Pre- and postischemic effects of the NMDA receptor antagonist dizocilpine maleate (MK-801) on collateral cerebral blood flow

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The authors studied the effects of pre- and postischemic administration of dizocilpine maleate (MK-801) on collateral and regional cerebral blood flow (CBF). The ischemic penumbra appears to benefit most from the neuroprotective effects of MK-801. The precise mechanism by which MK-801 provides this neuroprotection remains controversial. Alterations in CBF have been demonstrated with MK-801 administration, but whether the response is an increase or decrease in flow has remained unclear.

A left-sided craniectomy was performed in 20 dogs. A branch of the middle cerebral artery (MCA) was cannulated and collateral blood supply–dependent tissue (CDT) was identified using the “shadow flow” technique. Regional CBF was measured using radiolabeled microspheres. Six dogs received MK-801 (1 mg/kg administered intravenously) before they underwent MCA branch occlusion; the remaining 14 dogs received MK-801 after they underwent MCA occlusion. Cerebral blood flow and vascular pressures were measured 30 and 60 minutes after MK-801 administration. In animals that received MK-801 before MCA occlusion, CBF did not change significantly from baseline values before or after occlusion. In contrast, in animals that received MK-801 after MCA occlusion, CBF was significantly reduced in all regions of the brain, including the CDT. Collateral blood supply–dependent tissue showed a 51.7% reduction in flow, whereas normal CBF was reduced by 29.7%. The MK-801 induced cerebral vasoconstriction in both groups. The neuroprotective effects of MK-801 do not appear to be caused by the augmentation of collateral or global cerebral circulation and, in fact, may block the glutamate-mediated vasodilation that occurs during ischemia.

KEY WORDS • collateral cerebral blood flow • microsphere • glutamate antagonist • stroke • dog
ion with siliconized PE-50 tubing (10 cm in length). Back pressure was monitored continuously during each experiment. The accuracy of back pressure was confirmed by measuring pressure in the cannulated MCA branch with a glass micropipette and a servonull device. The tip diameter of the micropipette is small (2–4 μm), which allows a nonoccluding, relatively atraumatic puncture of vessels. The micropipette was removed from the vessel after each measurement of pressure and the pressure in the neighboring noncannulated MCA branch was measured.

Measurement of CBF and Shadow Flow

A No. 7 French pigtail catheter was introduced into the left ventricle through a femoral artery for injection of radioactive microspheres to measure CBF. Isotopes were randomly selected from a stock pool (cerium-141, tin-113, niobium-95, strontium-85, cobalt-57, and scandium-46), and the order of injection was randomized. To measure flow, 10³ microspheres (15 μm in diameter) were injected into the left ventricle and flushed with 5 ml of saline over 5 seconds. The number of spheres was sufficient to ensure approximately 400 spheres per tissue sample. Reference samples were withdrawn from the brachial and femoral arteries at 2.06 ml/minute for a total of 2.5 minutes starting 30 seconds before injection of the microspheres.

Collateral blood supply–dependent regions of the brain were determined by using the shadow flow technique. We have used this technique extensively and have described the method in detail previously. Autologous heparinized blood from a reservoir was perfused by using a pump through PE-50 tubing into the cannulated MCA branch. Vascular pressures were then measured by insertion of the micropipette into the neighboring noncannulated branch and then the cannulated MCA branch distal to the cannulation site (Fig. 1). Perfusion pressure from the pump was increased until pressure in the cannulated MCA branch was equal to the pressure in the noncannulated MCA branch. This required perfusion of approximately 2 ml of autologous blood per minute. The “shadow” CBF was then measured to identify the CDT. The external perfusion of the cannulated MCA branch perfused with microsphere-free blood prevents blood flow through collateral channels that supply the territory that is normally supplied by the occluded MCA branch (only overlap flow will be present during the shadow flow). Because the area at risk (that area identified morphologically to be supplied by the cannulated vessel) is perfused with microsphere-free blood at a pressure that prevents perfusion by collateral channels, values for CBF to the area at risk during the shadow flow reflect only overlap flow from adjacent vascular beds. By using this technique we have consistently identified an area of CDT within the MCA risk area in which shadow flow (overlap excluded) is less than 10 ml/minute/100 g. This area represents tissue that would experience profound ischemia if collateral flow was decreased or eliminated because it has almost no normal overlap flow from neighboring vascular beds. (The surrounding tissue, supplied by the ever-present overlap flow as well as by collateral flow, would not be so greatly affected, despite being within the “risk area,” and is thus not truly dependent on collateral vessels.) Thus, by studying tissue within the vascular distribution of the cannulated MCA branch in which flow was at least 10 ml/minute/100 g during shadow flow, we were able to identify tissue that received primarily collateral flow with minimum contamination from overlap flow. Following completion of the shadow flow, perfusion of the MCA branch was stopped so that the area at risk was once again perfused by both collateral and overlap flow.

Administration of MK-801 Before MCA Occlusion

In six dogs MK-801(1 mg/kg) was given intravenously before MCA branch occlusion. Baseline CBF, MAP, and MCA branch pressures were measured prior to MK-801 administration. The CBF and vascular pressures were measured again 30 minutes after MK-801 was administered. A branch of the MCA was then cannulated and CDT was identified by using the shadow flow technique. Blood flow and vascular pressures were measured again 60 minutes after the MK-801 had been given. Hypotension was then induced by the removal of blood until the MAP was reduced to 50 mm Hg, after

Fig. 1. Drawing showing a branch of the MCA that is occluded with an aneurysm clip and cannulated distally. External perfusion of the cannulated MCA branch is increased until vascular pressure measured at point A is equal to that measured previously at point B. This creates a CDT region that is temporarily without blood flow. A radioactive-free negative map is created during the shadow flow; this is the CDT. A second region of overlap flow containing an admixture of radioactive and nonradioactive blood is also identified.

Superfused with artificial cerebrospinal fluid (pH 7.3 changed significantly during the interventions.

Materials and Methods

Surgical Preparation of Animals

Twenty mongrel dogs, each weighing between 19 and 26 kg, were anesthetized with an intramuscular injection of ketamine hydrochloride (10 mg/kg) and an intravenous injection of thiopental (25 mg/kg) and were intubated endotracheally. Anesthesia was maintained with 1% halothane and a mixture of 45% O₂/55% N₂O. Core temperature, measured with a rectal temperature probe, was 37.5 ± 0.4˚C (mean ± standard deviation [SD]) and did not change significantly during the interventions. Catheters were placed in a brachial artery for measurement of mean arterial pressure (MAP) and in a femoral vein for administration of fluid and drugs. Blood was obtained from a brachial artery catheter to measure arterial blood gas levels. Baseline pH was 7.34 ± 0.04; PaO₂ 259 ± 5 mm Hg; and PaCO₂ 39.8 ± 1.3 mm Hg; none of these values changed significantly during the interventions.

A left frontotemporal craniotomy was performed, the dura over the sylvian fissure was opened, and the exposed cerebrum was superfused with artificial cerebrospinal fluid (pH 7.3 ± 0.08; CO₂ 41 ± 0.2 mm Hg; and O₂ 43 ± 12 mm Hg). Temperature and partial pressure of gases in the superfusate were determined at the beginning and conclusion of superfusion and were not significantly different. The superfusate temperature was maintained at body temperature (± 0.3˚C).

A large primary and a secondary branch (400–900 μm) of the MCA were identified. The primary branch was occluded proximally with an aneurysm clip and cannulated distally in antegrade fashion.

The superfusate temperature was maintained at body temperature (± 0.2˚C).
Effects of MK-801 on collateral blood flow

Fig. 2. Drawing showing coronal brain section of normal cerebrum (unshaded) and tissue at risk (shaded), which includes CDT and overlap flow. Tissue is sectioned and the radioactivity is measured for calculation of blood flow.

which CBF and vascular pressures were measured under these new conditions.

Administration of MK-801 After MCA Occlusion

In 14 dogs a branch of the MCA was first cannulated and CDT was identified by using the shadow flow technique. Baseline CBF, MAP, and MCA pressures were measured. The MK-801 (1 mg/kg) was administered intravenously and CBF, MAP, and MCA pressures were measured 30 and 60 minutes later. Hypotension was induced by the removal of blood until the MAP was reduced to 50 mm Hg, after which CBF and vascular pressures were measured under these new conditions.

Identification of the Area at Risk

At the end of each experiment the cannulated branch of the MCA was again perfused with autologous blood (similar to shadow flow), thereby preventing blood flow through collateral channels. Fifty milliliters of 4% neutral red dye was injected into the femoral vein and allowed to circulate for 2 minutes, creating a negative map of the area at risk. This approach is similar to that used previously. \cite{12,17,13,16,41}

The animals were killed with an intravenous injection of pentobarbital (300 mg/kg), and the cerebrum was removed, sectioned in the midline, and sliced into 3-mm coronal sections. The area at risk (defined morphologically by the absence of staining by neutral red) was dissected from the surrounding tissue and divided into tissue samples weighing 0.4 to 0.5 g (Fig. 2). Similar samples were taken from ipsilateral and contralateral normal cortex outside of the risk area. These tissue samples were weighed and, along with reference blood samples, were counted in a 3-in well-type gamma counter for 5 minutes each.

Calculation of Resistance

Small vessel resistance in the normal cerebrum was calculated using the equation: normal MCA branch pressure/rCBF to ipsilateral cerebrum. Collateral resistance was calculated from: (pressure in normal MCA branch − pressure in cannulated MCA branch)/rCBF to CDT region. Large artery resistance was calculated from: (aortic pressure − pressure in normal MCA branch)/rCBF to ipsilateral cerebrum.

Statistical Analysis

Values are expressed as means ± SDs. Internal controls were present in all animals because tissue samples from the opposite untreated hemisphere were analyzed and compared with the CDT and the surrounding normal zone of the experimental hemisphere. Tissue samples from both kidneys and ventricles also served as an internal control to ensure uniform microsphere distribution. External controls measuring the effects of the experimental condi-

tions over time on CBF, microvascular pressure, and MCA back pressure have been previously described. \cite{12,17,13,16,41}

Statistical comparisons were performed using a computerized Student’s paired t-test with Bonferroni corrections for rCBF, vascular pressures, and calculated resistance between baseline and all subsequent flows. A probability value of less than 0.05 was considered statistically significant.

Sources of Supplies and Equipment

Anesthesia in the rats was maintained using the Harvard pump respirator from Harvard Apparatus Co., Inc., Millis, MA. The Servo-nulling Pressure Measuring System (model 5), used to determine pressure in the cannulated MCA, was obtained from Instrumentation for Physiology and Medicine, Inc., San Diego, CA. Blood was perfused into the cannulated MCA branch using a pump available from Harvard Apparatus Co., Cambridge, MA. Neutral red dye, used to create the negative map of the area at risk, was obtained from Fisher Scientific Co., Fairlawn, NJ. Statistical analysis was performed using the Sigma Stat software package, available from Jandel Scientific Software, San Rafael, CA.

Results

Administration of MK-801 Before MCA Occlusion

The results from experiments in which MK-801 was administered before MCA occlusion are shown in Table 1. Blood flow to CDT and the normal cerebrum increased slightly, but not significantly, from baseline levels 30 minutes after MK-801 administration. Following MCA branch occlusion and 60 minutes after MK-801 administration, blood flow remained elevated in all cortical regions. Hypotension significantly reduced blood flow to CDT by 69.0%. The normal cerebrum was less affected by hypotension, with a reduction in flow of 17.6% and 25.1% to ipsilateral and contralateral cortex, respectively. The MAP, MCA branch pressures, and heart rate did not change significantly after MK-801 was administered.

Pressure in the precannulated MCA branch was reduced by 27.1% after it was occluded and cannulated, reflecting the back pressure from collateral vessels. Hypotension caused a significant drop in both cannulated and normal MCA branch pressures and an increase in heart rate. Small vessel resistance increased, whereas large vessel resistance decreased after MK-801 was administered; however, the change was not statistically significant in either case. The cannulated vessel resistance decreased after it was occluded, signifying vasodilation of collateral branches distal to the site of occlusion, which was confirmed by the low vascular resistance calculated for collateral vessels. A baseline collateral vascular resistance before MCA branch occlusion could not be calculated because CBF to the region was from a normal vessel and not from collateral vessels. During hypotension large and small vessel resistance decreased significantly, whereas collateral vessel resistance increased. The increase in collateral resistance was reflected in the reduced CBF to CDT. In summary, MK-801 appears to have caused a mild increase in CBF prior to MCA occlusion, presumably through a direct or indirect reduction in large cerebral vessel resistance. This response persisted even after MCA branch occlusion. Under hypotensive conditions this response was lost and blood flow to CDT was significantly reduced. Thus, MK-801 does not appear to preserve flow selectively to CDT under extreme ischemic conditions.
Administration of MK-801 After MCA Occlusion

The results of experiments in which MK-801 was administered after MCA occlusion are shown in Table 2. Cerebral blood flow to CDT was reduced by 51.7% from baseline levels 30 minutes after MK-801 was administered. Ipsilateral and contralateral CBF were reduced by 58.9% and 29.7%, respectively, during this same time period. At 60 minutes after MK-801 administration, CBF remained significantly reduced in all regions. Under hypotensive conditions, CBF was reduced by an additional 29.7% for the ipsilateral and 28.9% for the contralateral cerebral hemisphere. The MAP, MCA branch pressures, and heart rate did not change significantly after MK-801 was administered. A slight decrease in MAP and cannulated MCA branch pressure was noted 60 minutes after administration but it was not statistically significant. Collateral and cannulated vascular resistance were low during the baseline measurement, reflecting vasodilation distally in response to proximal occlusion. After MK-801 was administered, vascular resistance increased in all measured vessels at 30 minutes and was significantly increased in all vessels including collateral vessels by 60 minutes. Hypotension caused an additional increase in small vessel, cannulated vessel, and collateral vessel resistance, but was not determined to be significant in the small vessels. Large vessel resistance decreased presumably to provide maximum blood flow to the brain. Thus, MK-801 administered after MCA branch occlusion caused a significant increase in cerebrovascular resistance and a corresponding decrease in CBF. The CDT showed the greatest drop in CBF and did not appear to show any selective preservation of flow under mild or severe ischemic conditions with MK-801.

Discussion

Experimental Technique

Immediate input of collateral blood supply to the CDT is most likely derived from existing anatomical pathways that are caused by the development of differential pressures rather than by metabolic changes in the ischemic zone. We have shown that cerebral collateral vessels arise primarily at the level of large extraparenchymal arteries, not principally at the level of microvascular communications. In the nonischemic state, blood flow through potential collateral channels supplies the normal cerebrum; these normal vessels become collateral vessels after a neighboring artery is occluded. These anatomical collateral pathways include communications at the circle of Willis, in the large pial arteries (macrocommunications in the boundary zones of the anterior, middle, and posterior cerebral arteries), and microcommunications in minor pial arteries.

Our model differs from previous studies of cerebral collateral flow because it allows us to identify an area of tissue that is dependent on collateral flow (CDT) with minimal contamination from overlap flow within a morphologically identified “risk area” for MCA occlusion. Identification of this zone hinges on the separation of overlap flow (present at all times) from true collateral flow (present only on demand) within the area at risk for occlusion of an MCA branch. This model, in which a radiolabeled microsphere-based shadow flow technique is used, was initially refined in the coronary circulation and prior to our studies had not been applied to CBF.

23-25,28 Because the area at risk (that area identified morphologically to be supplied by the cannulated vessel) is perfused with microsphere-free blood at a pressure that prevents perfusion by collateral channels, values for CBF to the area at risk during the shadow flow reflect only overlap flow from adjacent vascular beds. To our knowledge, no other investigators have evaluated collateral flow in this way and all previous studies of collateral flow most likely included admixtures of overlap and collateral components, thus limiting the investigators’ ability to assess true collateral failure and reserve.
Effects of MK-801 on collateral blood flow

TABLE 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Shadow</th>
<th>Baseline</th>
<th>30 Min</th>
<th>60 Min</th>
<th>Hypotension</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood flow (ml/min/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDT</td>
<td>7.8 ± 3.5</td>
<td>13.0 ± 76.7</td>
<td>63.0 ± 28.7†</td>
<td>63.7 ± 28.7†</td>
<td>26.2 ± 12.7‡</td>
</tr>
<tr>
<td>ipsilateral cerebrum</td>
<td>130.4 ± 48.3</td>
<td>134.7 ± 50.7</td>
<td>94.7 ± 24.5‡</td>
<td>92.3 ± 29.5‡</td>
<td>49.7 ± 18.6‡</td>
</tr>
<tr>
<td>contralateral cerebrum</td>
<td>164.4 ± 74.7</td>
<td>161.3 ± 88.1</td>
<td>114.7 ± 43.9‡</td>
<td>102.3 ± 45.3‡</td>
<td>55.4 ± 19.0‡</td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>121.8 ± 21.0</td>
<td>120.7 ± 16.3</td>
<td>116.7 ± 11.7</td>
<td>112.0 ± 12.7</td>
<td>49.6 ± 3.0‡</td>
</tr>
<tr>
<td>normal MCA branch</td>
<td>45.1 ± 27.4</td>
<td>41.5 ± 29.4</td>
<td>41.6 ± 21.1</td>
<td>47.1 ± 27.0</td>
<td>33.1 ± 14.1‡</td>
</tr>
<tr>
<td>cannulated MCA branch</td>
<td>30.1 ± 19.9</td>
<td>30.5 ± 19.2</td>
<td>35.4 ± 26.0</td>
<td>26.9 ± 18.4</td>
<td>17.2 ± 4.6‡</td>
</tr>
<tr>
<td>heart rate</td>
<td>142.3 ± 27.1</td>
<td>140.3 ± 44.1</td>
<td>146.0 ± 30.2</td>
<td>145.3 ± 26.7</td>
<td>170.4 ± 16.7‡</td>
</tr>
<tr>
<td>resistance (mm Hg/ml/min/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>small vessel</td>
<td>0.316 ± 0.210</td>
<td>0.268 ± 0.200</td>
<td>0.457 ± 0.200†</td>
<td>0.628 ± 0.361†</td>
<td>0.673 ± 0.261</td>
</tr>
<tr>
<td>cannulated vessel</td>
<td>NA</td>
<td>0.210 ± 0.202</td>
<td>0.482 ± 0.402†</td>
<td>0.413 ± 0.262†</td>
<td>0.643 ± 0.252‡</td>
</tr>
<tr>
<td>collateral vessels</td>
<td>NA</td>
<td>0.143 ± 0.152</td>
<td>0.170 ± 0.163</td>
<td>0.316 ± 0.312†</td>
<td>0.656 ± 0.131‡</td>
</tr>
<tr>
<td>large vessel</td>
<td>0.851 ± 0.588</td>
<td>0.710 ± 0.572</td>
<td>1.061 ± 0.740†</td>
<td>1.052 ± 0.863†</td>
<td>0.441 ± 0.163‡</td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± SD. Abbreviation: NA = not applicable; true collateral vessel resistance could not be calculated because shadow flow was moving through the vessel.
† p < 0.05 compared with baseline.
‡ p < 0.05 compared with 60-minute value.

There are several limitations of the present model. First, measurement of pressure in branches of the MCA limits the precision of the calculated value of collateral vessels. Such values include not only the resistance of collateral vessels, but also resistance of arteries between the point of measurement and the origin of collateral vessels. Second, in using this method we make the assumption that pressure in a single pial artery is representative of pressure in other vessels of that size and can be used to calculate segmental vascular resistance. This assumption cannot be tested directly, but has been made routinely and is considered valid by others. Third, changes in small vessel resistance were calculated; however, because the segment of vessels that causes small vessel resistance is heterogeneous (and includes arterioles, capillaries, and venules), we could not precisely localize where changes in small vessel resistance occurred. Finally, it is not clear whether findings in dogs can be extrapolated to humans. In these experiments, blood flow to the cerebrum was not affected when a branch of the MCA was occluded. This response is different in humans, who are prone to develop infarctions with large arterial occlusions.

Role of MK-801 in Neuroprotection

In the current study, NMDA receptor blockade with MK-801 did not lead to relative sparing of CBF within the CDT after MCA branch occlusion or severe hypotension. Furthermore, rCBF was reduced in all areas when MK-801 was administered after MCA branch occlusion. Cerebral vasoconstriction appeared to be the major contributor to the reduction in blood flow observed. These data confirm previous findings of reduced CBF secondary to vasoconstriction after MK-801 administration. There is still much to be learned about the effects of MK-801 on blood flow, but it is clear that MK-801 can increase CBF in the normal cerebral arteries. Increases in CBF with MK-801 have been reported in several studies.50,43

It would appear that the increases in CBF from MK-801 occur only in the normal cortex and through mechanisms yet to be discovered. Regulation of CBF prior to MCA occlusion does not appear to depend on NMDA activation and neuronal NO production to maintain basal levels of flow.

The efficacy of MK-801 as a neuroprotective agent has been well documented in both ischemic and NMDA models of brain injury.3,26,30,39,42 The primary mechanism of this protection is through the regulation of intracellular metabolism after ischemic injury. The MK-801 reduces glucose use and oxygen consumption, accelerates recovery of cerebral high-energy phosphates and intracellular pH, inhibits perifusion cerebral protein synthesis and immediate-early gene production, blocks cortical spreading depression–like activity, limits tissue lactic acidosis, and limits the release of metabolites of arachidonic acid believed to affect CBF by their effect on platelet aggregation and involvement in cerebral ischemia, edema, and vasospasm.1,17,43

A second potential mechanism of protection is inhibition of postischemic hyperemia.15,43 Glutamate activation of neuronal NO synthase results in vasodilation and increase in CBF through the local release of neuronal NO. Blockade of the NMDA receptors responsible for activating neuronal NO production should theoretically reduce in part postischemic vasodilatation and hyperemia. Our results support the concept that postischemic activation of NMDA receptors is partially responsible for maintaining CBF during ischemia, presumably through production of neuronal NO, which may contribute to postischemic hyperemia.46 The vasoconstriction and reduced CBF response after postischemic administration of MK-801, whether actually protective against hyperemia or actually detrimental, appear to be minor compared with the overall benefits achieved by attenuation of the intracellular cytotoxic cascade.

A third proposed mechanism is selective preservation
of blood flow to ischemic tissue by MK-801. Our laboratory, which was in the unique position of being able to test the effects of MK-801 on collateral vessels, was unable to show a selective preservation of flow to CDT. Collateral blood flow was reduced under hypotensive conditions irrespective of the time it was administered. Selective augmentation of blood flow by MK-801 to the ischemic penumbra therefore appears highly unlikely.

The consistently strong neuroprotective effects of MK-801, despite its widely variable hemodynamic response, make it unlikely that the neuroprotection offered by this compound is achieved through regulation of blood flow. We reiterate the importance of having a thorough understanding of the pathophysiological steps leading to cell death and the importance of timing in the administration of neuroprotective agents.

Conclusions

Depending on whether it is administered before or after MCA branch occlusion, MK-801 produces variable hemodynamic responses. These responses in part appear to be regulated through mechanisms that are dependent on NMDA receptor activation and presumed neuronal NO production. Collateral CBF does not appear to be selectively preserved by MK-801. The MK-801 appears to exert its neuroprotective effects primarily as a direct cytoprotectant that reduces cell injury and the cytotoxic cascade while having little beneficial effect on CBF except for the possible attenuation of postischemic hyperemia.

References


S. C. Robertson, et al.
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Manuscript received April 28, 1997.
Accepted in final form July 15, 1997.
This study was supported by a Merit Review Grant from the Department of Veterans Affairs to Dr. Loftus.
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