\( \sigma_1 \)-Receptor Ligand 4-Phenyl-1-(4-Phenylbutyl)-Piperidine Affords Neuroprotection From Focal Ischemia With Prolonged Reperfusion

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**Background and Purpose**—We previously showed that the intravenous administration of the potent \( \sigma_1 \)-receptor ligand 4-phenyl-1-(4-phenylbutyl)-piperidine (PPBP) provides neuroprotection against transient focal cerebral ischemia and that the protection depends on treatment duration. We tested the hypothesis that PPBP would provide neuroprotection in a model of transient focal ischemia and 7 days of reperfusion in the rat as assessed with neurobehavioral outcome and infarction volume.

**Methods**—Under the controlled conditions of normoxia, normocarbia, and normothermia, halothane-anesthetized male Wistar rats were subjected to 2 hours of middle cerebral artery occlusion (MCAO) with the intraluminal suture occlusion technique. We used laser Doppler flowmetry to assess MCAO. At 60 minutes after the onset of ischemia, rats were randomly assigned to 1 of 4 treatment groups in a blinded fashion and received a continuous intravenous infusion of control saline or 0.1, 1, or 10 \( \mu \)mol \( \cdot \) kg\(^{-1} \) \( \cdot \) h\(^{-1} \) PPBP for 24 hours. Neurobehavioral evaluation was performed at baseline (3 to 4 days before MCAO) and at 3 and 7 days of reperfusion. Infarction volume was assessed with triphenyltetrazolium chloride staining on day 7 of reperfusion in all rats.

**Results**—Triphenyltetrazolium chloride–determined infarction volume of ipsilateral cortex was smaller in rats treated with 10 \( \mu \)mol \( \cdot \) kg\(^{-1} \) \( \cdot \) h\(^{-1} \) PPBP (n=15, 68±12 mm\(^3\), 18±3% of contralateral structure, \( P<0.05 \)) (mean±SEM) compared with corresponding rats treated with saline (n=15, 114±11 mm\(^3\), 31±3% of contralateral structure). PPBP did not provide significant neuroprotection in the caudoputamen complex. Although MCAO was associated with several alterations in behavior, the treatment with PPBP had no effect on behavioral outcomes.

**Conclusions**—The data demonstrate that the potent \( \sigma_1 \)-receptor ligand PPBP decreases cortical infarction volume without altering neurobehavior after transient focal ischemia and prolonged reperfusion in the rat. *(Stroke. 2000;31:976-982.)*

**Key Words:** cerebral infarction ■ cerebral ischemia, focal ■ excitotoxicity ■ ligands ■ rats

Numerous studies have demonstrated that \( \sigma \) site–mediated drug effects modulate glutamate receptor function, neurotransmitter release, behavior, and cognition.\(^1,2\) Furthermore, there is mounting experimental evidence from in vitro and in vivo studies that \( \sigma \)-receptors play a role in the modulation of ischemic neuronal injury. Several possible mechanisms for this neuroprotection have been postulated; they include inhibition of presynaptic glutamate release,\(^3\) buffering of postsynaptic glutamate-evoked Ca\(^{2+} \) influx, modulation of neuronal responses to pharmacological N-methyl-D-aspartate (NMDA) receptor stimulation,\(^4,5\) inhibition of dopamine release,\(^6\) prevention of cortical spreading depression,\(^7\) and attenuation of basal and NMDA-evoked nitric oxide production in vivo.\(^8\)

We previously demonstrated that the short-term administration of the potent and highly selective \( \sigma_1 \)-receptor ligand 4-phenyl-1-(4-phenylbutyl)-piperidine (PPBP) prevents early alteration in behavior after transient focal ischemia and prolonged reperfusion in the rat.\(^9\) We postulated that this may be due to neurotoxicity secondary to prolonged interaction with the NMDA/receptor complex and undesirable suppression of glutamate-triggered events that subserve vitally important metabolic, neurotransmitter, and neurotrophic functions.\(^10\) However, we did not observe cytotoxic effects histopathologically with prolonged treatment with PPBP in naive rats,\(^11\) as has been shown with NMDA antagonist toxicity.\(^12\)

Experimental pharmacological neuroprotection is rarely assessed at 3 to 4 days of reperfusion as an end point after transient focal ischemia. However, prolonged continuous infusion with PPBP, beyond 24 hours, provides no neuroprotection after transient focal ischemia in the rat.\(^11\) We postulated that this may be due to neurotoxicity secondary to prolonged interaction with the NMDA/receptor complex and undesirable suppression of glutamate-triggered events that subserve vitally important metabolic, neurotransmitter, and neurotrophic functions.\(^12\) However, we did not observe cytotoxic effects histopathologically with prolonged treatment with PPBP in naive rats,\(^11\) as has been shown with NMDA antagonist toxicity.\(^12\)

Experimental pharmacological neuroprotection is rarely assessed at 3 to 4 days of reperfusion as an end point after transient focal ischemia.
transient focal ischemia. Furthermore, studies have predominantly used morphometric analysis of infarction volume as the primary outcome. Although improvements in complex behavior such as memory without histological correlation have been reported after global forebrain ischemia and traumatic brain injury, neuroprotective studies that correlate infarction volume and neurobehavior after focal ischemic stroke are limited. The purpose of the present study was to determine whether 24 hours of continuous infusion of PPBP affords neuroprotection by decreasing infarction volume and improves functional outcome (sensory capabilities, coordination, and anxiogenic effect) in a dose-specific manner after transient middle cerebral artery occlusion (MCAO) and 7 days of reperfusion.

Materials and Methods

General Preparation and Animal Surgery

The experimental protocol was approved by the Institutional Animal Care and Use Committee and conforms to the National Institutes of Health guidelines for the care and use of animals in research. Adult male Wistar rats weighing 260 to 340 g (n = 103) were anesthetized with halothane (1.0% to 2.0%) in O2-enriched air (fraction of inspired O2 0.35% to 0.40%) to maintain the following conditions: arterial pH 7.35 to 7.40, PaCO2 35 to 45 mm Hg, and Pao2 100 to 160 mm Hg. With aseptic surgical techniques, the right femoral artery was cannulated to monitor arterial blood pressure and arterial blood gases. The right femoral vein was cannulated for the administration of drugs and fluids. After cannulation, both femoral arterial and venous catheters were tunneled subcutaneously, exteriorized in the posterior mid thorax, and suture fixed onto a swivel. The swivel affords neuroprotection by decreasing infarction volume and neurobehavior after focal ischemic infarction and neurobehavior after focal ischemic behavior such as memory without histological correlation have been reported after global forebrain ischemia and traumatic brain injury, neuroprotective studies that correlate infarction volume and neurobehavior after focal ischemic stroke are limited. The purpose of the present study was to determine whether 24 hours of continuous infusion of PPBP affords neuroprotection by decreasing infarction volume and improves functional outcome (sensory capabilities, coordination, and anxiogenic effect) in a dose-specific manner after transient middle cerebral artery occlusion (MCAO) and 7 days of reperfusion.

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Focal Ischemia and Reperfusion

The adequacy of vascular occlusion was documented with laser Doppler flowmetry (LDF). An area 2 to 3 mm in diameter at the right parietal bone (2 mm posterior and 6 mm lateral to bregma) was thinned for the placement of an LDF probe (model MBF3D; Moor Instruments Ltd) as previously described. Transient focal ischemia (2 hours) was produced with MCAO according to an intraluminal suture technique as previously described with some modification. An area 2 to 3 mm in diameter at the right parietal bone (2 mm posterior and 6 mm lateral to bregma) was thinned for the placement of an LDF probe (model MBF3D; Moor Instruments Ltd) as previously described. Transient focal ischemia (2 hours) was produced with MCAO according to an intraluminal suture technique as previously described with some modification. At the end of ischemia (2 hours), reperfusion was produced through withdrawal of the intraluminal suture; this was associated with rapid restoration of the LDF signal. Rats that did not demonstrate a significant reduction (to at least 40% of baseline) during MCAO or rapid restoration of the LDF signal during reperfusion were excluded from the study. LDF measurements were obtained during 5-minute periods at 5, 15, 30, 60, 90, and 120 minutes after MCAO and 15 minutes of reperfusion.

Treatment Groups

Rats were randomly assigned in a blinded fashion to 1 of 4 treatment groups receiving 0.1, 1, or 10 μmol · kg⁻¹ · h⁻¹ PPBP (National Institute on Drug Abuse) or an equivalent volume of saline. The infusion was initiated at 1 hour of MCAO and continued for 24 hours. Infusion volume was 20 mL at a rate of 0.5 mL/h for 24 hours. Using HPLC, we have previously shown that PPBP remains stable in 0.9% saline for ≥96 hours at 37°C. At 15 minutes after the onset of reperfusion, rats were allowed to emerge from anesthesia and were provided ad libitum access to food and water. On day 7 after MCAO, rats were deeply anesthetized with 5% halothane and decapitated. The brain was harvested and sliced into seven 2-mm-thick coronal sections for staining with 1% triphenyltetrazolium chloride (TTC) in saline at 37°C for 30 minutes as previously described. Infarction volume was measured with digital imaging (Digital Camera 40; Eastman Kodak Co) and analysis software (SigmaScan Pro; Jandel). The infarcted area (unstained) was numerically integrated across each section and over the entire ipsilateral hemisphere. Infarction volumes were measured separately in the cerebral cortex and caudoputamen and expressed as a percentage of the volume of the ipsilateral side.

Neurobehavioral Testing

Baseline behavioral tests were conducted 3 to 4 days before MCAO (baseline) and then repeated at 3 (day 3) and 7 days (day 7) after MCAO. To assess the effect of repeated testing, a separate group of experimentally naïve animals (n = 15) were subjected to a similar behavioral testing protocol. The trials were scored by a single, experimentally uninformed observer. The behavioral tests were conducted in a quiet room, during the light phase (between 1 and 3 PM Eastern Standard Time). Between trials, the apparatus were cleaned with a 75% ethanol solution.

Visual Placement

For evaluation of visual acuity, the rats were suspended by their tails and slowly lowered toward the edge of a laboratory bench top. A positive score was recorded if the animal extended its forepaws toward the bench top before contact.

Locomotor Balance and Coordination

Rats were placed at the center of a wooden bridge or pole that was suspended ~60 cm above a foam pillow. Animals were tested in random order with a 2-cm-wide square bridge, and a wooden pole with a diameter of 2 cm. The latencies (seconds) to fall off the bridges and pole were recorded. Animals that did not fall received a score of 120 seconds.

Turning on an Inclined Screen

A mesh screen, maintained at a 45° angle, was used to assess strength and agility. Rats were placed in the middle of the screen facing downward. The time required for each rat to complete a 90° turn was recorded.

Turning in an Alley

The ability to turn in an alley was used as a measure of coordinated muscle movement and agility. The width of the alley was 12 cm. The animals were placed in the alley facing the back wall. The time that the animals required to turn around and face the open end of the alley was recorded.

Latency to Fall From Wire

Forelimb strength and grasping ability were evaluated by suspending the rats by their forelimbs on a wire. The wire was stretched between 2 posts at a height of 60 cm. A foam pillow was positioned beneath the apparatus. The time (seconds) until the animal fell was recorded. A score of zero was assigned to animals that did not grasp the wire or fell immediately. The trials lasted a maximum of 90 seconds.

Elevated Plus-Maze

The elevated plus-maze was used to assess anxiogenic behavior. The maze consisted of 4 intersecting arms and was constructed from varnished wood and opaque black Plexiglas. The maze was elevated ~1 m above the floor. The arms of the maze were 45 cm long and 10 cm wide; the closed arms had walls on 3 sides that were 20 cm high, and the open arms did not have walls on any of the sides. At the beginning of the test, the rat was placed in the center of the maze facing 1 of the open arms. An observer, who was seated 1 m from the apparatus at a height sufficient to observe the animal in all 4 arms of the maze, recorded the number of entries into each arm of the maze and the time spent in each arm. The time that the experimental animal spent grooming was also recorded.

Statistical Analysis

All values are expressed as mean ± SEM. Physiological parameters were subjected to repeated measures ANOVA. Differences in infarc-
Results
Mean arterial blood pressure, \( P_{aCO_2} \), \( P_{aO_2} \), pH, and temporalis muscle temperatures were within normal physiological ranges in all groups at baseline (preischemia), during ischemia, and after ischemia (postischemia) (Table 1). Death occurred before the end of the experimental protocol in 4 of 26 control rats treated with saline, 2 of 21 rats treated with 0.1 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) PPBP, 2 of 19 rats treated with 1 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) PPBP, and 4 of 21 rats treated with 10 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) PPBP. The number of animals that were excluded because the residual LDF signal did not decrease to 40% of baseline signal and did not remain below this value during the period of MCAO was 7 saline-treated control rats, 4 rats treated with 0.1 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) PPBP, 2 rats treated with 1 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) PPBP, and 2 rats treated with 10 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) PPBP. Thus, 15 rats in each group successfully completed the experimental protocol. The residual LDF signal during PPBP. Thus, 15 rats in each group successfully completed the experimental protocol. The residual LDF signal during PPBP treatment, \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \), was determined with 1-way ANOVA. Post-hoc analysis was performed with Dunnett’s test. The criterion for statistical significance was \( P<0.05 \). Behavioral data from the pole and wire were analyzed via ANOVA for repeated measures. The experimental errors of the data were not normally distributed, so the data were transformed \( \log_{10}(y+1) \) before analysis. Transformation was not successful in normalization of the data from the 2-cm bridge, inclined screen, alley, and elevated plus-maze, so these data were analyzed with Kruskal-Wallis 1-way ANOVA on ranks. If there was no significant treatment effect on any of the test days, the groups were collapsed, and a Kruskal-Wallis ANOVA on ranks was conducted across time with post-hoc comparisons with the use of Dunn’s method. Data points that were \( >3 \) SDs from the mean were removed before analysis (pole \( n=2 \), incline \( n=4 \), alley \( n=2 \), wire \( n=4 \), elevated plus-maze \( n=3 \)).

### Table 1. Summary of Selected Physiological Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saline</th>
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<tr>
<td>MAP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>86±1</td>
<td>82±1</td>
<td>87±2</td>
<td>86±2</td>
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<td>Ischemia</td>
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<td>88±1</td>
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<tr>
<td>Postischemia</td>
<td>90±2</td>
<td>89±2</td>
<td>88±2</td>
<td>80±1</td>
</tr>
<tr>
<td>Hemoglobin g/dL</td>
<td>12.5±0.3</td>
<td>12.1±0.2</td>
<td>12.6±0.2</td>
<td>12.7±0.1</td>
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<tr>
<td>Glucose, mg/dL</td>
<td>89±3</td>
<td>101±3</td>
<td>91±4</td>
<td>93±4</td>
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<tr>
<td>Rectal temperature, °C</td>
<td>37.9±0.1</td>
<td>37.9±0.1</td>
<td>38.0±0.1</td>
<td>37.9±0.1</td>
</tr>
<tr>
<td>Temporalis muscle temperature, °C</td>
<td>36.8±0.1</td>
<td>36.9±0.1</td>
<td>36.9±0.1</td>
<td>36.8±0.1</td>
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</table>

MAP indicates mean arterial blood pressure. Values are mean±SEM.
Infarction volume in the cerebral cortex, caudoputamen complex, and ipsilateral hemisphere in the 4 treatment groups (n=15 per group). *P<0.05 saline-treated control rats vs 10 μmol · kg⁻¹ · h⁻¹ PPBP—treated rats.

in 10 μmol · kg⁻¹ · h⁻¹ PPBP-treated rats). On withdrawal of the monofilament, the LDF signal returned rapidly to baseline values within 5 minutes in all groups. After 15 minutes of reperfusion, LDF signal was 116±13% in saline control rats, 104±8% in 0.1 μmol · kg⁻¹ · h⁻¹ PPBP-treated rats, 106±11% in 1 μmol · kg⁻¹ · h⁻¹ PPBP-treated rats, and 104±10% in 10 μmol · kg⁻¹ · h⁻¹ PPBP-treated rats.

TTC-determined infarction volume measured at 7 days of reperfusion of ipsilateral cortex was smaller in rats treated with 10 μmol · kg⁻¹ · h⁻¹ PPBP (n=15, 68±12 mm³, 18±3% of contralateral structure; P<0.05) (mean±SEM) than in corresponding rats treated with saline (n=15, 114±11 mm³, 31±3% of contralateral structure) (Figure). Infarction volume of the caudoputamen complex was not statistically different between saline control rats and PPBP-treated rats at the 3 different doses.

Behavioral Data

There were no significant behavioral differences (P>0.05) between animals treated with PPBP or the vehicle at any time point measured in any of the behavioral tests. However, MCAO was associated with functional alterations in several of the behavioral tests when pre-MCAO baseline performance was compared with performance after 3 or 7 days of reperfusion (Table 2). Latency to fall from the pole was significantly longer at 7 days but not at 3 days post-MCAO compared with baseline (F3,165=4.2, P<0.05). Latency to fall from the 2-cm bridge was significantly longer at 3 and 7 days after MCAO than at baseline (H=25.4, df=2, P<0.01). Latency to turn on an inclined screen was significantly longer at 7 days, but not at 3 days, after MCAO than at baseline (H=16.5, df=2, P<0.01). Latency to fall from the wire was significantly longer at both 3 and 7 days after MCAO than at baseline (F3,163=21.2, P<0.01). There was a significant decrease in the total number of arm entries in the elevated plus-maze at 3 days after MCAO (H=35.9, df=2, P<0.01) and an increase in the amount of time spent autogrooming in the maze on day 7 (F3,163=5.8, P<0.01) compared with baseline. Because of the extreme MCAO-induced decrease in locomotor (exploratory) behavior observed in the elevated plus-maze, time spent in the open versus closed arms is not a valid measure of anxiogenic-like behavior; therefore, these data are not presented. There was no significant effect (P>0.05) of MCAO on visual placement or turning in an alley.

Discussion

The main findings of the present study with a model of focal ischemia with prolonged reperfusion model in the rat are that (1) continuous 1-day intravenous infusion PPBP administered 1 hour from the onset of MCAO decreases cortical but not striatal infarction volume and that (2) PPBP treatment does not alter neurobehavioral outcome within a 7-day observation period. These data suggest that PPBP-associated neuroprotection is region specific but robust in the mature as well as early ischemic lesion.

Effects on Infarction Volume

Focal ischemic injury volume is dependent on the temporal end point of the study. Most previous studies of neuroprotection have used morphometric analysis of infarction volume at 3 to 4 days of reperfusion after transient focal ischemia as their end point. Less is known about the outcome of these agents after prolonged reperfusion. In our previous study, the continuous infusion of PPBP ameliorated the effects of transient focal ischemia at the dosage of 1 μmol · kg⁻¹ · h⁻¹ when the infarction volume was assessed 4 days after MCAO. In the present study, we assessed infarction volume at 7 days of reperfusion after MCAO. Although some investigators have demonstrated that brain infarction after mild transient focal ischemia can develop in a delayed fashion (>3 days after the original insult), others have shown that cerebral edema can cause an artifactual increase in infarction volume as assessed with TTC staining. Lin et al found that edema was maximal at 24 hours after MCAO with a subsequent reduction at 3 days. In addition, the total volume of the infarcted cortex in the injured hemisphere was smaller than the noninfarcted hemisphere at 7 days after MCAO. Other variables, such as drug stability and penetration into the brain, may influence infarction volume in studies that investigate neuroprotective agents in focal ischemia. It has been shown

<table>
<thead>
<tr>
<th>TABLE 2. Behavioral Outcomes Affected by MCAO</th>
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<tr>
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<tr>
<td>Latency to fall from 2-cm bridge, s</td>
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<td>Latency to fall from pole, s</td>
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<tr>
<td>Latency to fall from wire, s</td>
</tr>
<tr>
<td>Latency to turn on inclined screen, s</td>
</tr>
<tr>
<td>Total arm entries in elevated plus-maze, n</td>
</tr>
<tr>
<td>Time spent autogrooming in elevated plus-maze, s</td>
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</table>

*P<0.05 vs pre-MCAO baseline value.
that PPBP penetrates the brain very readily when administered intravenously with a slow in vivo clearance from the brain. In addition, we previously demonstrated that PPBP remains stable in solution for ≥96 hours at 37°C.11

Temperature is an important variable and modulator in ischemic brain injury,22 and it is well established that temperature affects outcome and infarction volume in focal cerebral ischemia.23,24 In a previous study in the cat, this neuroprotective effect of PPBP could not be explained by either a more favorable redistribution of cerebral blood flow or an effect on brain temperature.10 Small differences in brain temperature during and immediately after a period of transient focal ischemia can critically influence neuropathological outcome.25–27 In our study, rectal and temporalis muscle temperatures were monitored during preischemia, MCAO, and immediate reperfusion (Table 1). We10,20 and others22 have demonstrated that α-receptor ligands afford neuroprotection without affecting body temperature. We attempted to control temporalis muscle temperature during the acute experimental period in our study, but we did not monitor temperature on a continuous basis beyond the immediate reperfusion period. Thus, it is conceivable that variations in body and brain temperature could have affected infarction size in the experimental groups.

Behavioral Outcome

In the present study, transient MCAO was associated with functional alterations in the outcomes of several behavioral tests (Table 2). For example, rats exhibited a motor deficit as measured by the number of arm entries made during a 5-minute test in the elevated plus-maze. The number of arms entered decreased to 37% of baseline on the third day after MCAO and recovered to ≈72% of baseline by the seventh day after MCAO. Alterations in motor behavior also were likely to be the cause of the significant increases in latency to fall from a 2-cm bridge, to fall from the pole, and to turn on an inclined screen that were observed after MCAO. During the baseline testing on the 2-cm bridge and the pole, the rats explored the entire length of the bridge and the pole; however, after MCAO, the rats tended to remain where they were placed at the beginning of the trial. Because they were moving less after MCAO, the rats were less likely to misstep and fall, thus accounting for the increase in latency to fall from the bridge and the pole after MCAO. After cerebral ischemia, the latency to turn on the inclined screen also increased as a result of slower initiation of movement after being placed on the apparatus. Many factors could contribute to the locomotor deficits that were observed in the ischemic rats, including an increase in the prevalence of “sickness behaviors,” which commonly accompany an inflammatory response.29,30 Additional studies are necessary to determine whether rats subjected to MCAO exhibit a generalized suppression in spontaneous locomotor activity or whether the locomotor deficits are condition specific. After MCAO, the latency to fall while suspended from a wire also was ≈3 times longer when tested several days after MCAO as opposed to several days before surgery (baseline). The increase in latency to fall is attributed to an abnormal grasp reflex in the forelimbs that most likely resulted from damage to the frontal cortex during MCAO. Abnormal grasp reflexes also have been identified in humans with cortical damage.31 Repeated testing is not likely to be a contributing factor to any of the MCAO-induced behavioral changes described earlier, because the behavior of the experimentally nonmanipulated animals did not change with repeated behavioral testing in any of these measures. The only behavior that did change with repeated testing in both the experimentally manipulated and the nonmanipulated animals was the amount of time spent autogrooming in the elevated plus-maze. Once spontaneous locomotor recovery has occurred in rats that underwent MCAO, the use of additional behavioral tasks such as passive avoidance or amphetamine-induced rotation to assess striatal function may have revealed an effect of PPBP on behavioral outcome.

PPBP treatment was associated with histological protection in the cortex in the present study, but the compound did not offer protection from MCAO-associated behavioral deficits. However, the decreases in locomotor activity that were noted in the elevated plus-maze and on the bridge, pole, and screen prevented a clear assessment of anxiety, coordination, and balance in this study. It is possible that extension of the behavioral testing of the animals beyond 1 week may have allowed time for locomotor recovery to occur, which in turn would have allowed us to more accurately assess anxiety, coordination, and balance in the PPBP-versus vehicle-treated animals. Alterations in locomotor activity, coordination, balance, and anxiety are commonly identified confounding factors in the interpretation of data from complex behavioral and cognitive tests. Although the series of locomotor tests conducted in the present study do not specifically assess cortical or striatal function, they do indicate that the rats have significant sensorimotor deficits after MCAO that would prevent clear interpretation of the data from additional behavioral tests that could be used to assess cortical or striatal function. Taken together, the behavioral data presented in this study indicate that a recovery period of >7 days after MCAO will be necessary before the incorporation of complex behavioral and cognitive tests in future studies.

Although some studies of cerebral ischemia have reported a close correlation between histology and behavior,32,33 other studies have reported a dissociation between histology and sensorimotor behavior or cognitive function.34,15,34–37 Several factors could account for the lack of consistent correlation between histology and behavior, including the timing of the behavioral tests (as described earlier) and the use of histological techniques that provide low resolution. The most frequently used methods to quantify infarction volume after MCAO are not capable of measuring diffuse morphological changes,33 which could affect behavior. It is also possible that neurons that have been spared through pharmacological intervention are still not capable of functioning at the level of effective communication with other cells. In contrast, it is possible for the recovery of some sensorimotor functions to occur “spontaneously” several days after MCAO in the absence of accompanying histological improvement; presumably, these behavioral improvements are due to compensatory changes that occur elsewhere in the brain. Clearly, until there is a better understanding of how histological...
changes affect behavior, it will be necessary to continue assessment of both histological and functional outcomes of potential treatments of stroke.

In conclusion, this study demonstrates that the continuous intravenous infusion of the potent \(\sigma_1\)-receptor ligand PPBP improves histological outcome after transient focal ischemia and prolonged reperfusion without altering neurobehavioral outcome.

Acknowledgments

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References

Previous work has suggested that σ-receptors may modulate neuronal injury following ischemia. The goal of this study was to examine the effect of 24-hour infusion of a σ₁-receptor ligand, 4-phenyl-1-(4-phenylbutyl) piperdine (PPBP), on brain injury and neurological function in a model of focal ischemia with relatively prolonged reperfusion (7 days). A major strength of the study relates to its use of an array of neurological tests.

The results suggest that following focal ischemia with prolonged reperfusion, PPBP decreases cortical, but not caudoputamen, infarct volume. Despite the apparent selective reduction in brain injury, treatment with PPBP did not result in any detectable improvement in neurobehavioral outcome. Thus, the correlation between the degree of tissue injury, as determined by histological assessment of infarct volume, and neurological function was not perfect.

One limitation of this study is that it does not provide insight into the mechanism by which treatment with PPBP exerts a protective effect on infarct volume in this model. Possibilities include alterations in changes in gene expression that occur in response to ischemia as well as changes in neuronal activity, neurotransmitter release, or effects of glutamate (including effects on NMDA receptors).1–3

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