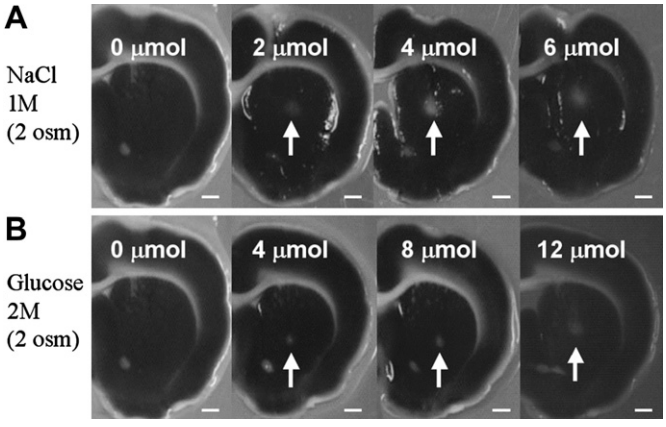

Fig. 4. Triphenyltetrazolium chloride (TTC)-stained brain slices show that infusion (0.05 μL/min) of various doses of high osmolar (A) sodium chloride (NaCl) and (B) glucose solutions into the striatum of chloral hydrate (0.4 g/kg). Anesthetized rats produced only tiny lesions (arrows) that can be ignored. The doses were adjusted by injection time as in Fig. 2. The rats were sacrificed 18 h after the infusion for TTC staining of brain slides. 0 μmol indicates vehicle (normal saline) infusion for 2 h. Scale bar, 1 mm.

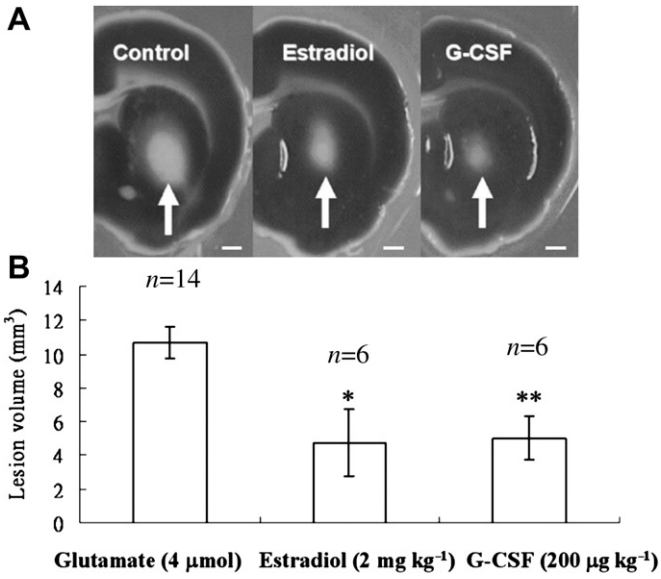

Fig. 5. The effects of estradiol or granulocyte colony-stimulating factor (G-CSF) on striatal lesion sizes (A) and volumes (B) produced by intrastriatal infusion of glutamate (4 μmol, 1 M, 0.05 μL/min) in rats anesthetized with chloral hydrate (0.4 g/kg). Estradiol or G-CSF was administered intravenously 30 min before the glutamate infusion and the rats were sacrificed 18 h later. Brain slices were stained with TTC (A). Arrows indicate the lesion sizes. The histogram summarizes the experimental results (B). *, $p < 0.05$, **, $p < 0.01$ versus the control group (glutamate and vehicle), unpaired t test. Scale bar, 1 mm.

The infusion rate did not appear to affect the lesion size or volume, since lesion sizes caused by the same dose (4 μmol in 4 μL) of 1 M glutamate infused at different rates (0.3 μL/min and 0.05 μL/min, $n = 3$) showed no significant differences (data not shown).

3.3. Negligible effect of high osmolar solutions on lesion size

The glutamate (1 M) used in this experiment was 2 Osm. This osmolarity was much higher than that of plasma or extracellular fluid (0.29 Osm). It was tested whether this high concentration of glutamate causes osmotic damage to the brain. Fig. 4 shows that microinfusion of a 2 Osm solution of 1 M sodium chloride (NaCl) or

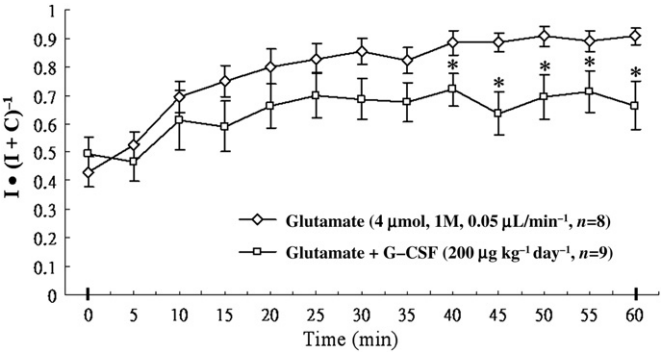

Fig. 6. Effects of granulocyte colony-stimulating factor (G-CSF) on rotational behavior produced by unilateral intrastriatal infusion of glutamate (4 μmol, 1 M, 0.05 μL/min). G-CSF was administered subcutaneously immediately before glutamate infusion and every day until the day the animals were sacrificed. The glutamate infusion induced motor disturbance as judged by a rotational behavior test 1 week after the lesion was induced. Numbers of complete ipsiversive and contraversive rotations were recorded every 5 min for 60 min. I/(I + C) indicates ipsiversive rotations/(ipsiversive rotations and controversive rotations), reflecting changes in rotational behavior. *, $p < 0.05$ versus the control group (glutamate and vehicle), unpaired t test.

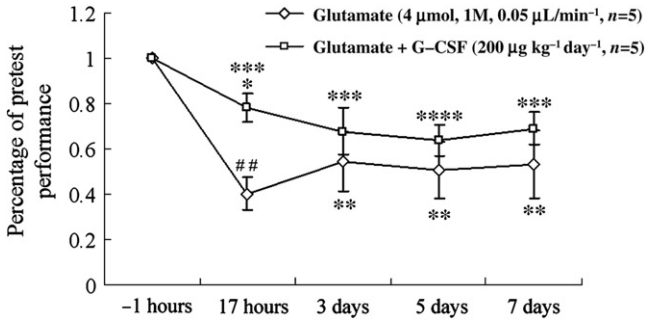

Fig. 7. Effects of granulocyte colony-stimulating factor (G-CSF) on rotarod performance in rats subjected to intrastriatal infusion of glutamate (4 μmol, 1 M, 0.05 μL/min). G-CSF was administered subcutaneously right before glutamate infusion and every day until the day the animals were sacrificed. Rotarod performance was tested 1 h before (−1 h) glutamate infusion and 17 h, 3 days, 5 days, and 7 days after glutamate infusion. *, $p < 0.01$ versus the glutamate group (paired t test); **, $p < 0.05$ versus −1 h (paired t test); ***, $p < 0.05$ versus −1 h (paired t test); ****, $p < 0.01$ versus −1 h (paired t test); ##, $p < 0.01$ versus −1 h (paired t test).

2 M glucose produced only very small (negligible) lesion sizes. These results suggest that the 1 M (2 Osm) glutamate used in this experiment does not produce significant osmotic damage.

3.4. Validity of the model for studying neuroprotecting agents

In order to test whether this animal model was valid for studying neuroprotective agents, the effects of G-CSF and estradiol on unilateral glutamate-induced lesion sizes and volumes were examined (Fig. 5). Pretreatment with either drug significantly reduced the glutamate-induced striatal lesion size and volume. These findings indicate that this model is valid for testing neuroprotective agents.

3.5. Motor function assessed by rotational behavior test and rotarod test

A unilateral intrastriatal injection of glutamate (4 μmol, 1 M, 0.05 μL/min) induced motor disturbance as judged by the rotational behavior test 1 week after induction of the lesion (Fig. 6). Motor disturbance was characterized by increased (biased) ipsiversive rotation activity as indicated by an increase in the ratio of I/(I + C) in the glutamate and vehicle group, with a decrease in the glutamate and G-CSF group.

Fig. 7 shows that a unilateral striatal lesion caused motor disturbance as judged by the rotarod test. Motor disturbance improved with subcutaneous G-CSF administration immediately before glutamate infusion and every day until the day the animals were sacrificed.

These findings indicate that the current model is valid for evaluating neuroprotective agents with assessment of motor function impairment.

4. Discussion

4.1. Major findings

This investigation demonstrated for the first time a novel rat model in which dose-dependent increases in striatal lesion sizes or volumes were produced by intrastriatal infusion of controlled doses of sodium glutamate (2–6 μmol, 1 M; Figs. 2 and 3). Although a glutamate solution of 1 M or 2 Osm has a much higher osmolarity than brain extracellular fluid (0.3 Osm), the osmolarity itself did not create a significant lesion (Fig. 4). This model is valid in studying the effects of neuroprotective agents, as shown by findings that glutamate-induced striatal lesions were reduced by two neuroprotective agents, estradiol and G-CSF (Fig. 5). Furthermore, the lesion-induced increase in ipsiversive rotational activity was attenuated by G-CSF (Fig. 6) and the lesion-induced decrease in motor performance on the rotarod test was improved by G-CSF (Fig. 7). Therefore, this study established a novel focal striatal lesion model in which local intrastriatal infusion of controlled doses of glutamate produced controllable and reliable lesion sizes and volumes. This model is easy and convenient for application in studying neuroprotective agents.

4.2. Glutamate-induced lesion size is controllable and reliable

In this experiment, glutamate doses in the range of 2–6 μmol (1 M) were able to produce dose-dependent and controllable lesion sizes (Fig. 2). The lesion size caused by the same (equal) dose of glutamate, however, varied in a somewhat wider range. This could be attributed to individual animal variation and not to technical problems, because highly reliable (less varied) lesion sizes were created by intrastriatal infusion of the same dose of glutamate into both sides of the same rat brain (Fig. 3A), and markedly dose-dependent lesion sizes were produced by intrastriatal infusion of different doses of glutamate into both sides of the same rat brain (Fig. 3B). Increasing the number of experimental animals to 14 or 15 could easily solve the problem of the wide variation in lesion sizes (Fig. 2). These findings indicate the reliability of the present technique.

Other models such as the middle cerebral artery occlusion (MCAO), middle cerebral artery ligation, endothelin-1 [14,17], collagenase infusion [18], and blood infusion models [18] have displayed unstable results, such as unexpected lesion sizes or damages, although all operative procedures and conditions were strictly standardized. To solve this problem, data selection was used in these models to minimize the standard error of the mean. Because the MCAO model usually has an infarct volume up to 400 mm^3, animals with no or minimal infarcts (<60 mm^3) in one study were excluded from the analysis before unblinding, but included in the treatment group [14]. In this experiment, the lesion size varied somewhat, but there were never unexpectedly large or small lesions. Nevertheless, exclusion of data from analyses was avoided by simply increasing the number of animals to 14 or 15 (Fig. 2).

4.3. High-concentration glutamate and its osmotic effects

Microdialysis studies have confirmed the release of various excitatory amino acids in the extracellular space in rat brains subjected to MCAO [19]. MCAO induced a rapid and marked increase in the extracellular concentrations of glutamate up to 14 fold of the physiological level, <1–10 μM [20]. In this study, microinjection of sodium glutamate at a concentration lower than 0.34M, or 0.68 Osm (1.0 μmol or 2.0 μmol, infused at 0.3 μL/min for 3.3 min) failed to produce a lesion (data not shown). The failure was probably due to faster clearance of the glutamate, very slow infusion of the glutamate, or glutamate-induced increase in blood flow (Fig. 1). Therefore, a high concentration 1 M sodium glutamate, about $1–10 \times 10^6$ times higher than the physiological level, was used to produce striatal lesions.

Using high concentration glutamate (1 M) to produce lesions in this experiment inevitably involved an osmotic effect on the lesion size. The osmolarity of the glutamate was IM or 2 Osm (2 μmol, 3 μmol, 4 μmol, and 6 μmol in 2 μL, 3 μL, 4 μL, and 6 μL, respectively). This was higher than the plasma osmolarity of 0.29 Osm. An infusion of 2 Osm NaCl (1 M) or glucose (2 M) into the striatum, however, did not produce significant lesions (Fig. 4). This indicates that a 1 M

concentration of glutamate could produce only small or negligible lesions, and the lesions produced by various doses of glutamate can be almost attributed to the excitotoxic effect of glutamate.

4.4. The model is valid for investigation of neuroprotective agents

G-CSF, a member of the cytokine family of growth factors, protects against glutamate-induced excitotoxicity in cell culture and reduces infarction volume after focal cerebral ischemia [21]. Estradiol, a female hormone, possesses a wide spectrum of protective mechanisms against multiple toxic insults, as shown in not only an *in vitro* cell culture model [22] but also in an *in vivo* model of global and transient ischemia [23]. In this study, pretreatment with G-CSF or estadiol reduced the size and volumes of glutamate-induced striatal lesions (Fig. 5). Furthermore, pretreatment with oroxylin-A, an antioxidant biphenolic compound, also reduced lesion size (data not shown). These pretreatment experiments show the validity of the present model for assessing the effect of neuroprotective drugs.

4.5. The model is valid for evaluation of motor function impairment

An ideal *in vivo* brain damage model has to be valid for evaluating motor function impairment in addition to lesion sizes so the effects of neuroprotective agents can be properly assessed. In this experiment, the lesion involved primarily the rostral striatum, which relays motor control signals of the basal ganglia. An increased (biased) rotational activity to the lesion side (ipsiversive rotational behavior) was detected 7 days after the lesion (Fig. 6). Impairment of rotarod performance was detected at 17 hours, 3 days, 5 days, and 7 days after induction of lesions in the rats. G-CSF attenuated the increased ipsiversive rotational activity and improved the rotarod performance compared with vehicle-treated animals. Hence, our model is valid for evaluating motor function impairment.

5. Conclusion

This study established a novel focal striatal lesion model in which local intrastriatal infusion of controlled doses of glutamate produced stable, controllable, and reliable focal striatal lesions. This model is easy and convenient for application in studying the neuroprotective mechanisms of neuroprotective agents and the pathophysiological mechanisms in glutamate-induced brain damage.

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