Anesthesiology
82:205–213, 1995

Ketamine Inhibits Glutamate-, N-Methyl-D-Aspartate-, and Quisqualate-stimulated cGMP Production in Cultured Cerebral Neurons

Jerry M. Gonzales, M.D.,* Alex L. Loeb, Ph.D.,† Peter S. Reichard, M.D.,‡ Steven Irvine, B.S.§

Background: **Glutamatergic signaling has been linked to the recently discovered neurotransmitter/neuromodulator nitric oxide (NO), and several classes of anesthetics block some step in glutamatergic signaling. This study was designed to determine whether or not ketamine would prevent NO-dependent cGMP production stimulated by glutamate (GLU) and the GLU analogs NMDA, quisqualate (QUIS), and kainate (KAIN).**

Methods: **Primary cultures of cortical neurons and glia (prepared from 16-day gestational rat fetuses) were used after 12–16 days in culture. Reactions were carried out in magnesium-free buffer containing 100 μM 3-isobutyl-1-methylxanthine, and cGMP content of cultures was used as a bioassay of NO production.**

Results: **Cyclic GMP production stimulated by sodium nitroprusside (100 μM) occurred predominately in neurons and not in glia. Neurons were spontaneously active in these cultures; basal cGMP production was decreased by 50% in the presence of 1 μM tetrodotoxin (TTX). Glutamate (100 μM), NMDA (100 μM), QUIS (300 μM), and KAIN (100 μM) each increased cGMP content of neuronal cultures. L-NMMA (100 μM), a NO synthase inhibitor, prevented the stimulation of cGMP production by GLU or its analogs. Pretreatment with MK-801 (1 μM) or ketamine (10–100 μM) inhibited GLU-, NMDA-, and QUIS-stimulated cGMP production. Quisqualate-stimulated responses were the most sensitive to inhibition by ketamine and NMDA-stimulated responses were the least sensitive to inhibition. MK-801 and ketamine did not significantly inhibit KAIN-stimulated cGMP production. CNQX (10 μm) blocked KAIN-stimulated cGMP production only.**

Conclusions: **The authors' data demonstrate that ketamine inhibited NO synthesis stimulated by NMDA- and non-NMDA-receptor specific analogs. Our findings indicate that blockade of QUIS- as well as NMDA-subtypes of GLU- receptor may be important in the development of ketamine-induced anesthesia. (Key words: Agonist: NMDA. Amino acid, excitatory: kainate; quisqualate. Anesthetics, intravenous: ketamine. Antagonists: MK 801. Cultured neurons. Neurotransmitters: glutamate.)**

GLUTAMATE (GLU) is the major excitatory neurotransmitter in the vertebrate central nervous system. L-glutamate activates several subtypes of receptors leading to an increase in intracellular calcium ion concentration ($[Ca^{2+}]_i$). This increase in $[Ca^{2+}]_i$ occurs by at least three mechanisms. First, Ca^{2+} enters the cells from the extracellular space through ion channels that are integral to GLU receptors that are activated selectively by the GLU analog N-methyl-D-aspartate (NMDA).[1–3] Second, Ca^{2+} enters the cells through voltage-dependent calcium channels that are opened secondary to an influx of sodium ions through ligand gated channels that are activated selectively by the GLU analogs kainate (KAIN), quisqualate (QUIS), α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), or NMDA.[1,3,4] Third, metabotropic GLU receptors that are selectively activated by QUIS or (±)-1-aminocyclopentane-*trans*-1,3-dicarboxilic acid (ACPD) stimulate phospholipase C through a G-protein to produce inositol 1,4,5-trisphosphate (IP_3) and release of Ca^{2+} from intracellular stores.[5,6] Activation of GLU receptors and subsequent cellular responses are associated with the production of excitatory postsynaptic potentials (EPSPs) and long-term modulation of neuronal behavior, including changes in neuronal excitability, nerve cell architecture, and gene expression.[7–13]

Although many responses to GLU in the central nervous system can be directly attributed to these changes in membrane polarization and $[Ca^{2+}]_i$, it has been demonstrated recently that activation of GLU receptors in the central nervous system results in the synthesis of nitric oxide (NO). This response was first observed by

* Assistant Professor of Anesthesia.

† Assistant Professor of Anesthesia and Pharmacology.

‡ Resident in Anesthesia.

§ Research Assistant.

Received from the Department of Anesthesia, University of Pennsylvania, Philadelphia, Pennsylvania. Submitted for publication November 10, 1993. Accepted for publication September 2, 1994. Supported in part by awards from the Foundation for Anesthesia Education and Research (Starter Grant) with a grant from Stuart Pharmaceuticals (JMG), a Young Investigator Award from the Foundation for Anesthesia Education and Research (JMG), and USPHS Grant R01-GM43969 (ALL).

Address reprint requests to Dr. Gonzales: Department of Anesthesia, University of Pennsylvania Medical Center, 3400 Spruce Street, Philadelphia, Pennsylvania 19104-4283.

Garthwaite *et al.*[14,15] Nitric oxide produced after GLU stimulation acts as a paracrine/autocrine substance where it stimulates cyclic GMP (cGMP) accumulation,[16] and may have other actions, as well.[17,18]

There is increasing evidence that blocking the function of the GLU-Ca^{2+}-NO-cGMP pathways may be associated with the development of anesthesia. Riluzole (53274 RP), an inhibitor of GLU neurotransmission with both pre- and postsynaptic effects, has anesthetic action.[19] Noncompetitive antagonists at the NMDA receptor, including (+)-5-methyl-10,11-dihydro-5H-dibeno(*a,d*)cyclohepten-5,10-amine maleate (MK-801), (+)-N-allyl-normetazocine [(+)-SKF 10,047], phencyclidine, dextrorphan, and ketamine, increase the potency of a variety of general anesthetics and have analgesic and anesthetic properties.[20,21] The competitive antagonist at the NMDA receptor, CGS-19755, also has analgesic and anesthetic effects.[22,23] A competitive antagonist at AMPA receptors, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX), reduces the MAC for halothane in rats.[24] Because GLU has been associated with both direct and indirect postsynaptic responses (*e.g.,* ion flux, EPSP, and NO release), the possibility has been raised that the anesthetic properties of at least some drugs may be attributable to inhibition of NO synthesis/release. In support of this possibility, it has been shown that antagonists of NMDA receptors inhibit GLU-stimulated increases in NO synthesis,[25,26] and blocking the synthesis of NO increases the potency of the volatile anesthetic halothane.[27] Also, NO synthase inhibition has been shown to be antinociceptive,[28–33] although other work indicates that NO may mediate some forms of analgesia.[31,34–37] In addition, the administration of GLU or its analogs can reverse ketamine anesthesia.[38,39]

In vivo and *in vitro* studies from several laboratories indicate that various general anesthetics block the GLU-Ca^{2+}-NO-cGMP pathway at several sites. Martin *et al.*[40] demonstrated that enflurane inhibited GLU-stimulated MK-801 binding to NMDA receptors in membranes prepared from rat cerebral cortex; because MK-801 binds to the open channel, this indicated that enflurane decreased opening of the anion channel. Halothane and isoflurane reduce increases in $[Ca^{2+}]_i$ in response to GLU and NMDA in neurons cultured from rat hippocampus.[41] In cortical wedges from mice, electrophysiologic responses of neurons to AMPA and NMDA were reduced by chloroform, halothane, thiopental, diethyl ether, and isoflurane.[42] Enflurane also decreased cGMP content of the cerebellum of mice.[43]

Ketamine is the single anesthetic drug used in clinical practice that is believed to act primarily by blockade of GLU receptors.[44–47] Whether ketamine works exclusively by blockade of NMDA receptors, or whether it blocks the function of other subtypes of GLU receptor, is not known with certainty. In studies of cortical wedges from mice, ketamine blocked responses to NMDA but not to AMPA, but agonists at other subtypes of GLU receptors were not tested.[42] Studies of isolated spinal cord from one of the most primitive vertebrates, the lamprey, showed that ketamine specifically blocked NMDA responses and not responses to KAIN or QUIS.[48] Studies in another model system, locust muscle, indicate that ketamine can block a QUIS receptor.[49] The current study was designed to examine how ketamine affects the function of the various subtypes of GLU receptors. Our main experimental goal was to determine whether or not ketamine would prevent NO-dependent cGMP production stimulated by the GLU analogs QUIS and KAIN, as well as the responses to GLU and NMDA in cortical neurons cultured from the rat.

Materials and Methods

Materials

Chemicals used in the current study were obtained from the following sources: GLU, KAIN, NMDA, tetrodotoxin (TTX), cytosine-β-D-arabinofuranoside (Ara-C), 3-isobutyl-1-methylxanthine (IBMX), Hank's balanced salt solution (HBSS), Dulbecco's modified Eagle's medium with 25 mM HEPES (DMEM), and sodium nitroprusside (SNP) from Sigma (St. Louis, MO); Ham's F-12 and penicillin/streptomycin from JRH Biosciences (Lenexa, KS); (+)-quisqualate and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) from Research Biochemicals, Inc. (Natick, MA); ketamine HCl from Parke-Davis (Morris Plains, NJ); and N^G-monomethyl-L-arginine (L-NMMA) from Calbiochem (San Diego, CA). Fetal calf serum was obtained from HyClone (Logan, UT).

Cell Culture

Cultures of cerebral cortical neurons were prepared essentially as described by Dichter[50] and Atkinson and Minneman.[51] Whole cerebral hemispheres were collected in HBSS from fetuses of 16–17-day gestational Sprague-Dawley rats. They were washed twice in phosphate buffered saline, digested using trypsin (5 $mg \cdot ml^{-1}$) in DMEM, washed three times in HBSS, and

triturated with a "fire polished" pasteur pipette into DMEM containing 25 mM HEPES supplemented with 10% Ham's F12 media, 10% heat inactivated fetal calf serum, 100 $\mu g \cdot ml^{-1}$ streptomycin, and 100 $U \cdot ml^{-1}$ penicillin. Cells were plated at a density of 4×10^5 cells per 1.2-cm-diameter tissue culture well that had been pretreated with 10 $\mu g \cdot ml^{-1}$ poly-D-lysine. The cultures were grown in a 37°C humidified incubator containing 5% CO_2 in air, and were fed approximately every 2–3 days with fresh medium. On day 5, cells were treated with 10 $\mu g \cdot ml^{-1}$ Ara-C for 24 h to stop nonneuronal cell proliferation. Subsequent feeding was with the above-described media without Ham's F-12, to eliminate GLU from the feeding. Experiments were carried out after 12–16 days in culture. Preliminary experiments indicated that the capacity of the cultures to respond to GLU with an increase in cGMP was well developed by this time.

Glial cell cultures were prepared as described by Atkinson and Minneman[51] by trypsinizing cells at day 5 in culture and replating at a density of 8×10^4 cells per 1.2-cm-diameter well. The cultures were also fed every 2–3 days, but were not treated with Ara-C. This procedure resulted in preparations that were essentially devoid of neuronal cells. Similar to the neurons, experiments were performed after 12–16 total days in culture.

Measurement of cGMP Production

Experiments were performed at 37°C. Cells were washed three times in buffer (in mM: NaCl, 137; KCl, 0.4; $CaCl_2$, 1.3; KH_2PO_4, 0.44; Na_2HPO_4, 0.7; glucose, 6; HEPES, 20; *p*H 7.4) with a final addition of buffer as the reaction medium. Tetrodotoxin (1 μM) was added at this time in most experiments to block spontaneous action potentials and transmitter release. IBMX (100 μM), a phosphodiesterase inhibitor, various GLU antagonists, or vehicle were added at the beginning of the timed 10-min incubation period. After the incubation period, agonist or vehicle was added for 90 s, except as noted for the time course experiments. At this time, the supernatant was removed and 0.1 M HCl was added to stop the reaction and to extract cyclic nucleotides. Cyclic GMP was measured by radioimmunoassay.[52] Protein content was determined using the coomassie blue method.[53] Results were expressed as pmol $cGMP \cdot mg^{-1}$ protein.

Data Analysis

Data were expressed as mean ± SEM and differences between treatment groups were determined using Student's *t* test or ANOVA and *post hoc* analysis with the method of Bonferroni, as indicated in the figure legends. Differences were considered significant when $P < 0.05$.

Results

The primary cultures of mixed cerebral cortical cells used in these experiments consisted of a bed of glial cells on which the neurons grew. The glial cultures were similar, but without the overlying neuronal cells. To determine whether pure cultures of glia, as well as the mixed cultures containing mostly neurons, could respond to NO with an increase in cGMP, we determined the time course of cGMP accumulation on exposure to the NO donor sodium nitroprusside (SNP), as shown in figure 1. Although both types of cultures did respond to SNP with an increase in cGMP, the response in the neuronal cultures was of much greater magnitude, indicating that the preponderance of the cGMP response occurred in neurons. Figure 1 demonstrates that the increase in cGMP in response to 100 μM SNP was maximal by 90 s. Therefore, all subsequent experiments were terminated 90 s after the addition of agonist.

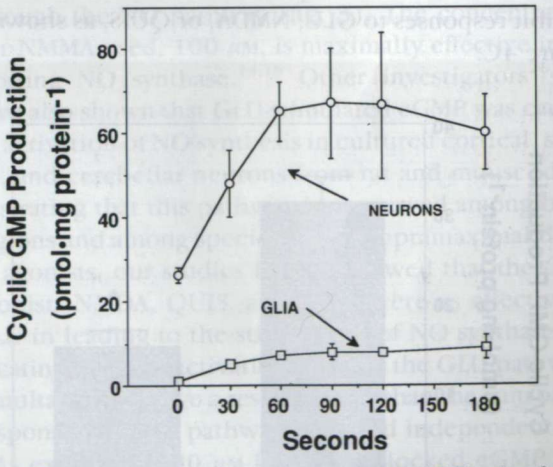

Fig. 1. Time course of sodium nitroprusside (SNP)-stimulated cGMP production in cultured neurons and glia from fetal rat brain. Primary cultures were prepared from the cerebral hemispheres of 16-day gestational Sprague-Dawley rats and experiments were carried out on day 12–16 in cultures. Cyclic GMP production was measured in mixed cultures of neurons and glia or glia alone at various time points after the addition of 100 μM SNP. IBMX (100 μM) was included in all reactions. Data represent the mean ± SEM for n = 2 experiments, each performed in triplicate.

Neuronal cells in culture form multiple complex intercellular contacts and are spontaneously active.[50] To determine whether spontaneous neuronal activity, including action potential-stimulated neurotransmitter release, was responsible for a portion of the basal cGMP content observed, the cells were treated with 1 μM TTX, a sodium channel blocker. As shown in figure 2, TTX-treated neurons had significantly reduced basal cGMP content. To prevent spontaneous generation of action potentials and, thus, transmitter release, TTX was included in all subsequent experiments.

Treatment of neuronal cultures with agonists at GLU receptors, including GLU (100 μM), NMDA (100 μM), QUIS (300 μM), or KAIN (100 μM), for 90 s each produced significant increases in cGMP content, as shown in figure 3. Pretreatment of cultures with the NO synthase inhibitor L-NMMA (100 μM) decreased cGMP production to below basal levels and prevented any increase in the presence of GLU or its analogs. These data demonstrated that GLU and its analogs stimulated cGMP production *via* the NO pathway and that any increase in cGMP observed in response to these agonists was subsequent to NO synthesis and release.

Pretreatment of cultures with ketamine (100 μM) or MK-801 (1 μM) blocked GLU-, NMDA-, and QUIS-stimulated cGMP production, as shown in figure 4A and B. The non-NMDA GLU-channel antagonist, CNQX (10 μM), blocked cGMP responses to KAIN, but did not inhibit responses to GLU, NMDA, or QUIS, as shown in figure 4C.

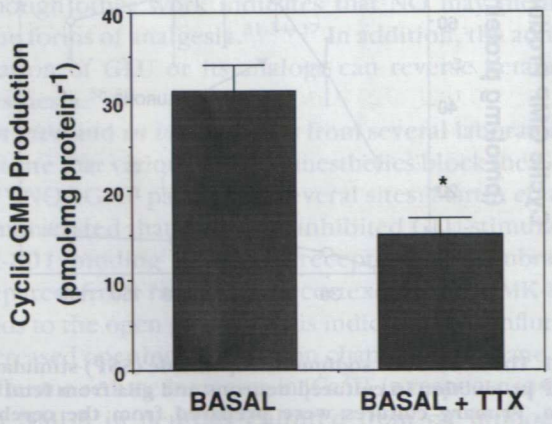

Fig. 2. Role of spontaneous neuronal activity on basal cGMP production in cultured neurons. Cyclic GMP production was determined in cultures of neurons after a 10-min exposure to 1 μM TTX or vehicle (control) in the absence of exogenous stimulating agents (basal). Data represent the mean ± SEM for n = 3 experiments, each performed in triplicate. *$P < 0.05$ control *versus* the presence of TTX (Student's *t* test).

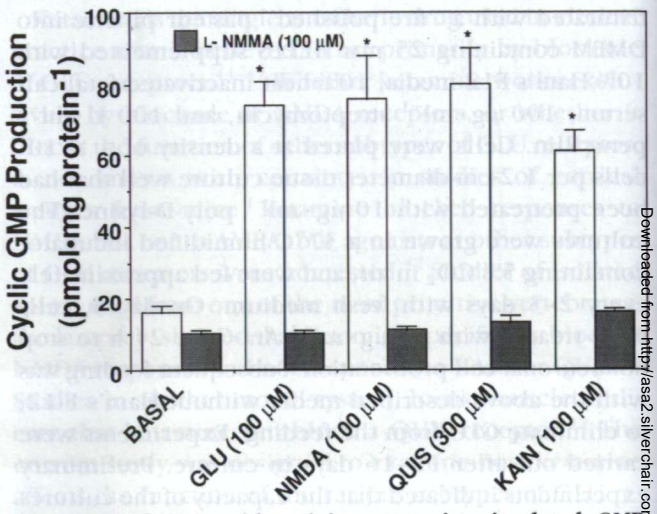

Fig. 3. Role of nitric oxide activity on agonist-stimulated cGMP production in cultured neurons. Cyclic GMP accumulation was determined in neuronal cultures stimulated by GLU and the GLU analogs NMDA, KAIN, and QUIS in the presence and absence of the NO synthase inhibitor L-NMMA (100 μM). Data represent the mean ± SEM for n = 3 experiments, each performed in triplicate. *$P < 0.05$ each *versus* basal, by ANOVA and the Bonferroni *post hoc* correction.

Additional experiments were carried out with cultures pretreated with lower concentrations of ketamine to determine the dose-response relationship, if any. As shown in figure 5, ketamine at concentrations as low as 10 μM blocked the response to QUIS. The response to NMDA, however, were not significantly affected by ketamine at concentrations less than 100 μM. The response to GLU was inhibited by ketamine with a sensitivity between that of QUIS and NMDA.

Discussion

Initial studies indicating that activation of GLU receptors in the central nervous system resulted in NO synthesis and increases in cGMP have implicated the NMDA receptor as the responsible subtype.[14,15] Subsequently, other GLU analogs have been shown to stimulate NO synthesis through activation of specific receptor subtypes.[25,26,54,55] Our results demonstrate that ketamine, and its sister compound MK-801, both drugs primarily believed to block the NMDA receptor channel, can also inhibit QUIS receptor mediated NO synthesis. However, ketamine and MK-801 had no significant effect on KAIN-stimulated NO synthesis.

We monitored changes in NO synthesis by measuring changes in cGMP content in cultures of cells from fetal

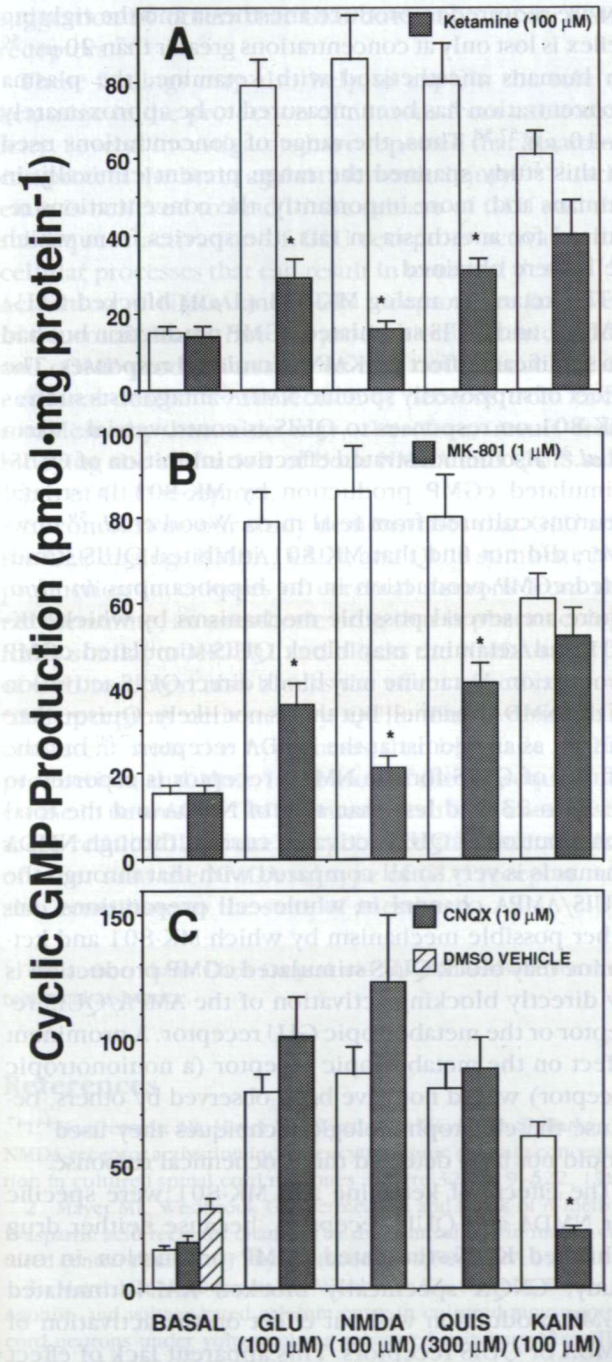

Fig. 4. Effect of GLU-receptor inhibitors on agonist-stimulated cGMP production in cultured neurons. The effect of the GLU-receptor antagonists ketamine (*A*), MK-801 (*B*), and CNQX (*C*) on cGMP production was determined in neuronal cultures stimulated by GLU and the GLU analogs NMDA, KAIN, and QUIS. Data represent the mean ± SEM for n = 5–18 experiments, each performed in triplicate. *$P < 0.05$ antagonist *versus* control without antagonist for each agonist, by ANOVA and Bonferroni *post boc* correction.

rat brain after agonist stimulation. Although both neurons and glia can respond to NO with an increase in cGMP, we found that preparations of mixed cells from brain that contain neurons respond to SNP with a much greater increase in cGMP than cultures of nonneuronal cells (fig. 1). These findings indicated that the primary site of response to NO was in neurons and, more importantly, that the changes in cGMP production that we observed were from changes occurring in neurons, in which anesthetic drugs are believed to work.

The experiments with and without TTX (fig. 2) showed that neurons in these cultures were spontaneously active. These data indicated that there was, most likely, a basal release of endogenous neurotransmitter in these cultures that was caused by this spontaneous depolarization that stimulated NO synthesis. This phenomenon has been described previously in electrophysiologic terms in cultured neurons,[50] but we know of no other report indicating that a substantial portion of this basal generation of NO is caused by this mechanism. To minimize spontaneous activity and release of endogenous transmitter, subsequent experiments were all carried out in the presence of TTX. Thus, the cGMP measured is almost exclusively caused by neuronal stimulation by exogenous agonist and is not caused by intrinsic or spontaneous activity.

L-NMMA pretreatment clearly demonstrated that the observed stimulation of cGMP production was indeed through the NO pathway (fig. 3). The concentration of L-NMMA used, 100 μM, is maximally effective in inhibiting NO synthase.[14,16] Other investigators[26,54,55] have also shown that GLU-stimulated cGMP was caused by activation of NO synthesis in cultured cortical, striatal, and cerebellar neurons from rat and mouse, demonstrating that this pathway is conserved among brain regions and among species. Using supramaximal doses of agonists, our studies further showed that the GLU-agonists NMDA, QUIS, and KAIN were as effective as GLU in leading to the stimulation of NO synthase, indicating that full activation of all of the GLU pathways simultaneously give a response less than the sum of the responses of each pathway activated independently.

As expected, 100 μM ketamine blocked cGMP production stimulated by the GLU analog NMDA. Blockade of NMDA receptor activation by ketamine has been shown previously using other indicators of NMDA-receptor stimulation[44–47] and is the basis for the view that NMDA-receptor activation is specifically coupled to the stimulation of NO synthase. In addition to acting at the NMDA-receptor channel, our data showed that

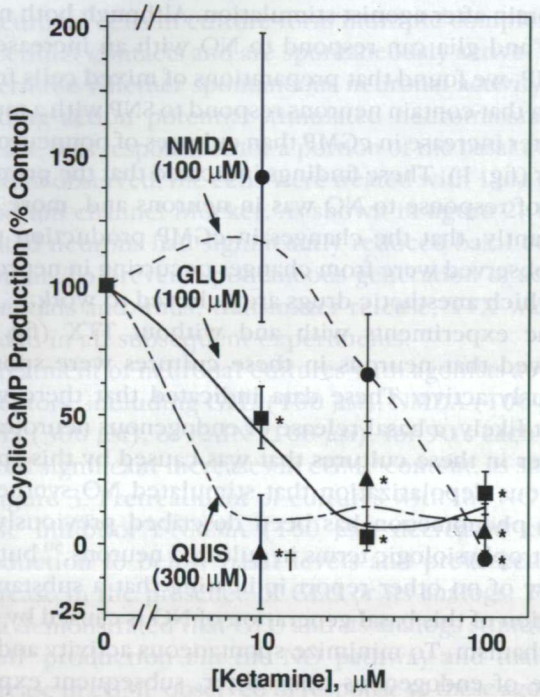

Fig. 5. Effect of ketamine on GLU-, NMDA-, and QUIS-stimulated cGMP production in cultured neurons. The effect of various concentrations of ketamine on cGMP production was determined in neuronal cultures stimulated by GLU and the GLU analogs NMDA and QUIS. Data represent the mean ± SEM for n = 5–27 experiments, each performed in triplicate. The results from individual experiments were normalized and the data expressed as the percent of the agonist-stimulated value in the absence of ketamine (100%) (control basal = 18 ± 2 pmol · mg protein^{-1}, GLU-stimulated control value = 96 ± 15 pmol · mg protein^{-1}, NMDA-stimulated control value = 127 ± 26 pmol · mg protein^{-1}, QUIS-stimulated control value = 90 ± 16 pmol · mg protein^{-1}). *$P < 0.05$ *versus* control without ketamine for that agonist, by ANOVA and Bonferroni *post hoc* correction. †$P < 0.05$ *versus* NMDA-stimulated value in presence of 10 μM ketamine.

100 μM ketamine blocked GLU- and QUIS-stimulated NO production. Quisqualate-stimulated NO production was more sensitive to ketamine blockade than NMDA-stimulated responses, as shown in figure 5. Ketamine at concentrations of 10, 30, and 100 μM prevented QUIS-stimulated cGMP production. This effect on QUIS-stimulated responses has not been reported previously.

The concentrations of ketamine used in this study are comparable with those measured in the plasma of rats and humans anesthetized by ketamine. The potency of ketamine is greater in humans compared with rats. In rats, plasma ketamine concentrations greater than 50 μM are required to produce anesthesia and the righting reflex is lost only at concentrations greater than 20 μM.[56] In humans anesthetized with ketamine, the plasma concentration has been measured to be approximately 5–10 μM.[57,58] Thus, the range of concentrations used in this study spanned the range present clinically in humans and, more importantly, the concentrations required for anesthesia in rats, the species from which cells were obtained.

The ketamine analog MK-801 (1 μM) blocked GLU-, NMDA-, and QUIS-stimulated cGMP production but had no significant effect on KAIN-stimulated responses. The effect of supposedly specific NMDA-antagonists such as MK-801 on responses to QUIS is controversial. Mari *et al.*[26] also demonstrated effective inhibition of QUIS-stimulated cGMP production by MK-801 in striatal neurons cultured from fetal mice. Wood *et al.*,[59] however, did not find that MK-801 inhibited QUIS-stimulated cGMP production in the hippocampus *in vivo*. There are several possible mechanisms by which MK-801 and ketamine may block QUIS-stimulated cGMP production. Ketamine may block direct QUIS activation of the NMDA channel, but this is not likely. Quisqualate can act as an agonist at the NMDA receptor,[7,60] but the affinity of QUIS for the NMDA receptor is reported to be 2- to 23-fold less than that of NMDA and the total contribution of QUIS-activated current through NMDA channels is very small compared with that through the QUIS/AMPA channel in whole cell preparations. Another possible mechanism by which MK-801 and ketamine may block QUIS-stimulated cGMP production is by directly blocking activation of the AMPA/QUIS receptor or the metabotropic GLU receptor. A prominent effect on the metabotropic receptor (a nonionotropic receptor) would not have been observed by others, because the electrophysiologic techniques they used[44–47] would not have detected this biochemical response.

The effects of ketamine and MK-801 were specific for NMDA and QUIS receptors, because neither drug inhibited KAIN-stimulated cGMP production in our study. CNQX specifically blocked KAIN-stimulated cGMP production without effect on the activation of NMDA or QUIS receptors. This apparent lack of effect of CNQX on QUIS responses in our study may be explained by either rapid desensitization of the AMPA/QUIS receptor, therefore preventing any agonist-mediated response through this receptor,[26] or by the fact that QUIS activation of the metabotropic receptor gives a maximal cellular response. The lack of blockade of the GLU-stimulated response by CNQX supports the

suggestion by others that GLU does not activate KAIN receptors.[7,61]

These findings may also help to explain the role of ketamine in the protection of neurons from excitotoxicity. Several investigators have reported that ketamine can protect the brain against ischemic injury.[62,63] Ischemia is believed to lead to the release of GLU and the subsequent activation of GLU receptors activates the cellular processes that can result in neuronal death.[64,65] Activation of NMDA and QUIS receptors are involved in neuronal death, but the production of nitric oxide and cGMP production may or may not be involved in neuronal death.[64,65] Protection of neurons from excitotoxicity by ketamine may, therefore, result from blocking the activation of both NMDA and QUIS subtypes of GLU receptors.

In conclusion, our study demonstrates that GLU and the GLU analogs NMDA, KAIN, and QUIS stimulate NO production in cultures of cerebral neurons from the rat. Ketamine, an anesthetic drug that is pharmacologically similar to MK-801 and blocks the NMDA-subtype of GLU receptor, also blocks the effect of activation of GLU receptors activated by QUIS. These results are consistent with the hypothesis that blockade of NO production, and, thus, inhibition of the subsequent increase in neuronal cGMP content, results in anesthesia; these results allow one to speculate that interruption of other than the NMDA subtype of GLU receptor may also contribute to the state of anesthesia.

The authors thank Chris Cuspud and Iris Méndez-Bobé for their technical assistance.

References

1. MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL: NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature 321:519–522, 1986

2. Mayer ML, Westbrook GL: Permeation and block of *N*-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. J Physiol (Lond) 394:501–527, 1987

3. Mayer ML, MacDermott AB, Westbrook GL, Smith SJ, Barker JL: Agonist- and voltage-gated calcium entry in cultured mouse spinal cord neurons under voltage clamp measured using arsenazo III. J Neurosci 7:3230–3244, 1987

4. Murphy SN, Miller RJ: Regulation of Ca^{++} influx into striatal neurons by kainic acid. J Pharmacol Exp Ther 249:184–193, 1989

5. Sugiyama H, Ito I, Hirono C: A new type of glutamate receptor linked to inositol phospholipid metabolism. Nature 325:531–533, 1987

6. Sugiyama H, Ito I, Watanabe M: Glutamate receptor subtypes may be classified into two major categories: A study on xenopus oocytes injected with rat brain mRNA. Neuron 3:129–132, 1989

7. Ascher P, Nowak L: The role of divalent cations in the *N*-methyl-D-aspartate responses of mouse central neurones in culture. J Physiol (Lond) 399:247–266, 1988

8. Lipton SA, Kater SB: Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. Trends Neurosci 12:265–270, 1989

9. Szekely AM, Barbaccia ML, Alho H, Costa E: In primary cultures of cerebellar granule cells the activation of *N*-methyl-D-aspartate-sensitive glutamate receptors induces c-fos mRNA expression. Mol Pharmacol 35:401–408, 1989

10. Izumi Y, Ito K, Miyakawa H, Kato H: Requirement of extracellular Ca^{2+} after tetanus for induction of long-term potentiation in guinea pig hippocampal slices. Neurosci Lett 77:176–180, 1987

11. Dunwiddie TV, Lynch G: The relationship between extra cellular calcium concentrations and the induction of hippocampal long-term potentiation. Brain Res 169:103–110, 1979

12. Malenka RC, Kauer JA, Zucker RS, Nicoll RA: Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science 242:81–84, 1988

13. Malenka RC, Kauer JA, Perkel DJ, Nicoll RA: The impact of postsynaptic calcium on synaptic transmission—Its role in long-term potentiation. Trends Neurosci 12:444–450, 1989

14. Garthwaite J, Charles SL, Chess-Williams R: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336:385–388, 1988

15. Garthwaite J: Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci 14:60–67, 1991

16. Bredt DS, Snyder SH: Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. Proc Natl Acad Sci U S A 86:9030–9033, 1989

17. Brüne B, Lapetina EG: Activation of a cytosolic ADP-ribosyl-transferase by nitric oxide-generating agents. J Biol Chem 264:8455–8458, 1989

18. Brüne B, Lapetina EG: Properties of a novel nitric oxide-stimulated ADP-ribosyltransferase. Arch Biochem Biophys 279:286–290, 1990

19. Mantz J, Chéramy A, Thierry AM, Glowinski J, Desmonts JM: Anesthetic properties of riluzole (54274 RP), a new inhibitor of glutamate neurotransmission. ANESTHESIOLOGY 76:844–848, 1992

20. France CP, Snyder AM, Woods JH: Analgesic effects of phencyclidine-like drugs in rhesus monkeys. J Pharmacol Exp Ther 250:197–201, 1989

21. Daniell LC: The noncompetitive N-methyl-D-aspartate antagonists, MK-801, phencyclidine and ketamine, increase the potency of general anesthetics. Pharmacol Biochem Behav 36:111–115, 1990

22. France CP, Winger GD, Woods JH: Analgesic, anesthetic, and respiratory effects of competitive *N*-methyl-D-aspartate (NMDA) antagonist CGS 19755 in rhesus monkeys. Brain Res 526:355–358, 1990

23. Daniell LC: Effect of CGS 19755, a competitive N-methyl-D-aspartate antagonist, on general anesthetic potency. Pharmacol Biochem Behav 40:767–769, 1991

24. McFarlane C, Warner DS, Todd MM, Nordholm L: AMPA receptor competitive antagonism reduces halothane MAC in rats. ANESTHESIOLOGY 77:1165–1170, 1992

25. Kiedrowski L, Costa E, Wroblewski JT: Glutamate receptor agonists stimulate nitric oxide synthase in primary cultures of cerebellar granule cells. J Neurochem 58:335–341, 1992

26. Marin P, Quignard JF, Lafon-Cazal M, Bockaert J: Non-classical glutamate receptors, blocked by both NMDA and non-NMDA antagonists, stimulate nitric oxide production in neurons. Neuropharmacology 32:29–36, 1993

27. Johns RA, Moscicki JC, DiFazio CA: Nitric oxide synthase inhibitor dose-dependently and reversibly reduces the threshold for halothane anesthesia: A role for nitric oxide in mediating consciousness? ANESTHESIOLOGY 77:779–784, 1992

28. Babbedge RC, Hart SL, Moore PK: Anti-nociceptive activity of nitric oxide synthase inhibitors in the mouse: Dissociation between the effect of L-NAME and L-NMMA. J Pharm Pharmacol 45:77–79, 1993

29. Moore PK, Oluyomi AO, Babbedge RC, Wallace P, Hart SL: L-N^G-nitro arginine methyl ester exhibits antinociceptive activity in the mouse. Br J Pharmacol 102:198–202, 1991

30. Morgan CVJ, Babbedge RC, Gaffen Z, Wallace P, Hart SL, Moore PK: Synergistic anti-nociceptive effect of L-N^G-nitro arginine methyl ester (L-NAME) and flurbiprofen in the mouse. Br J Pharmacol 106: 493–497, 1992

31. Kawabata A, Umeda N, Takagi H: L-arginine exerts a dual role in nociceptive processing in the brain: Involvement of the kyotorphin-Met-enkephalin pathway and NO-cyclic GMP pathway. Br J Pharmacol 109:73–79, 1993

32. Przewlocki R, Machelska H, Przewlocka B: Inhibition of nitric oxide synthase enhances morphine antinociception in the rat spinal cord. Life Sci 53:PL1–PL5, 1993

33. Malmberg AB, Yaksh TL: Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain 54:291–300, 1993

34. Duarte ID, Ferreira SH: The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine-nitric oxide-cGMP pathway. Eur J Pharmacol 221:171–174, 1992

35. Kumar A, Raghubir R, Srimel RC, Dhawan BN: Evidence for involvement of nitric oxide in pretectal analgesia in rat. Neuroreport 4:706–708, 1993

36. Tseng LF, Xu JY, Pieper GM: Increase of nitric oxide production by L-arginine potentiates i.c.v. administered beta-endorphin-induced antinociception in the mouse. Eur J Pharmacol 212:301–303, 1992

37. Kawabata A, Fukuzumi Y, Fukushima Y, Takagi H: Antinociceptive effect of L-arginine on the carrageenin-induced hyperalgesia of the rat: Possible involvement of central opioidergic systems. Eur J Pharmacol 218:153–158, 1992

38. Irifune M, Shimizu T, Nomoto M, Fukuda T: Ketamine-induced anesthesia involves the *N*-methyl-D-aspartate receptor-channel complex in mice. Brain Res 596:1–9, 1992

39. Jensen TS, Yaksh TL: The antinociceptive activity of excitatory amino acids in the rat brainstem: An anatomical and pharmacological analysis. Brain Res 569:255–267, 1992

40. Martin DC, Abraham JE, Plagenhoef M, Aronstam RS: Volatile anesthetics and NMDA receptors. Enflurane inhibition of glutamate-stimulated [^{3}H]MK-801 binding and reversal by glycine. Neurosci Lett 132:73–76, 1991

41. Puil E, El-Beheiry H, Baimbridge KG: Anesthetic effects on glutamate-stimulated increase in intraneuronal calcium. J Pharmacol Exp Ther 255:955–961, 1990

42. Carlà V, Moroni F: General anaesthetics inhibit the responses induced by glutamate receptor agonists in the mouse cortex. Neurosci Lett 146:21–24, 1992

43. Vulliemoz Y, Verosky M, Alpert M, Triner L: Effect of enflurane on cerebellar cGMP and on motor activity in the mouse. Br J Anaesth 55:79–84, 1983

44. Harrison NL, Simmonds MA: Quantitative studies on some antagonists of *N*-methyl-D-aspartate in slices of rat cerebral cortex. Br J Pharmacol 84:381–391, 1985

45. MacDonald JF, Bartlett MC, Mody I, Pahapill P, Reynolds JN, Salter MW, Schneiderman JH, Pennefather PS: Actions of ketamine, phencyclidine and MK-801 on NMDA receptor currents in cultured mouse hippocampal neurones. J Physiol (Lond) 432:483–508, 1991

46. Thomson AM, West DC, Lodge D: An N-methylaspartate receptor-mediated synapse in rat cerebral cortex: A site of action of ketamine? Nature 313:479–481, 1985

47. Anis NA, Berry SC, Burton NR, Lodge D: The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. Br J Pharmacol 79:565–575, 1983

48. Yamamura T, Harada K, Okamura A, Kemmotsu O: Is the site of action of ketamine anesthesia the *N*-methyl-D-asparate receptor? ANESTHESIOLOGY 72:704–710, 1990

49. Macdonald AG, Ramsey RL, Shelton CJ, Usherwood PNR: Single channel analysis of ketamine interaction with a quisqualate receptor. Eur J Pharmacol 210:223–229, 1992

50. Dichter MA: Rat cortical neurons in cell culture: Culture methods, cell morphology, electrophysiology, and synapse formation. Brain Res 149:279–293, 1978

51. Atkinson BN, Minneman KP: Multiple adrenergic receptor subtypes controlling cyclic AMP formation: Comparison of brain slices and primary neuronal and glial cultures. J Neurochem 56:587–595, 1991

52. Brooker G: Improvements in the automated radioimmunoassay for cAMP or cGMP. Methods Enzymol 159:45–50, 1988

53. Read SM, Northcote DH: Minimization of variation in the response to different proteins of the coomassie blue G dye-binding assay for protein. Anal Biochem 116:53–64, 1981

54. Agulló L, García A: Different receptors mediate stimulation of nitric oxide-dependent cyclic GMP formation in neurons and astrocytes in culture. Biochem Biophys Res Commun 182:1362–1368, 1992

55. Wood PL, Emmett MR, Rao TS, Cler J, Mick S, Iyengar S: Inhibition of nitric oxide synthase blocks *N*-methyl-D-aspartate-, quisqualate-, kainate-, harmaline-, and pentylenetetrazole-dependent increases in cerebellar cyclic GMP in vivo. J Neurochem 55:346–348, 1990

56. Cohen ML, Chan S-L, Way WL, Trevor AJ: Distribution in the brain and metabolism of ketamine in the rat after intravenous administration. ANESTHESIOLOGY 39:370–376, 1973

57. Chang T, Glazko AJ: A gas chromatographic assay for ketamine in human plasma. ANESTHESIOLOGY 36:401–404, 1972

58. White PF, Ham J, Way WL, Trevor AJ: Pharmacology of ketamine isomers in surgical patients. ANESTHESIOLOGY 52:231–239, 1980

59. Wood PL, Ryan R, Li M: Excitatory amino acid signal transduction in the hippocampus: Role of noradrenergic afferents and nitric oxide in cGMP increases *in vivo*. Life Sci 51:601–606, 1992

60. Grudt TJ, Jahr CE: Quisqualate activates N-methyl-D-aspartate receptor channels in hippocampal neurons maintained in culture. Mol Pharmacol 37:477–481, 1990

61. Gunderson CB, Miledi R, Parker I: Messenger RNA from human

brain induces drug- and voltage-operated channels in Xenopus oocytes. Nature 308:421–424, 1984

62. Church J, Zeman S, Lodge D: The neuroprotective action of ketamine and MK-801 after transient cerebral ischemia in rats. ANESTHESIOLOGY 69:702–709, 1988

63. Hoffman WE, Pelligrino D, Werner C, Kochs E, Albrecht RF, Schulte am Esch J: Ketamine decreases plasma catecholamines and improves outcome from incomplete cerebral ischemia in rats. ANESTHESIOLOGY 76:755–762, 1992

64. Beal MF: Mechanisms of excitotoxicity in neurologic diseases. FASEB J 6:3338–3344, 1992

65. Dawson VL, Dawson TM, Bartley DA, Uhl GR, Snyder SH: Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. J Neurosci 13:2651–2661, 1993