Methamphetamine Potentiates Ischemia/Reperfusion Insults After Transient Middle Cerebral Artery Ligation

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Background and Purpose—Previous studies have indicated that both methamphetamine (MA) and ischemia/reperfusion injuries involve reactive oxygen species formation and activation of apoptotic mechanism. That MA could have a synergistic or additive effect with stroke-induced brain damage is possible. The purpose of the present study was to investigate whether administration of MA in vivo would potentiate ischemic brain injury.

Methods—Adult CD-1 mice were pretreated with MA or saline. Each animal later was anesthetized with chloral hydrate and placed in a stereotaxic frame. A subset of animals received intracerebral administration of glial cell line–derived neurotrophic factor (GDNF). The right middle cerebral artery and bilateral carotids were transiently occluded for 45 minutes. Regional cerebral blood flow was measured by laser Doppler. Animals were sacrificed for triphenyltetrazolium chloride staining and p53 mRNA Northern blot assay after 24 hours of reperfusion. Cortical and striatal GDNF levels were assayed by ELISA.

Results—We found that pretreatment with MA increased ischemia-induced cerebral infarction. Ischemia or MA alone enhanced p53 mRNA expression. Moreover, MA potentiated expression of p53 mRNA in the ischemic mouse brain. MA pretreatment decreased GDNF levels in ischemic striatum. Intracerebral administration of GDNF before ischemia reduced MA-facilitated infarction.

Conclusions—Our data indicate that MA exacerbates ischemic insults in brain, perhaps through the inhibition of GDNF-mediated pathways and suggest that MA may antagonize endogenous neuroprotective pathways as part of its mechanism of action. (Stroke. 2001;32:775-782.)

Key Words: amphetamines ■ apoptosis ■ cerebral ischemia, focal ■ transforming growth factors

Several clinical studies have indicated that patients with a chronic or acute methamphetamine (MA) abuse history develop cerebral hemorrhages in striatum and infarction in the middle cerebral arterial (MCA) distribution area, which suggests that the use of MA may be associated with brain ischemia. However, it is still not known whether administration of MA predisposes to ischemic brain insults and, if so, the potential mechanisms by which this occurs.

MA causes formation of reactive oxygen species, and others have previously reported that ischemia and reperfusion can induce formation of reactive nitrogen compounds. Because reactive oxygen and reactive nitrogen species form a toxic metabolite ONOO-, which activates poly(ADP-ribose) synthetase and damages cells, MA could facilitate the formation of ONOO- during stroke and cause increased neuronal damage. A recent study has also indicated that MA or amphetamine increases neuronal cell death associated with endonucleosomal DNA cleavage and differential expression of antiapoptotic and proapoptotic bcl-x (L/S) splice variants. Furthermore, p53 knockout mice have reduced MA-induced toxicity. Taken together, these data support the hypothesis that apoptotic pathways are involved in amphetamine-induced neurotoxicity.

Recent studies have indicated that glial cell line–derived neurotrophic factor (GDNF) reduces MA-mediated toxicity. GDNF antagonized MA-induced reductions in potassium-evoked overflow of dopamine, and dopamine and serotonin (5-HT) content in striatum. Similarly, pretreatment with GDNF decreases ischemia-induced brain infarction, brain edema, density of terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling (TUNEL) (+) neurons, and immunoreactivity of caspase-1 and caspase-3 in the ischemic cortex, which suggests that GDNF may reduce ischemic insults by attenuating apoptosis and/or necrosis. Kokaia et al reported that GDNF mRNA was upregulated after short-term ischemia. Upregulation of GDNF may be a
result of activation of endogenous neuroprotective processes during these insults.

In the present study, we acutely treated CD-1 mice with MA before ischemia/reperfusion induced by distal MCA ligation. We sought to determine whether MA augmented the extent of ischemic damage and whether such changes could be correlated with alterations in endogenous proapoptotic or antiapoptotic mechanisms.

Subjects and Methods

Animals

A total of 153 adult male CD-1 mice (Charles River Laboratories) were used for the present study. Animals were treated with 4 doses (2 hours apart) of MA (1.0 mg/mL×10 mL/kg IP) or 4 doses of saline (10 mL/kg IP). Animals were subjected to right MCA ligation (see below) 30 minutes after the last dose of MA or saline. All procedures were performed in accordance with institutional guidelines.

MCA Ligation and Histology

Each mouse was anesthetized with chloral hydrate (400 mg/kg IP). Ligation of the right MCA and bilateral common carotid arteries (CCAs) was performed using methods previously described. Bilateral CCAs were identified and isolated through a ventral midline cervical incision. CCAs were ligated with nontraumatic arterial clips. A craniotomy of approximately 4 mm² was made in the right squamous bone. Right MCA was ligated with a 10–0 suture. After 45 minutes of ischemia, the suture on the MCA and arterial clips on CCAs were removed to allow reperfusion. Body temperature was monitored with a thermistor probe and maintained at 37°C with a heating pad during anesthesia. After recovery from the anesthesia, body temperature was further maintained at 37°C with a heat lamp. Twenty-four hours after onset of reperfusion, animals were euthanatized. Brain tissue was then removed and sliced into 1.0-mm sections with a mouse brain matrix (Harvard model 52-8893). Brain slices were incubated in 20 g/L of tripolyphosphate chloride (TTP) dissolved in saline for 15 minutes at 37°C and then transferred to 1.25 mol/L formaldehyde solution for fixation. Area of infarction in each slice was measured with a digital scanner. Volume of infarction in each animal was obtained from the product of average slice thickness (1 mm) and sum of infarction areas in all brain slices.

Intracerebral Injection of GDNF

After the fourth MA injection, animals were anesthetized with chloral hydrate and placed in a stereotactic frame. Human recombinant GDNF (6×10⁻⁶ g in 3×10⁻⁸ L) was injected directly into the right cortex and striatum (1 to 2 mm below brain surface) through a Hamilton syringe adjacent to the first bifurcation of MCA. The injection was made 15 minutes before MCA ligation. The needle was retained in place for 5 minutes after each injection.

P53 mRNA Assays

Mice were killed 24 hours after ischemia/reperfusion. Brains were removed rapidly from skulls, dissected, and stored at −70°C.

P53 Analyses

Total RNA was extracted from freshly dissected frontal cortex and striatum. For Northern blot analysis, total RNA (10×10⁻⁶ g per lane) was denatured with 12.5 mol/L formamide and 2.17 mol/L formaldehyde, electrophoresed on a 10 g/L denaturing agarose/formaldehyde gel, and transblotted directly onto a nylon membrane (Hybond N, Amersham). Blotted RNAs were UV cross-linked. The level of p53 was normalized compared with the 18S rRNA. PCRs of the p53 mRNA probe with a sequence complementary to bases 206 to 1138 were radiolabeled with 3²P-dCTP using Random Primed DNA labeling kit (Boehringer Mannheim Biochemica). The oligonucleotide probe was used for detection of 18S rRNA. The probe was labeled with 3²P-dCTP with 3²-terminal deoxynucleotidyl transferase (Amersham) and hybridized to the membrane.

Measurement of GDNF by ELISA and Acidic Treatment

One day after MCA ligation or injection of MA or saline, animals were killed and brains quickly removed. After cerebellum was removed, the brain tissue was dissected and homogenized with a Teflon homogenizer in a lysis buffer (137×10⁻³ mol/L NaCl, 20×10⁻³ mol/L Tris [pH 8.0], 16.6×10⁻³ mol/L NP40, 1.1 mol/L glycerol, 1×10⁻³ mol/L phenylmethyl sulfonyl fluoride, 1.5×10⁻⁶ mol/L aprotinin, 4.3×10⁻⁶ mol/L leupeptin, and 1.0×10⁻² mol/L sodium vanadate). Homogenates were centrifuged at 12 000 g for 20 minutes. Pellets were discarded, and the supernatant was acidified. Acidification has been reported to enhance detection of neurotrophic factors. Samples were neutralized to pH 7.4 and adjusted with buffer to contain the same amount of protein (2 g/L). Protein concentrations were measured by use of the BCA kit (Pierce). Samples were assayed for neurotrophic factors by ELISA. For measurement of GDNF, mouse monoclonal anti-GDNF antibody (R&D System) was used as a capture antibody and biotinylated goat anti-GDNF antibody (R&D System) was used for detection. NP-40, aprotinin, and sodium vanadate were purchased from Calbiochem. PMSF and leupeptin were obtained from Sigma. A THERMOMax 96-well microplate reader was used to measure optical intensities.

Cerebral Blood Flow

Cortical and striatal blood flow was continuously measured with a laser Doppler flowmeter (PF-5010, Periflux system, SE). Animals were anesthetized and placed in a stereotactic frame. Two burr holes (1 mm diameter) were made on the right and left frontoparietal region to allow placement of the photodetectors. Two probes (probe 411, 0.45 mm in diameter) were stereotactically placed in the cortex (1.3-mm posterior and 2.8 mm lateral to bregma and 1.0 mm below dura) and in striatum (0.02 mm posterior and 2.0 mm lateral to bregma and 2.5 mm below dura).

Blood Pressure, Heart Rate, Calcium, and Blood Gas Measurements

Physiological parameters were measured in mice treated with 1 or 4 doses of either MA or saline. Animals were anesthetized 10 minutes after the last dose of MA, and a polyethylene catheter (Dural Plastics and Engineering Pty Ltd) was inserted into the right CCA. Mean arterial pressure was recorded through a strain-gauge transducer (Statham P23 ID) and recorded on a strip-chart recorder. Arterial blood (0.3 mL) was withdrawn from the artery after the fourth dose of MA or saline injection. Blood was heparinized; blood pH,
concentrations of CO₂ and O₂, and hemoglobin levels were analyzed with a blood gas analyzer (NOVA Biomedical).

**Hematoxylin and Eosin Staining**

Each mouse treated with 4 doses of MA or saline was killed for staining with hematoxylin and eosin. The animal was anesthetized and perfused with 4% paraformaldehyde. The brain was blocked and embedded in paraffin. Tissues were sectioned at a thickness of 4×10⁻⁶ m. Slices were stained with hematoxylin and eosin.

**Statistics**

Data were expressed as mean±SEM. Student’s t test, ANOVA, and post hoc Newman-Keuls test were used for statistical analysis. Probability values <0.05 were considered significant.

**Results**

**Cerebral Blood Flow**

A total of 34 mice were used for cerebral blood flow analysis and divided into 4 groups as follows: (A) saline-treated (n=8), (B) MA-treated (n=7), (C) saline-treated plus stroke (n=9), and (D) MA-treated plus stroke (n=10). In the (A) saline and (B) MA groups, cerebral cortical and striatal blood flows were measured continuously from 30 minutes before the first dose to 1 hour after the fourth dose of MA (10 mg/kg) or saline. Typical cortical and striatal flow recordings before and after the first injection are shown in Figure 1A1 and 1A2. Blood flow in each animal was normalized by comparison of average blood flow before and after each drug application (average blood flow 0 to 15 minutes before MA or saline/average blood flow 0 to 15 minutes before MA or saline). We found that cortical blood flow was significantly reduced 0 to 15 minutes after the first MA administration, as compared with saline injection (saline 105.1±4.2%, n=8, versus MA 85.0±3.4%, n=7; P=0.003, t test). Cortical blood flow returned to the predrug level 15 to 30 minutes after the first MA injection (Figure 1A1). After repeated injection of MA, the difference in cortical blood flow between MA and saline injection was gradually attenuated (Figure 1A3). MA did not significantly reduce cortical blood flow after the fourth drug administration (saline 101.7±3.5%, n=3; MA 92.6±3.6%, n=7; P=0.168, t test). These data suggest that the MA-induced decrease in cortical blood flow is short acting and shows tachyphylaxis.

Striatal blood flow was also measured from the same animals receiving repeated injections of MA (n=7) or saline (n=8). We found that MA did not alter striatal blood flow after the first (Figure 1A2, saline 103.6±3.2, n=8, versus MA 99.7±4.0, n=7; P=0.454, t test) or fourth injection (Figure 1A4, saline 91.0±3.1, n=3, versus MA 92.9±3.4, n=7; P=0.751, t test), suggesting that attenuation of blood flow induced by MA is regionally specific.

Previous studies have indicated that transiently occluding both bilateral CCAs significantly augments reduction of blood flow in the cortex of the right MCA distribution after permanent right MCA ligation and also induces consistent brain infarction in rats. MCA ligation alone did not induce infarction in the present model. Blood flow was also measured in the stroke animals after MA or saline injection. In the saline plus stroke group (C), 9 mice were anesthetized with chloral hydrate after 4 doses of saline. The right MCA and both CCAs were ligated for 45 minutes. We found that cortical blood flow was significantly reduced to 8.2±0.7% in the right (ischemic) cortex supplied by right MCA in 5 control saline-treated mice. A typical recording is demonstrated in Figure 1B1. Blood flows in contralateral cortex (35.5±8.3%, n=4) and striatum (43.8±10.6%, n=4) were also reduced during the 45 minutes cerebral ischemia in the other 4 mice. A total of 10 mice were included in the MA + stroke group (D). These animals received MCA ligation and CCA occlusion after 4 doses of MA. We found that MA pretreatment did not further reduce the blood flow in the right (ischemic) cortex (MA 9.5±0.6%, n=4, versus saline 8.2±0.7%, n=5; P=0.238, t test). Similarly, cerebral blood flow was not significantly further reduced in the contralateral cortex (Figure 1B2; MA 37.3±7.9%, n=6, versus saline 35.5±8.3%, n=4, P=0.860; t test) or striatum (MA 39.5±6.3%, n=6, versus saline 43.7±10.6%, n=4, P=0.660; t test) by MA in the stroke animals.

**TTC Staining**

**Volume of Infarction**

A total of 46 mice were used for TTC analysis. Animals were divided into 5 groups. For group A, the MA group (n=4), mice treated with 4 doses of only MA did not develop striatal or cortical infarction 24 hours after the last injection. For group B, the MA plus CCA occlusion group (n=7), animals received bilateral CCA clamping for 45 minutes after MA administration and (as with group A) did not develop infarction 24 hours after removing the clamps. These data suggest that MA pretreatment and transient CCA occlusion do not induce cerebral infarction. For group C, mice treated with saline plus MCA-CCA ligation (n=12), mice pretreated with 4 doses of saline were used for MCA ligation and CCA occlusion. The right MCA was ligated and bilateral CCAs were clamped for 45 minutes. Infarction was found on the ischemic (right) side of the cerebral cortex after 24-hour reperfusion. No infarction occurred in the striatum. Typical TTC staining is shown in Figure 2. Volume of infarction was 15.9±4.4 mm³ (mean±SEM, Figure 3A). For group D, the MA plus MCA-CCA ligation group (n=16), animals were pretreated with MA and received MCA ligation and CCA clamping, which induced a much higher degree of infarction (33.8±6.0 mm³; Figure 2 and 3A) in these animals. Nine of the 16 mice pretreated with MA showed bilateral infarction either in the striatum or cortex. Incidence of bilateral infarction was significantly higher than that in the saline-treated animals (P<0.003; Fisher Exact Test). Analysis of volume of infarction was further separated by measurements in right and left hemispheres. MA pretreatment significantly increased volume of infarction on the lesioned side (Figure 3A; P<0.001, F(0.50)=10.149, 1-way ANOVA, P<0.05, post hoc Newman-Keuls test). For group E, the MA plus MCA-CCA ligation plus GDNF (n=7) group, animals received intracebral administration of GDNF (6×10⁻⁶ g) after 4 doses of MA. Right MCA and CCAs were later ligated for 45 minutes. Volume of infarction, examined after 24 hours’ reperfusion, was reduced to 17.4±3.6 mm³.
We further compared the area of infarction in different brain slices (Figures 3B and 4A). Animals pretreated with MA developed a more extensive infarction compared with those treated with saline. Area of infarction was significantly augmented in the MA-treated versus saline-treated mice (Figure 3B; $P<0.001$, $F=23.555$, 3-way ANOVA; $P<0.05$, post hoc Newman-Keuls test; Figure 4A; $P<0.001$, $F_{(2,244)}=12.111$, 2-way ANOVA; $P<0.05$, post hoc Newman-Keuls test). Pretreatment with GDNF significantly reduced the area of infarction in MA-treated mice (Figure 4A; $P<0.001$, $F_{(2,244)}=12.111$, 2-way ANOVA; $P<0.05$, post hoc Newman-Keuls test).

**Area of Infarction in Different Brain Slices**

We further compared the area of infarction in different slices (Figures 3B and 4A). Animals pretreated with MA developed a more extensive infarction compared with those treated with saline. Area of infarction was significantly augmented in the MA-treated versus saline-treated mice (Figure 3B; $P<0.001$, $F=23.555$, 3-way ANOVA; $P<0.05$, post hoc Newman-Keuls test; Figure 4A; $P<0.001$, $F_{(2,244)}=12.111$, 2-way ANOVA; $P<0.05$, post hoc Newman-Keuls test). Pretreatment with GDNF significantly reduced the area of infarction in MA-treated mice (Figure 4A; $P<0.001$, $F_{(2,244)}=12.111$, 2-way ANOVA; $P<0.05$, post hoc Newman-Keuls test).
P53 mRNA Expression
Twenty-nine mice were used for p53 mRNA analysis. The level of p53 was normalized by comparison to the mean p53 value in nonstroke mice receiving 4 doses of saline. We found that ischemia, with or without MA, significantly increased the expression of p53 mRNA in right cortex (Figure 5A; \( P < 0.014 \), \( F(3,28) = 4.329 \), 1-way ANOVA; \( P < 0.05 \), post hoc Newman-Keuls test) and in right striatum (Figure 5A; \( P < 0.001 \), \( F(3,28) = 9.417 \), 1-way ANOVA; \( P < 0.05 \), post hoc Newman-Keuls test). Compared with saline pretreatment, MA also increased p53 mRNA in striatum (Figure 5A; \( P < 0.05 \), post hoc Newman-Keuls test). MA did not further potentiate P53 mRNA level in the stroke animals.

Ischemia and MA–induced p53 mRNA expression was also analyzed in left cortex and striatum. Similar to what occurred in the right hemisphere, ischemia with MA significantly increased levels of p53 mRNA in left cortex (Figure 5B; \( P < 0.002 \), \( F(3,26) = 6.979 \), 1-way ANOVA; \( P < 0.05 \), post hoc Newman-Keuls test) and in left striatum (Figure 5B; \( P < 0.05 \), post hoc Newman-Keuls test). p53 mRNA levels in left cortex and striatum of animals that received both MA and ischemia are significantly higher than those that received the ischemia or MA alone (\( P < 0.05 \); ANOVA plus post hoc Newman-Keuls test). These data suggest that MA potentiates ischemia–induced p53 expression contralateral to the ischemic hemisphere.

GDNF Levels
Nineteen mice were used for GDNF measurement. Level of GDNF was normalized by comparison to the mean GDNF value in nonstroke rats that received 4 doses of saline. Compared with saline, we found that MA significantly reduced the level of GDNF in striatum both in nonstroke (Figure 4B; saline 100.0±6.6% versus MA 76.1±4.5%, \( P < 0.05 \), t test) and stroke animals (saline 100.0±14.2% versus MA 70.7±4.6%, \( P < 0.05 \), t test). Such reduction was not seen in cortex.

Physiological Responses to MA
Physiological parameters were measured after 1 or 4 doses of MA or saline in 19 anesthetized mice. We found that mean arterial pressure was significantly increased after the first dose of MA (without MA, 89±3 mm Hg; \( n = 13 \), versus with MA, 104±7 mm Hg, \( n = 6 \), \( P = 0.037 \); t test). In contrast, systemic blood pressure was significantly decreased after 4 doses of MA (67±6 mm Hg; \( n = 6 \), \( P = 0.002 \), t test). Heart rate was not altered at these 2 time points (first dose, MA 636±15 versus saline 600±19 bpm; \( P = 0.172 \), t test; fourth dose, MA 660.0±18.5 versus saline 648.0±22.4; \( P = 0.689 \), t test).

MA did not change hemoglobin, serum calcium, \( \mathrm{PaO}_2 \) and \( \mathrm{PaCO}_2 \) levels. Arterial pH and \( \mathrm{HCO}_3^- \) were significantly reduced after the fourth dose of MA (Table, \( P < 0.05 \), t test), which suggests that animals may experience a mild metabolic acidosis after repeated MA application.

Hematoxylin and Eosin Staining
We found no obvious hemorrhage in the brain after MA treatment. Mice treated with 4 doses of MA (\( n = 3 \)) or saline (\( n = 3 \)) were killed for hematoxylin and eosin staining. No red blood cells were found in the cortex or striatum, which suggests that no microscopic hemorrhage occurred after MA administration.

Discussion
In the present study, we evaluated the interaction of MA treatment and transient brain ischemia in mice. We found that repeated administration of MA or transient CCA clamping did not induce infarction. Similar to results of previous reports,\(^{14} \) we found that transient distal MCA and bilateral...
CCA occlusion elicited infarction in the ipsilateral cerebral cortex in CD-1 mice. However, MA pretreatment extended the area of infarction in these ischemic animals to both the ipsilateral striatum as well as the contralateral cortex and striatum. These data suggest that infarction induced by MCA ligation and bilateral CCA occlusion is potentiated by the pretreatment with MA.

We found that the potentiation of infarction by MA pretreatment in the stroke animals is probably not related to changes in cerebral blood flow. MA transiently reduced cerebral blood flow in cortex in nonstroke animals. Such responses lasted for only 15 to 30 minutes and showed tachyphylaxis. Moreover, striatal blood flow was not altered by MA alone, which suggests that the increase in infarction, at least in contralateral striatum, is not due to a decrease in blood flow produced by MA. Previous studies have indicated that transient CCA occlusion or MCA ligation alone reduces cerebral blood flow but does not induce brain infarction. Concomitant MCA ligation and bilateral CCA occlusion significantly reduced blood flow in the cortex in the area of MCA perfusion and induced unilateral infarction in rats. Our data also indicate that MCA ligation and CCA occlusion decreased cerebral blood flow to 10% of control levels in the MCA territory and induced unilateral brain infarction. MA did not potentiate the attenuation of cortical blood flow ipsilateral to the ischemia. Blood flows in the contralateral cortex or striatum decreased to 30% to 40% of control level during the 45-minute arterial occlusion, which did not induce infarction in the saline-treated mice. MA pretreatment did not further attenuate blood flow in the contralateral cortex and striatum. However, MA did induce infarction. Taken together, these data indicate that MA-facilitated infarction...
mediated toxicity was attenuated by [\(\text{d-Ala}^2,\text{d-Leu}^4\)]enkephalin through sequestration of free radicals. \(^{15}\) Studies have shown that expression of p53 mRNA is associated with and has been used as one of the indices of apoptosis. \(^{16,17}\) In the present study, we found that ischemia increased p53 mRNA levels in the ipsilateral cortex and striatum. MA did not further upregulate its expression. It is possible that p53 expression has reached its maximal level in the ipsilateral hemisphere after ischemia/reperfusion and cannot be further modulated by MA. On the other hand, we found ischemia itself did not enhance expression of p53 mRNA in the contralateral cortex and striatum in the stroke mice, whereas MA pretreatment significantly potentiated p53 mRNA levels during stroke in these areas. Because upregulation of p53 mRNA may be related to apoptosis, these data may suggest that ischemia-mediated apoptosis, especially contralateral to the ischemic side, is facilitated by MA. More detailed study with histological markers of apoptosis is necessary to determine further whether the increase in apoptosis after MA administration results in the increased area of infarction seen in the present study after ischemia.

We and others have previously reported that pretreatment with GDNF decreased reactive oxygen species formation in the ischemic cortex and the extent of cortical infarction in rats. \(^{6}\) The protective effect of GDNF against ischemia was significantly decreased in mice with GDNF receptor-\(\alpha_1\) (GFR\(\alpha_1\)) deficiency, which suggests that the protection is mediated through GFR\(\alpha_1\). \(^{18}\) Because ischemia activates expression of GDFN mRNA, \(^{11}\) upregulation of GDNF could be a result of activation of endogenous neuroprotective or neuroregenerative processes during insults. Previous reports have indicated that GDNF mRNA is present in adult mouse brain and endogenous GDNF can be measured more readily with acidic treatment, which may induce the dissociation of ligands from the receptors or binding proteins. After acid treatment, GDNF was found in adult mouse brain and human CSF. \(^{13}\) In the present study, which used the same technique, we found that MA significantly decreases GDNF levels in the striatum. It is possible that less endogenous GDNF-induced neuroprotection occurs after MA treatment, which increases vulnerability to ischemic insults. This hypothesis is further supported by our finding that the exogenous administration of GDNF before ischemia reduced the MA-induced increment in cerebral infarction.

Studies have reported that unilateral ischemia can elicit insults in the contralateral hemisphere. For example, expression of \(N\)-methyl-\(D\)-aspartate receptors was found to extend to the contralateral hemisphere after focal ischemia. \(^{19}\) Unilateral MCA occlusion also enhanced not only ipsilateral but also contralateral side monoamine release. \(^{20}\) Thus, MA pretreatment possibly could potentiate the ischemic insults and exacerbate the degree of infarction bilaterally.

We found that MA induced differential blood pressure responses after the first and fourth MA injections. BP was elevated after the first dose of MA and was reduced after the fourth dose of MA. Similar reports have indicated that administration of amphetamine or MA induced acute hypertension followed by hypotension in barbiturate-anesthetized rats \(^{21}\) and in human patients. \(^{22}\) The decrease in blood pressure
may result from depletion of presynaptic norepinephrine stores after repeated high doses of MA. Because cerebral blood flow and blood pressure were differentially altered by MA injection, these 2 responses could be independently regulated. We found that hemoglobin, serum calcium, PaO₂, and PaCO₂ were not altered after repeated MA treatment. Previous studies have indicated that anesthetized mice, with artificial ventilation, have arterial blood pH 7.27. In our study, mice anesthetized with chloral hydrate for >6 hours and receiving repeated saline injections, have pH 7.23, which suggests that saline injection did not alter arterial pH in anesthetized mice. We also found that animals that received repeated MA injections developed a significantly lower blood pH. Several clinical studies have indicated that patients with acute MA intoxication present with metabolic acidosis, during which arterial pH can drop to 7.12. These data suggest that the animal model we used is relevant to clinical situations with acute MA intoxication. We have previously reported that GDNF administration did not alter blood pressure, blood pH, and blood gas levels. Thus, the neuroprotection induced by GDNF seen in the present study probably is not mediated through normalization of these systemic parameters.

Previous studies have suggested that MA, given after ischemia, improves stroke symptoms through the enhancement of neurite outgrowth. How MA induces these changes is still not clear. In contrast to these reports, our data suggest that MA, given before ischemia, exacerbates infarction, increases apoptotic gene expression, and reduces the level of the neuroprotective protein GDNF. Our data thus suggest that the use of MA promotes more damage during ischemic insults. The present data further support the clinical observations that patients with a chronic or acute MA abuse history can develop cerebral hemorrhages in striatum and infarction in MCA distribution. In conclusion, our data suggest that the use of MA exacerbates stroke-induced damage in the brain and that GDNF may be involved in this response.

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References

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