Cortical spreading depression confounds concentration-dependent pial arteriolar dilation during N-methyl-D-aspartate superfusion

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Ayata, Cenk, and Michael A. Moskowitz. Cortical spreading depression confounds concentration-dependent pial arteriolar dilation during N-methyl-D-aspartate (NMDA) superfusion. Am J Physiol Heart Circ Physiol 290: H1837–H1841, 2006. First published November 18, 2005; doi:10.1152/ajpheart.01102.2005.—Pial arterioles do not express N-methyl-D-aspartate (NMDA) receptors but dilate in response to topical NMDA application. We explored the mechanism underlying NMDA-mediated responses in murine pial arterioles (11–31 μm), using a closed cranial window preparation, and found that arteriolar dilation was not concentration dependent. Pial arteriolar diameter abruptly increased within 3 min of superfusing 50 or 100 μM NMDA. Dilation reached a peak within 1 min (46 ± 14%) and then declined to a plateau (28 ± 13%) for the duration of superfusion. Whereas a higher concentration (200 μM) did not produce further dilation, lower concentrations (1–10 μM) did not dilate the arterioles at all. MK-801 (10 μM) abrogated the dilation response, whereas Nω-nitro-L-arginine (1 mM) attenuated the peak and abolished the sustained dilation during NMDA superfusion. We determined that NMDA-induced pial arteriolar responses were evoked by cortical spreading depression, because abrupt vasodilation during 50 or 100 μM NMDA superfusion was associated with a large negative slow potential shift and electrocorticogram suppression that spread from the superfusion window to distant cortical areas. Our data suggest that the responses of pial arterioles to NMDA are caused in part by neurovascular coupling due to cortical spreading depression.

NMDA RECEPTOR ACTIVATION dilates pial arterioles in a number of species, including rats, rabbits, and piglets (6, 15–18, 36). The mechanism by which this powerful excitatory agent dilates cerebral vessels has been the subject of several studies because NMDA has no effect on the caliber of isolated vessels (22). NMDA receptors are cation-permeable ion channels that, when activated, strongly depolarize neurons, increase intracellular Ca2+, and stimulate synthesis and release of nitric oxide (NO) (19). Hence, vasodilation may be mediated by factors released from cortical neurons and/or astrocytes on NMDA receptor activation. However, in several reports (15, 16, 18), the magnitude of NMDA-induced dilation was highly variable and the dose–response relationship was steep. Furthermore, Faraci and Heistad (18) reported that NMDA produced a transient response in rats, and after an initial large dilation, vessel diameter returned toward control values despite the presence of NMDA.

The purpose of this study was to investigate NMDA receptor-dependent vasodilation in pial arterioles in mice by examining their responses using both cranial windows and electrophysiological recordings. We provide evidence showing that NMDA triggers cortical spreading depression (CSD) in mouse cortex and that pial arteriolar dilation in response to NMDA is mediated in part by CSD.

METHODS

Mice (SV-129, male, 23–28 g, n = 27) were anesthetized with halothane (2% for induction and 1% for maintenance in 70% N2O-30% O2) and intubated transorally. Femoral artery and vein were catheterized for the measurement of the arterial blood pressure (ETH-400 transducer) and administration of drugs. After surgical procedures were completed, anesthesia was switched to α-chloralose (1%, 80–100 mg/kg iv), and halothane was discontinued. Supplemental doses of α-chloralose were injected as needed to maintain a stable level of anesthesia, checked by the absence of cardiovascular changes in response to tail pinch. Mice were paralized (pancuronium bromide, 0.4 mg/kg iv, q 45 min), mechanically ventilated (SAR-830, CWE, Ardmore, PA), and end-tidal CO2 monitored by a microcapnometer (Columbus Instruments, Columbus, OH). Ventilation parameters were adjusted to maintain normal end-tidal CO2. Arterial blood gases and pH were measured at the end of each experiment (Corning 178 blood gas/pH analyzer, Ciba Corning, Medford, MA) and used to verify the end-tidal CO2. Arterial PO2 was maintained between 100–170 mmHg. Rectal temperature was kept at 36.8–37.1°C using a thermostatically controlled heating mat (YSI, Yellow Springs, OH). Mean arterial blood pressure (99 ± 11 mmHg), arterial PaO2 (32.5 ± 9.4 mmHg), PaO2 (143 ± 28 mmHg), and pH (7.25 ± 0.11) were consistent with previously reported values and within physiological limits for SV-129 mice (10). All experiments complied with the guidelines of and were approved by the Massachusetts General Hospital’s Subcommittee on Research Animal Care.

The effects of topical NMDA superfusion on pial arteriolar diameter were studied by using a closed cranial window, as described previously (30). The volume under the window was ∼0.1 ml. Artificial cerebrospinal fluid (aCSF) was superfused by an infusion pump (0.4 ml/min) via polyethylene tubing (PE-100) connected to a window port. Intracranial pressure was maintained at 5–8 mmHg by adjusting the outlet tubing to an appropriate height. Pial vessels were visualized continuously through the cranial window by an intravital microscope (Leitz, Germany), equipped with a video camera (C2400, Hamamatsu Photonics, Hamamatsu, Japan) and a video width analyzer (C3161, Hamamatsu, Japan).

The electrophysiological effects of drug superfusion on extracellular slow (DC) potential and electrocorticogram (ECOg) were recorded by using an open cranial window (constructed as above but left uncovered), a microelectrode amplifier (Axotape 1A, Axon Instruments), and a glass micropipette (tip resistance, 1–2 MΩ, at a depth of 400 μm). In addition, the spread of electrophysiological events was recorded by a second glass micropipette through a small (0.3 × 0.3 mm) distant craniotomy at the frontal pole ∼4 mm away from the superfusion window (2 mm anterior and 1 mm lateral from bregma).
An Ag/AgCl reference electrode was placed under the scalp on the contralateral side. The open cranial window was used for aCSF superfusion, and the distant craniotomy was covered with mineral oil to prevent cortical drying. Mice were allowed to stabilize for 30 min after surgical preparation.

The following experimental protocols were used. First, to test the concentration response for NMDA-induced vessel diameter changes, NMDA was superfused in a closed cranial window at 1, 10, 50, and 100 μM for 3–8 min each, until a stable diameter was reached (n = 10). A higher concentration of 200 μM was tested in four mice only. To test the involvement of NO, the nonselective NO synthase inhibitor Nω-nitro-L-arginine (L-NNA, 1 mM, n = 7 mice) was superfused for 60 min before NMDA superfusion. This relatively high concentration of L-NNA was chosen to ensure adequate inhibition of cortical NO synthase under the superfusion window and has previously been used by us and others (2, 12, 24, 30, 31, 35, 37) in cranial window preparations in mice and rats. NMDA receptor dependency was confirmed by MK-801 superfusion (10 μM, n = 5 mice) for 30 min before NMDA. Second, to record the NMDA-induced cortical DC potential shifts using open cranial window, NMDA was superfused for 3–8 min at each concentration until a large DC shift occurred (n = 5 mice). At the end of the experiments, KCl (100 μM) was superfused for 1 min to induce CSD.

Maximum pial arteriolar diameter increases were measured for each NMDA concentration. When present, the abrupt large vasodilation response was measured at baseline and four deflection points (as shown in Fig. 3): baseline (a, b), early diameter increase (c), trough of subsequent constriction (d), peak of abrupt large dilation (e), and plateau (f). The data are expressed as means ± SD. Statistical testing was performed by using one- or two-way ANOVA for repeated measures, Mann-Whitney’s rank sum test, or paired t-test.

RESULTS

NMDA superfusion at concentrations of 1–10 μM did not change vessel diameter (Fig. 1). At higher concentrations, pial arterioles precipitously dilated to a peak (46 ± 14%) and then decreased to a plateau (28 ± 13%; n = 10 mice). This abrupt dilation occurred at 50 μM in six and at 100 μM in four mice (Fig. 1B). Increasing NMDA concentrations did not produce further dilation; instead, the diameter gradually decreased despite continued NMDA superfusion. The magnitude of peak or plateau dilation was not dose dependent. In some experiments, an initial vasoconstriction was superimposed on the dilation (Fig. 1A, arrow). When NMDA superfusion was discontinued, vessel diameter decreased below baseline and remained constricted for 30–45 min (7% decrease compared with baseline, P < 0.05). Prior superfusion with MK-801 (10 μM, n = 5 mice) abolished the arteriolar dilation to NMDA (4 ± 8%).

Electrophysiological recordings showed that NMDA superfusion produced a large negative DC shift (19 ± 2 mV) at 50 or 100 μM but not at lower concentrations (n = 5, Fig. 2B). ECoG variably suppressed during the DC shift. A CSD was detected at the distant recording site with a latency of ~2 min, suggesting that the depolarization spread from the cranial window (Fig. 2B). After a prolonged aCSF washing, the DC shift could again be elicited at the same NMDA concentration (not shown). At the end of each experiment, KCl (100 mM) was superfused to evoke a CSD in the superfusion window that spread to the distant recording site identical to that observed after NMDA superfusion (Fig. 2C).

Fig. 1. N-methyl-D-aspartate (NMDA) superfusion causes an abrupt vasodilation in pial arterioles. A: NMDA-induced vessel diameter changes in mouse pial arteriole. NMDA (1–100 μM) was superfused in a closed cranial window. An abrupt, large, and transient dilation was observed at 50 μM. This precipitous increase in arteriolar caliber was superimposed on a more persistent dilation throughout superfusion that tended to diminish over time despite increasing NMDA concentration. After NMDA superfusion was discontinued, vessel diameter decreased below baseline and remained constricted for 30–45 min (not shown). In some experiments, a brief vasoconstriction was superimposed on early phase of dilation (arrow). aCSF, artificial cerebrospinal fluid. B: maximum vessel diameter (%change from baseline) in response to each NMDA concentration in individual experiments (n = 10 mice). Shown superimposed is the average of all 10 experiments (±SD). Although the dilation to NMDA displayed an all-or-none relationship in individual experiments (circles with straight lines), when averaged, the data suggest an apparent concentration-dependent response (triangles with curved line). Statistical analysis showed that although dilation to 50, 100, and 200 μM NMDA was significantly >1 and 10 μM, these higher concentrations did not statistically differ among themselves (one-way repeated-measures ANOVA, followed by Holm-Sidak’s multiple comparisons test).
Superfusion of L-NNA (1 mM, n = 7 mice) for 60 min did not change baseline diameter. L-NNA produced a small but significant inhibition in peak dilation (P < 0.05) and completely inhibited the plateau in response to NMDA (P < 0.01; Fig. 3). Furthermore, the brief initial vasoconstriction became more prominent in the presence of L-NNA (Fig. 3, A and B, arrow). L-NNA did not change the minimum NMDA concentration, causing CSD (10 μM in one mouse, 50 μM in two mice, and 100 μM in four mice; P > 0.05 vs. the threshold in control experiments).

**DISCUSSION**

Our results indicate that NMDA superfusion triggers CSD and abruptly dilates pial arterioles in mouse cortex. Electrophysiologically, NMDA superfusion at concentrations of 50–
100 μM evoked a prolonged cortical depolarization accompanied by a wave of negative DC shift that spread to other areas of the cortex with a speed, amplitude, and duration similar to CSD (Fig. 2). Below these concentrations, changes in vessel diameter were not observed. Increasing concentrations did not further dilate the vessels; therefore, arteriolar response to NMDA exhibited a threshold-maximum effect rather than concentration dependency (Fig. 1).

NMDA receptor activation is a potent depolarizing stimulus for neurons and is known to facilitate the induction and spread of CSD (32). NMDA can cause CSD when directly applied to the hippocampus (C. Ayata, unpublished observations), cerebral cortex, or cerebellum (29, 38). CSD was a major contributor to NMDA-induced vasomotor responses in our experiments, because 1) NMDA-induced vascular changes were abrupt, and the peak dilation was transient; 2) there was often a superimposed early and brief decrease in vessel caliber, reminiscent of the hypoperfusion observed in mouse and rat cortex during KCl-induced CSDs (3, 14); 3) this vasoconstriction was augmented after L-NNA superfusion as previously reported for CSD-induced CBF changes in mice and other species (3, 8, 9, 13, 14); and 4) on washout of NMDA, a delayed and prolonged vasoconstriction was observed, possibly reflecting post-CSD oligemia.

However, there were also notable differences between NMDA-induced dilation and the previously described hemodynamic responses to CSD in mice (3). For example, the early brief vasoconstriction during NMDA superfusion was much smaller in amplitude and duration compared with the initial hypoperfusion during KCl-induced CSD in mice (3). Furthermore, in contrast to CSD-induced dilation that lasts <1 min in mice (3) and only 2 min in rats and cats (7, 40, 45, 47), vessels remained dilated throughout NMDA superfusion for >10 min. Therefore, although CSD caused the large abrupt vasodilation when the threshold concentration of NMDA was reached, it was not the sole mediator of vasodilation to NMDA. Rather, CSD-induced dilation appeared to be superimposed on a longer-lasting vasodilation response (Fig. 1A). This persistent vasodilation was probably mediated by NO release in response to NMDA receptor activation, lasting longer than the CSD (11, 16, 19) because L-NNA completely abolished the persistent dilation while causing only a small reduction in the magnitude of initial peak dilation (Fig. 3B). More work is needed to determine the contribution of other potential mechanisms of NMDA-induced vasodilation, including ATP- or calcium-sensitive potassium channels, adenosine, carbon monoxide, and P-450 epoxygenase (1, 4, 25, 39, 43).

Although the link between NMDA-induced vasodilation and NO is firmly established (6, 11, 15–18, 36), CSD may have contributed to the arteriolar response to NMDA in other species as well. In one study in the rat, the dilation provoked by relatively large NMDA concentrations (100 μM) was reportedly precipitous and large and did not sustain during the superfusion period (18). The response was blocked by MK-801, but MK-801 blocks both CSD and NMDA receptor-coupled NO increases. Furthermore, a relatively steep concentration-response relationship for NMDA-induced dilation was reported (11, 15, 16, 18). We believe CSD may have caused an apparent, but not real, concentration-dependent vasodilation to NMDA. We demonstrated this by measuring the maximum vessel diameter in response to each NMDA concentration in individual experiments and then by averaging them to generate a concentration-response curve. Although individual experiments clearly showed an all-or-none response to NMDA (Fig. 1, A and B), when maximum increases in vessel diameter were averaged for each NMDA concentration tested, there was an apparent concentration dependency (Fig. 1C, triangles). However, statistical analysis showed that the dilation response did not differ between the two subthreshold concentrations of 1 and 10 μM or among the suprathreshold concentrations of 50, 100, and 200 μM NMDA. Therefore, the precipitous, large, and transient dilation at threshold NMDA concentrations confounded the detection of a possible concentration-dependent dilation, perhaps mediated by NO.

There are factors modulating the sensitivity of cortex to CSD, which may have contributed to the dose dependency of NMDA response in previous studies. First, the choice of species influences the susceptibility to CSD. Although, to the best of our knowledge, there has been no systematic comparison of species for susceptibility to CSD, it is generally accepted that gyrencephalic species are less susceptible to CSD. Therefore, rats and mice may be more prone to develop CSD during NMDA superfusion compared with cats and piglets. Second, the choice of anesthetic also influences the susceptibility to CSD. Inhalational anesthetics and ketamine suppress CSD, whereas α-chloralose and barbiturates do not. Therefore, α-chloralose, an anesthetic favored in cerebrovascular studies, may render the cortex more prone to CSD during NMDA superfusion (5, 23, 26, 27, 33, 34, 44, 46). Third, the degree of cortical maturation influences CSD susceptibility (20, 21, 28, 41, 42, 48), and many studies of NMDA-induced vasodilation have traditionally been performed in immature cortex, such as in piglets.

In conclusion, our data demonstrate that NMDA superfusion causes CSD and attendant vasomotor changes that confound NMDA receptor-induced NO-dependent dilation. Previous studies investigating the vasomotor effects of topical superfusion of glutamate receptor agonists in other species have not controlled for the occurrence of CSD; therefore, data must be interpreted with caution. Future studies using this technique must incorporate electrophysiological monitoring to detect CSD.

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