P-450 epoxygenase and NO synthase inhibitors reduce cerebral blood flow response to N-methyl-D-aspartate

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measured at the site of drug delivery by the hydrogen-clearance technique. Two structurally dissimilar epoxide oxygenase inhibitors with different mechanisms of action were used: miconazole, which acts on the heme moiety of cytochrome P-450, and N-methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH), a selective substrate inhibitor (49). We tested the hypothesis that miconazole and MS-PPOH inhibit the increases in CBF during NMDA administration without inhibiting NO production. Results were compared with the use of the NOS inhibitor, N\(^{\text{\textregistered}}\) nitro-L-arginine (L-NNA). The NMDA antagonist MK-801 was also evaluated to assure that the NMDA-evoked response could be antagonized by a specific NMDA receptor antagonist.

**MATERIALS AND METHODS**

*Surgical procedures.* The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University and conforms to the National Institutes of Health Guidelines for the Care and Use of Animals in Research. Adult male Wistar rats weighing 300–450 g were initially anesthetized with 4% halothane and were tracheotomized. Anesthesia was maintained with halothane (1.0–2.0%) in oxygen-enriched air (35–40%) to maintain arterial pH (7.35–7.40) and arterial partial pressures of CO\(_2\) (P\(_{\text{CO}_2}\) = 35 mmHg) and O\(_2\) (P\(_{\text{O}_2}\) = 150 mmHg). Cannulas were inserted into the tail artery to monitor arterial blood pressure, heart rate, and arterial blood gases. Temperature was monitored with a rectal probe and maintained at 37–38°C with a heating lamp. Care was taken to avoid direct heating of the head, which would alter the normal thermal gradients between the brain and the core.

The rat’s head was placed in a Kopf stereotaxic frame for placement of microdialysis cannulas into the striata (0.5 mm anterior and 2.5 mm lateral to the bregma; depth 6 mm from the dura). A 2×2-mm area of the skull was removed with a variable-speed drill. A thin layer of bone was left intact and removed with forceps under microscopic observation to minimize trauma to the cortex. Cannulas were advanced bilaterally to predetermined coordinates with a micromanipulator and were fixed in position with dental cement. The animals were then removed from the stereotaxic apparatus and allowed a 60-min postsurgical equilibration period before the experiment began.

*Modified microdialysis with hydrogen-clearance technique for CBF measurements.* Dialysis probes were used as described previously (9). The probes consisted of a single hollow dialysis fiber, one end of which was sealed with epoxy. The dialysis membrane diameter was 300 μm and had a molecular mass cutoff of 5 kDa. Two hollow silicon tubes (150-μm outer diameter, 75-μm inner diameter) were inserted into the dialysis fiber so that their ends were 3 mm apart. The distance between the tips constituted the effective dialyzing area of the cannula (46). Starting 1 h after insertion, the cannulas were perfused at a rate of 1 μl/min. The concentration of the artificial cerebrospinal fluid (aCSF) was as follows (in mmol/l): 131.8 NaCl, 24.6 NaHCO\(_3\), 2.0 CaCl\(_2\), 3.0 KCl, 0.65 MgCl\(_2\), 6.7 urea, and 3.7 dextrose. The aCSF was filtered, warmed to 37°C, and bubbled with 95% N\(_2\)-5% CO\(_2\) until O\(_2\) and CO\(_2\) tensions were similar to those of normal CSF.

The use of the hydrogen-clearance technique for measurement of CBF in the area surrounding the dialysis cannula has been described in detail (46). The coating from a platinum wire (bare diameter 10 μm) was removed 1–2 mm from the end. The wire was inserted into the dialysis membrane adjacent to the silica inflow and outflow tubes. The wire was polarized to 475 mV relative to a reference electrode on the scalp. Hydrogen-clearance curves were generated by adding 10% H\(_2\) to the inspired gas mixture for ~2–3 min to achieve stable tissue concentrations before stopping ventilation with H\(_2\). Local CBF (in ml·min\(^{-1}\)·100 g\(^{-1}\)) was calculated from the time \(t\) (min) required for the tissue H\(_2\) concentration to decrease from 90 to 40% of maximum by using the formula

\[
\text{CBF} = 100 \ln(90/40)/t
\]

The 90% time value permitted time for H\(_2\) desaturation from arterial blood. Limiting the analysis of the decay to 40% of the maximum value reduces the influence of diffusion of H\(_2\) from low-flow compartments into striatal gray matter.

*Estimation of NO production.* We estimated NO production using modifications (9) of the assay described by Bredt and Snyder (12). Arginine is converted to equimolar concentrations of citrulline and NO by the action of NOS. During continuous infusion of aCSF containing 3 μmol/l L-[\(^{14}\)C]arginine, 20-μl effluent dialysate samples were collected during 20-min epochs and assayed for L-[\(^{14}\)C]citrulline content. Samples were diluted with 2 ml buffer containing 30 mmol/l HEPES (pH 5.2), 3 mmol/l EDTA, and 1 ml water. Radioactivity of the flow through the column was quantified by liquid scintillation spectroscopy. To determine resin efficiency of arginine trapping, 20 μl aCSF containing 3 μmol/l L-[\(^{14}\)C]arginine (not used for dialysis) was diluted in 200 μl water, poured over a column, and washed as above. Specific activity was corrected for counting efficiency and background activity and was expressed as femtomoles per minute of perfusion. As an internal control, 100 μl aCSF not used for dialysis was directly assayed for activity to ensure that consistent concentrations of L-[\(^{14}\)C]arginine were added to the aCSF.

*Experimental protocol.* Microdialysis probes in both the right and left striata were perfused with aCSF for 1 h during which time CBF was measured at three 20-min intervals. Thereafter, one probe was perfused with an inhibitor while the contralateral side was perfused with vehicle dissolved in aCSF for a 2-h period. The inhibitor was randomly assigned to the right or left side. At 1 h of vehicle/drug perfusion, NMDA was added to the perfusate on both sides. This experimental design permitted a paired analysis between vehicle and drug administration under identical conditions of arterial H\(_2\) concentration, arterial blood pressure, blood gases, temperature, and other physiological parameters.

CBF was measured in five groups of rats: *group 1* received 20 μmol/l miconazole and 0.3 mmol/l NMDA (n = 5); *group 2* received 20 μmol/l miconazole and 3 mmol/l NMDA (n = 7); *group 3* received 20 μmol/l MS-PPOH and 3 mmol/l NMDA (n = 7); *group 4* received 1 mmol/l L-NNA and 3 mmol/l NMDA (n = 7); and *group 5* received 0.1 mmol/l MK-801 and 3 mmol/l NMDA (n = 8). The vehicle for miconazole was 0.5% DMSO, and the vehicle for MS-PPOH was 0.5% ethanol. L-NNA and MK-801 were dissolved directly in aCSF. CBF was measured at 4, 12, 20, 40, and 60 min after switching the perfusate to the vehicle/inhibitor and to NMDA. To test for selectivity of MS-PPOH in inhibiting vasodilation, a sixth group of rats (*group 6, n = 7*) was studied in which 1 mmol/l of sodium nitroprusside was perfused bilaterally instead of NMDA after perfusion with vehicle on one side and 20 μmol/l MS-PPOH on the second side.
Table 1. Mean arterial blood pressure and PaCO2 in groups with blood flow measurements

<table>
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<th>Start Inhibitor</th>
<th>Start NMDA or SNP</th>
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PaCO2, mmHg

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Values are means ± SE; n, number of rats. PaCO2, arterial partial pressure of CO2; aCSF, artificial cerebrospinal fluid; NMDA, N-methyl-D-aspartate; SNP, sodium nitroprusside. Group 1, miconazole, low-dose NMDA; group 2, miconazole, high dose NMDA; group 3, N-methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH); group 4, N-nitro-l-arginine (L-NNA); group 5, MK-801; and group 6, MS-PPOH and SNP.

Production of NO was estimated by measuring labeled citrulline in the dialysis effluent bilaterally in separate groups of rats. Probes were perfused with labeled arginine for a 3-h loading period. Vehicle was added bilaterally during the last hour of the loading period to establish baseline values and then 20 μmol/l miconazole (n = 7) or 20 μmol/l MS-PPOH (n = 7) was added to one side while the contralateral side continued to receive vehicle. One hour later, 3 mmol/l NMDA was added bilaterally to the perfusate containing labeled arginine plus vehicle or drug. At the completion of the experiment, rats were killed with potassium chloride while still anesthetized, and dialysis probes were perfused with methylene blue. The brains were stored in 4% paraformaldehyde for 48 h and then dissected to confirm the probe placement in striatum.

Materials. L-[14C]arginine (317 mCi/mmol) was obtained from Amersham, and DMSO, miconazole, L-NNA, NMDA, and MK-801 were obtained from Sigma Chemical. MS-PPOH was synthesized as described previously (49).

Statistical analysis. Within each group, data were analyzed by two-way ANOVA; the two treatments delivered to the two striata were one within-subject factor, and time was a second within-subject factor. If the overall effect of treatment or the treatment × time interaction was significant, comparisons of mean values between the two treatments at individual time points were made with Dunn’s procedure for multiple comparisons. If time or treatment × time interaction was significant, data at a single probe site were subjected to one-way repeated-measures ANOVA. If the F value was significant, values during vehicle/drug perfusion were compared with baseline values at 60 min of aCSF perfusion by Dunnett’s test. The CBF data during NMDA and sodium nitroprusside perfusion were logarithmically transformed because the standard deviations increased with the mean values. These values were compared with the baseline value at 60 min of vehicle/drug perfusion by Dunnett’s test to determine whether there was a significant change during NMDA or sodium nitroprusside perfusion. P < 0.05 was considered significant. Data are presented as means ± SE.

RESULTS

Arterial blood pressure and PaCO2 remained stable during the course of the blood flow measurements (Table 1). The ranges of the average values of other variables were 7.35–7.42 for arterial pH, 119–165 mmHg for PaCO2, and 37–38°C for rectal temperature.

In group 1, basal CBF during aCSF perfusion was similar in both striata, and there were no differences in CBF when the perfusates were switched to 0.5% DMSO vehicle or 20 μmol/l miconazole (Fig. 1). With concurrent perfusion of 0.3 mmol/l NMDA, there were no significant increases in CBF with either vehicle or miconazole perfusion. In group 2, perfusion with 20 μmol/l miconazole produced an ~20% decrease in CBF (Fig. 2). Upon addition of 3 mmol/l NMDA, CBF doubled by 12 min on the side perfused with vehicle. The increase remained statistically significant through 30 min. On the side perfused with miconazole, CBF failed to increase during NMDA perfusion, and CBF remained significantly less than that on the vehicle side throughout the 60-min NMDA perfusion period.

In group 3, perfusion with 20 μmol/l MS-PPOH or the 0.5% ethanol vehicle had no effect on striatal CBF (Fig. 3). With concurrent perfusion of 3 mmol/l NMDA, CBF increased on the side perfused with vehicle but remained unchanged on the side perfused with MS-PPOH. Flow was significantly different between the vehicle and MS-PPOH sides at 12, 20, and 30 min of NMDA perfusion.

In group 4, microdialysis perfusion of 1 mmol/l L-NNA reduced striatal CBF by ~40% (Fig. 4). Subsequent perfusion of 3 mmol/l NMDA did not significantly increase CBF on the side perfused with L-NNA, and flow remained significantly less than that on the aCSF control side.

In group 5, perfusion with 0.1 mmol/l MK-801 decreased striatal CBF by ~35% (Fig. 5). The increase in

Fig. 1. Cerebral blood flow (CBF) measured bilaterally in striatum during microdialysis perfusion for 1 h with artificial cerebrospinal fluid (aCSF), 1 h with either vehicle (0.5% DMSO) or 20 μmol/l miconazole, and 1 h with 0.3 mmol/l N-methyl-D-aspartate (NMDA) plus either vehicle or miconazole. Values are means ± SE (n = 5). There were no significant effects of time or differences between sides.
CBF with 3 mmol/l NMDA perfusion was blocked by MK-801.

In group 6, perfusion with 20 μmol/l MS-PPOH or vehicle did not change CBF. With concurrent perfusion of 1 mmol/l of sodium nitroprusside, CBF increased on both sides (Fig. 6). There was no difference in CBF between the vehicle and MS-PPOH-treated sides during nitroprusside perfusion.

In group 7, there was no difference in basal citrulline recovery between sides receiving 0.5% DMSO vehicle and 20 μmol/l miconazole (Fig. 7). Bilateral addition of 3 mmol/l NMDA increased citrulline recovery on both sides, and there was no significant difference between sides. Likewise, in group 8, there were no differences in labeled citrulline recovery between the sides perfused with 0.5% ethanol vehicle and 20 μmol/l MS-PPOH under basal conditions or during 3 mmol/l NMDA perfusion (Fig. 8).

**DISCUSSION**

The results of this study demonstrate that increases in striatal blood flow evoked by local administration of NMDA were blocked by the cytochrome P-450 epoxygenase inhibitors, miconazole and MS-PPOH. Neither...
drug inhibited the evoked increases in the conversion of labeled arginine to labeled citrulline, thereby indicating that the mechanism of action of these drugs did not involve inhibition of NO production. Nevertheless, the NOS inhibitor L-NNA also attenuated the NMDA-evoked increase in striatal blood flow, consistent with studies using different experimental paradigms (20, 34, 37, 39). Therefore, our data imply that both NOS activity and epoxygenase activity are required for full expression of the blood flow response to NMDA receptor activation.

We found that delivery of 3 mmol/l NMDA produced a robust increase in CBF in all groups by 12 min of perfusion, whereas 0.3 mmol/l failed to produce a consistent increase. Recovery across these dialysis membranes is about 10% (9). Consequently, the tissue concentration in the immediate vicinity of the probe is expected to be an order of magnitude less than the delivered concentration. Thus the blood flow response to NMDA in striatum appears to require an NMDA concentration in the 10^{-2} M range. This concentration range is consistent with the study of Faraci and Breese (20) in mature rabbits in which 100 μmol/l NMDA produced 10% pial arteriolar dilation, whereas 300 μmol/l NMDA produced 30% dilation. A 30% dilation in all arteriolar segments would result in an approximate doubling of CBF. Both the pial arteriolar response (20) and the striatal flow response to NMDA were blocked by MK-801. These results indicate that NMDA at these concentrations are acting via NMDA receptors rather than through a nonspecific mechanism of action. Furthermore, the magnitude of the striatal blood flow response measured by hydrogen clearance is comparable to that measured in cerebral cortex by iodoantipyrine during topical application of 10 mmol/l NMDA (50).

Functional NMDA receptors have not been demonstrated on cerebral blood vessels (7, 20, 36) or on glia (25, 42). Thus NMDA is presumed to act on neurons. One view of the sequence of flow coupling to NMDA activation that has arisen over recent years is that NMDA receptor activation in neurons leads to calcium influx, stimulation of neuronal NOS, diffusion of NO from neurons to vascular smooth muscle, activation of guanylyl cyclase in smooth muscle, and vasodilation. The present results with epoxygenase inhibitors indicate that the mechanism is more complex.

Epoxygenase activity is present in brain slices (6) and is presumed to be based in astrocytes because glial cell cultures generate EETs (5) and express P-450 2C11, an enzyme that possesses epoxygenase activity (3). Also, other cytochrome P-450 enzymes have been localized in astrocytes (27, 29). Although the endothelium is a source of EETs in some vascular beds (14),
this source has not been documented in cerebral endothelium. EETs hyperpolarize cerebral vascular smooth muscle (3, 23) and produce vasodilation (6, 30). An increase in intracellular calcium in astrocytes is thought to mobilize arachidonic acid and stimulate EET production. Because of the lack of functional NMDA receptors on astrocytes, the increase in astrocytic calcium is assumed to be indirectly linked to neural activity evoked by NMDA. For example, application of NMDA to cerebellar slices produces increases in calcium in Bergmann glia inhibitable by TTX (41), thereby implying that glia sense an increase in neural activity. The signaling mechanism between neurons and glia is unclear. Astrocytic influx of potassium during increased neuronal firing is one possible signal. Another potential signal is neuronal release of ATP or adenosine. Stimulation of astrocytic purinergic receptors causes mobilization of arachidonic acid (13) and increases in calcium (40) that can be propagated over 100-μm distances in astrocytes (15). Thus neuronal release of ATP or adenosine may enable coordinated vasodilation in microcirculatory units via calcium wave propagation through an astrocytic network. In addition to neurotransmitter release of purinergic agonists, NMDA can stimulate oxidative metabolism (50), which could promote adenosine release. Another consideration is that application of NMDA can cause neuronal release of glutamate (35, 52), which could then act on astrocytic metabotropic and kainate/α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors to increase calcium (25, 48) and mobilize arachidonic acid (43). Thus there are several potential mechanisms by which NMDA receptor activation on neurons might lead to increased calcium and EET formation in astrocytes.

Because epoxygenase and NOS inhibitors individually blocked the NMDA flow response, it appears that both EETs and NO are required for the response. However, the mechanism of interaction of the NOS and epoxygenase pathways in this response remains speculative at this time. Nevertheless, there are a few findings in the literature that might have a bearing on this interaction. High levels of NO promote glutamate release from synaptosomes (33), and NOS inhibition prevents NMDA-evoked release of glutamate and noradrenaline (35). Thus one possible mechanism of an NOS-epoxygenase signaling interaction is that NOS inhibition interferes with NMDA-evoked neurotransmitter release necessary for astrocytes to sense increased neural activity. Another potential site of interaction is at the vascular smooth muscle. In renal arterioles, NO inhibits cytochrome P450 ω-hydroxylase production of 20-hydroxyeicosatetraenoic acid (20-HETE), which in turn inhibits potassium channels (44). In cerebral arteries, part of the vasodilation elicited by NO donors is mediated by a decrease in 20-HETE (4). Thus with NOS inhibition, it is possible that 20-HETE production would increase and mitigate hyperpolarization evoked by EETs. Hence, NO may permit full expression of vasodilation by EETs by inhibiting 20-HETE production.

Products of cyclooxygenase metabolism may also contribute to the evoked hyperemia. Because 5,6-EET can be metabolized by cyclooxygenase and because pial arteriolar dilation to 5,6-EET can be inhibited in the rabbit by indomethacin (19), it is possible that the epoxygenase-dependent hyperemia seen with NMDA is mediated by cyclooxygenase activity. However, Lefler and Fedinec (30) reported that, in piglets, indomethacin inhibited pial arteriolar dilation to all four regioisomers of EETs even though three of the four are not metabolized by cyclooxygenase. A small concentration of iloprost (10^{-12} M) restored vasodilation to 5,6-EET after indomethacin treatment. Moreover, 5,6-EET application to the pial surface failed to increase 6-keto-prostaglandin F_{1α} in perivascular fluid. They concluded that prostacyclin may play a permissive role in EET vasodilation rather than a mediatory role. Thus the interaction between the epoxygenase and cyclooxygenase pathways may be complex and are beyond the scope of the present study.

The CBF response to NMDA delivery via microdialysis reached a peak at about 20 min and subsided by 60 min, whereas labeled citrulline recovery remained elevated beyond 60 min. There are several factors to consider in the dissimilar time course. First, efflux of labeled citrulline out of cells and diffusion back to the dialysis membrane will lag behind the delivery of NMDA to the surrounding tissue. Second, the volume of tissue subtended by the hydrogen-clearance and citrulline-recovery measurements may differ over time. Dykstra et al. (18) reported that labeled sucrose spreads to a 1-mm-diameter cylinder by 14 min of perfusion, whereas we found that labeled arginine spread to about 20 min would act to increase the labeled citrulline in the immediate vicinity of the probe beyond 20 min. Third, the recovery of CBF at 60 min of continued NMDA delivery implies either a downregulation of the electrophysiological response to NMDA, counterregulation by release of a vasoconstrictor substance, or neuronal damage. However, in a similar experimental paradigm, we did not detect neuronal necrosis (11). Finally, there was some variability in the delay until CBF increased after starting microdialysis perfusion of NMDA and in the time of the peak response. Such variability was not unexpected based on the aforementioned evidence of the time required to increase concentrations to critical levels by radial diffusion throughout the tissue subtended by the hydrogen-clearance technique.

It is important to consider the specificity of action of MS-PPOH and miconazole at the infused concentration of 20 μmol/l. Because recovery across the microdialysis...
membrane is ~10%, the effective tissue concentrations of MS-PPOH and miconazole are expected to be ~2 μmol/l. MS-PPOH was found to inhibit cyclooxygenase activity by 40% at 50 μmol/l and to have no effect on cytochrome P-450 ω-hydroxylase activity at 200 μmol/l (49). Thus the effective tissue concentration is estimated to be well below that necessary to inhibit these other pathways. However, the effective concentration is also estimated to be below the IC50 of 13 μmol/l for epoxygenase activity in renal microsomes (49). Because percent inhibition by a substrate inhibitor depends on the duration of exposure, the 60 min of MS-PPOH perfusion before NMDA perfusion may have permitted greater than 50% inhibition. Miconazole acts on the cytochrome P-450 heme moiety and is more potent in inhibiting epoxygenase activity (IC50 = 0.3 μmol/l) than cytochrome P-450 ω-hydroxylase activity (IC50 = 3 μmol/l) in renal microsomes (54). Because the 20-HETE product of cytochrome P-450 ω-hydroxylase activity is a cerebral vasoconstrictor (22), we attribute the suppression of NMDA-evoked vasodilation by miconazole to epoxygenase inhibition.

Other effects of miconazole have been described. For example, at 10 μmol/l, miconazole decreases NOS sensitivity to calmodulin (51) and decreases endothelial NOS catalytic activity ~10% (17). In contrast, 20 μmol/l had no detectable effect on NOS catalytic activity in rat brain homogenate (1). In the present study, miconazole had no effect on basal citrulline recovery or on the increase in citrulline during NMDA perfusion. The same was true for MS-PPOH. Thus it is highly unlikely that the suppressed blood flow response to NMDA by miconazole and MS-PPOH is attributable to NOS inhibition. Direct effects of miconazole on K+ channels (47) and Ca2+ transport (28, 32) have been inferred in some isolated systems, although in some cases the evidence was indirect (28) or high doses of miconazole (100 μmol/l) were used (47). Because we found that MS-PPOH, which is structurally different from miconazole, was as effective as miconazole in blocking the NMDA hyperemia, miconazole is most likely acting on cytochrome P-450 epoxygenase activity.

Previous work has shown that miconazole does not inhibit the CBF response to sodium nitroprusside (1). Likewise, in the present study, we demonstrated that MS-PPOH does not inhibit the CBF response to sodium nitroprusside. Thus the inhibition of the NMDA flow response by these agents does not represent a nonspecific effect on cerebral vessels or an interference with the ability of the smooth muscle to respond to NO.

Basal CBF decreased substantially during microdialysis perfusion of l-NNA or MK-801 before NMDA perfusion. Because l-NNA and MK-801 markedly reduced labeled citrulline recovery in a similar experimental design (11), tonic activation of NMDA receptors and tonic NOS activity contribute to basal CBF in striata of halothane-anesthetized rats. Whether EETs contribute to basal blood flow in our experiments is not as clear. MS-PPOH had no effect on baseline flow in either group 3 or group 6. Miconazole decreased flow ~20% in group 2 but had no effect in group 1 before NMDA administration. To increase the statistical power, data in group 1 (n = 5) and group 2 (n = 7) were combined on a post hoc basis. However, ANOVA indicated no effect of miconazole treatment over time or between vehicle and miconazole-treated sides with the combined data (n = 12). Hence, if there is a true effect of miconazole on basal striatal blood flow, the effect is probably small. Therefore, basal production of NO appears to exert a greater effect on basal vascular tone than basal production of EETs in striata under the conditions of our experiment. If the basal production of EETs is very low, it is unlikely that a small, constant production of EETs serves in a permissive role in the vasodilation evoked by NMDA. Rather, it is more likely that the epoxygenase inhibitors block a large increase in EETs and that EETs play an obligatory role in the NMDA flow response.

The lack of a substantial effect of miconazole on CBF in striatum is different from that reported with subdural superfusion of 20 μmol/l miconazole in pentobarbital sodium-anesthetized rats in which laser-Doppler flowmetry indicated a 30% reduction in cortical perfusion (1). This difference may be attributed to differences in brain region, tissue concentration of miconazole, anesthesia, or blood flow methodology. In newborn piglets, 1 μmol/l miconazole superfusion had no effect on baseline pial arteriolar diameter (31).

In summary, inhibition of cytochrome P-450 epoxygenase activity and NOS individually block the blood flow response to NMDA receptor activation in rat striatum. We conclude that both the epoxygenase and NOS pathways are involved in the vasodilatory response to NMDA receptor activation in striatum, and that the signaling pathway is more complex than simply diffusion of NO from neurons to vascular smooth muscle.

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