Responses of Cerebral Arterioles to Kainate

Frank M. Faraci, PhD; Keith R. Breese; D.D. Heistad, MD

**Background and Purpose** Neurons release nitric oxide in response to glutamate. Glutamate acts via activation of different receptor subtypes, including N-methyl-D-aspartate and kainate receptors. This study examined the hypothesis that kainate produces dilatation of cerebral arterioles that is dependent on the formation of nitric oxide.

**Methods** Diameters of cerebral arterioles were measured by means of a closed cranial window in anesthetized rabbits. Kainate, quisqualate, acetylcholine, and N^-nitro-L-arginine (L-NNA, an inhibitor of nitric oxide synthase) were applied locally in the cranial window. We also examined whether kainate elicited direct vascular effects by the use of isolated cerebral arteries in vitro.

**Results** Under control conditions, topical kainate (100 μmol/L) increased the diameter of arterioles by 20±5% (mean±SE), 27±7%, and 31±7% at 3, 5, and 9 minutes of application, respectively. After topical application of L-NNA (300 μmol/L), kainate dilated cerebral arterioles by 8±4%, 9±5%, and 8±6% at 3, 5, and 9 minutes, respectively (P<.05 versus the control response). In contrast, quisqualate (100 and 300 μmol/L) did not alter the diameter of cerebral arterioles. In rings of the middle cerebral artery studied in vitro, kainate had no effect on vascular tone, which suggests that cerebral vessels lack receptors for kainate. Thus, cerebral vasodilator effects of kainate do not appear to be due to the direct effect of the excitatory amino acid on cerebral vessels.

**Conclusions** These findings suggest that kainate produces dilatation of cerebral arterioles in vivo that is mediated by release of nitric oxide from an extracellular source. (Stroke. 1994;25:2080-2084.)

**Key Words** cerebral arteries • glutamate • nitric oxide • rabbits

---

The excitatory amino acid glutamate affects neurons by activation of several receptor subtypes. These subtypes, which have been defined based on specificity of agonist binding, include N-methyl-D-aspartate (NMDA), kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA, formerly quisqualate). Studies using whole brain, brain slices, cultured neurons, and synaptosomal preparations suggest that neurons produce nitric oxide and release nitric oxide extracellularly in response to NMDA. Because nitric oxide is a potent vasodilator and it is highly diffusible, it seems likely that release of nitric oxide by neurons may affect the local cerebral microcirculation.

Topical application of NMDA in vivo produces dilatation of cerebral arterioles that is dependent on neuronal activation and production of nitric oxide. It is not known if activation of non-NMDA receptors also produces dilatation of cerebral arterioles. Kainate as well as NMDA has been reported to increase the activity of nitric oxide synthase and to produce nitric oxide–mediated increases in cyclic guanosine monophosphate (cGMP) in neurons. Thus, the first goal of this study was to test the hypothesis that kainate dilates cerebral arterioles and that vasodilatation in response to kainate is dependent on production of nitric oxide. Some studies suggest that activation of quisqualate receptors also produces nitric oxide–mediated increases in brain cGMP levels. Thus, our second goal was to determine if quisqualate produces dilatation of cerebral arterioles.

**Materials and Methods**

**Animal Preparation** Experiments were performed on 24 New Zealand White rabbits (weight, 2.5 to 3.5 kg) that were anesthetized with pentobarbital sodium (35 to 40 mg•kg^-1•IV). Additional pentobarbital was given at approximately 10 mg•kg^-1•h^-1. The trachea was cannulated, and the animals were ventilated mechanically with air and supplemental oxygen. A catheter was placed into a femoral artery for measurement of systemic pressure and to sample arterial blood. A femoral vein was cannulated for infusion of drugs.

Rabbits were placed in a head-holder, and a closed cranial window was placed over the parietal cortex as described in detail. The cranial window was filled with artificial cerebrospinal fluid (CSF) warmed to 37°C. Diameters of cerebral arterioles were measured with a microscope equipped with a television camera coupled to a video monitor. Images were recorded on videotape, and vessel diameters were measured later with an image analyzer. We studied one vessel per animal. Flushing the window with fresh CSF maintained at 37°C did not alter the baseline diameter of cerebral arterioles. In all animals, responses of cerebral arterioles to acetylcholine (1 and 10 μmol/L) were measured initially to establish reactivity of the vessels.

**Experimental Protocol** Three groups of animals were studied. In group 1 (n=8) we determined if kainate produced cerebral vasodilatation and whether vasodilatation in response to kainate was inhibited by N^-nitro-L-arginine (L-NNA), an inhibitor of nitric oxide synthase. Arteriolar diameter was measured under control conditions and 1, 3, 5, 7, and 9 minutes after filling the window with artificial CSF containing kainate (100 μmol/L). After 9 minutes, a higher concentration of kainate (300 μmol/L) was applied, and vessel diameter was measured at the same time.
Arterial blood gases and pH were similar in the various groups of animals (arterial \( {\text{P}CO}_2, 33 \pm 1 \text{ mm Hg} \); arterial pH, 7.46±0.01; and \( {\text{P}O}_2, 122 \pm 3 \text{ mm Hg} \)). Arterial blood pressure was 81±1 mm Hg. Arterial blood gases and pH were similar in all groups and averaged 91±5 μm (range, 46 to 127 μm). Topical application of kainate (100 and 300 μmol/L) produced dilatation of cerebral arterioles. For example, at 3 and 7 minutes, arteriolar diameter increased by 26±10% and 31±13% in response to 100 μmol/L kainate and 44±14% and 46±13% in response to 300 μmol/L kainate, respectively. After the 90-minute recovery period, during which time the arteriolar diameter returned to baseline levels, the response to kainate was reproducible. At 3 and 7 minutes of the second application, vessel diameter increased by 22±15% and 31±14% in response to 100 μmol/L kainate and 41±13% and 47±16% in response to 300 μmol/L kainate, respectively.

Dilatation of cerebral arterioles in response to both concentrations of kainate was inhibited significantly in the presence of L-NNA (Fig 1). The increase in diameter at 9 minutes in response to the low and high concentrations of kainate was inhibited by approximately 75% and 43%, respectively (\( P<.05 \)).

Pial arterioles ranging in diameter from 46 to 127 μm were used in this study. The magnitude of dilatation of cerebral vessels in response to kainate was dependent on vessel size. There was a negative correlation between vessel diameter under control conditions and the change in diameter in response to kainate (\( R^2=.68, P<.05 \)). Thus, although all vessels dilated, small vessels tended to dilate more than larger vessels in response to kainate.

In contrast to kainate, quisqualate had no effect on the diameter of cerebral arterioles. Vessel diameter increased by 2±1% and 3±3% in response to 100 and 300 μmol/L quisqualate, respectively. The same arterioles dilated by 22±7% and 45±6% in response to 1 and 10 μmol/L acetylcholine, which indicates that the vessels were responsive to vasoactive stimuli.

**Discussion**

The major new finding in the present study is that kainate produces dilatation of cerebral arterioles in vivo.
that is dependent on production of nitric oxide. Kainate does not appear to have direct effects on isolated, endothelium-intact, cerebral vessels. In contrast to kainate, quisqualate did not produce dilatation of cerebral arterioles in vivo. These findings suggest that activation of at least one type of non-NMDA receptor, the kainate receptor, produces cerebral vasodilatation that is mediated by nitric oxide.

Effects of Excitatory Amino Acids

Glutamate is the major excitatory neurotransmitter in the mammalian brain. Glutamate mediates its effects through activation of distinct receptor subtypes including NMDA, kainate, and AMPA (formerly quisqualate). Glutamate and agonists for these various glutamate receptors increase neuronal activity and the rate of oxygen consumption in brain.24

In studies using neuronal cell cultures, NMDA and kainate increase production of L-citrulline (which reflects activity of nitric oxide synthase)10 and cause nitric oxide–mediated increases in cGMP.5,5,6,10,18,25 Isolated neurons and synaptosomal preparations release nitric oxide extracellularly in response to activation of receptors for NMDA.3,11 Similar results have been obtained using brain slice preparations6,17,20 and in brain tissue in vivo.4,7,8,19 These findings suggest that activation of NMDA and kainate receptors stimulates neuronal production and release of nitric oxide.

In contrast to kainate and NMDA, the role of AMPA/quisqualate receptors in stimulating nitric oxide synthase in brain tissue is less clear. AMPA has been reported to have no effect18,20 or to produce increases5,8-20 in cGMP levels. Quisqualate also has been reported to have no effect5,18 or to produce an increase5,8,9,15 in levels of cGMP. Quisqualate does not increase citrulline production in cultured neurons.10 When increases in cGMP in response to AMPA or quisqualate have been described, however, they appear to be mediated by nitric oxide.4,9,20 In the present study quisqualate did not alter the diameter of cerebral arterioles, suggesting that receptors for quisqualate were not activated during topical application or that receptor activation did not alter local metabolism.

Local application of the excitatory amino acids glutamate and NMDA produces dilatation of cerebral arterioles in vivo,14,16,27 and this response to NMDA is inhibited by L-NNA.15 These findings suggest that cerebral vasodilatation in response to NMDA is dependent on production of nitric oxide.

In the present study kainate produced marked dilatation of cerebral arterioles that was inhibited by L-NNA, suggesting that vasodilatation in response to kainate is mediated by nitric oxide. It is unlikely that the effect of L-NNA on responses to kainate is through a nonspecific mechanism. L-NNA inhibits activity of nitric oxide synthase in brain.22,28-30 We have shown that L-NNA does not inhibit dilatation of cerebral arteries in response to sodium nitroprusside,15,31,32 and we have observed that inhibitory effects of L-NNA on responses to acetylcholine and NMDA are reversed by excess L-arginine in this model.15,33

We considered the possibility that kainate could have direct effects on cerebral blood vessels. In isolated cerebral arteries, however, kainate had no effect on vascular tone, which suggests that structures in the vessel wall were not activated directly. These findings are similar to our previous study in which NMDA had no direct effect on tone of isolated cerebral arteries.15 Vessels in the present study exhibited marked relaxation in response to acetylcholine and nitroprusside, which demonstrates that the vessels were capable of responding to vasoactive stimuli.

Our findings using the middle cerebral artery suggest that receptors for kainate are not present in large cerebral blood vessels. We considered the possibility that receptors for kainate might be present only in more distal blood vessels. This possibility seems unlikely, however, because glutamate does not alter the tone of isolated pial arteries and parenchymal arterioles.24,25

Possible Sources of Nitric Oxide

The source of nitric oxide that mediates vasodilatation in response to kainate is not known. Neurons are the most likely source because kainate stimulates production of nitric oxide by neurons.5,6,10,18,25 Studies using NADPH-diaphorase as a marker for nitric oxide synthase and immunocytochemistry suggest that some nitric oxide synthase–containing neurons are closely associated with microvessels in the brain parenchyma.36-38 Glial cells are also a potential source of nitric oxide because glia contain receptors for kainate39 (but not NMDA) and can produce nitric oxide in response to some stimuli.40 However, it appears that astrocytes do not produce nitric oxide in response to NMDA or kainate.6

Endogenous release of glutamate can potentially activate several receptor subtypes.1 Activation of NMDA and kainate receptors produces local nitric oxide formation6,9,10,17-20 and dilatation of cerebral arteries12 (present study). With respect to nitric oxide/cGMP production, there is relatively little information on possible interaction of NMDA and kainate receptors or on whether both receptor types are activated simultaneously. Recent evidence suggests that in adult cerebellum, increases in cGMP in response to glutamate are mediated by both NMDA and non-NMDA receptors.8

In summary, kainate produces nitric oxide–mediated dilatation of cerebral arterioles in vivo. Kainate receptors are distributed in a number of brain regions,
including the neocortex. These findings support the concept that release of glutamate and activation of kainate receptors may cause neuronal release of nitric oxide and increases in local cerebral blood flow. Thus, nitric oxide may function as a "coupler" of blood flow and neuronal activity in brain.

Acknowledgments

This study was supported by National Institutes of Health grants HL-38901 and NS-24621 and a Grant-In-Aid from the American Heart Association (92015170). Dr Faraci is an Established Investigator of the American Heart Association. We thank Kristen Orel for excellent technical assistance and Dr J.E. Brian, Jr, for critical evaluation of the manuscript.

References

Responses of cerebral arterioles to kainate.
F M Faraci, K R Breese and D D Heistad

Stroke. 1994;25:2080-2083
doi: 10.1161/01.STR.25.10.2080

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/25/10/2080

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/