Effects of Targeted Neuronal Nitric Oxide Synthase Gene Disruption and Nitro-G-L-Arginine Methylester on the Threshold for Isoflurane Anesthesia

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Background: Considerable evidence suggests that nitric oxide plays a role in synaptic transmission in the central and peripheral nervous system. Nonselective inhibition of nitric oxide synthase by nitro-G-L-arginine methylester (L-NAME) reduces the minimum alveolar concentration of halothane anesthesia. The effects of selective neuronal nitric oxide synthase inhibition on the anesthetic requirements in mice congenitally deficient in neuronal nitric oxide synthase (knockout mice) were examined.

Methods: Isoflurane minimum alveolar concentration and righting reflex ED50 (REDD50) were determined in knockout and wild-type mice. Subsequently, the effects of intraperitoneal L-NAME on minimum alveolar concentration and REDD50 of knockout and wild-type mice were examined. In a separate experiment, the effects of week-long administration of L-NAME were examined in wild-type mice. Isoflurane minimum alveolar concentration and REDD50 were measured on the 8th day and were repeated after an acute intraperitoneal dose of L-NAME.

Results: Targeted disruption of the neuronal nitric oxide synthase gene did not modify isoflurane minimum alveolar concentration and REDD50 of knockout mice. Acute intraperitoneal L-NAME decreased the minimum alveolar concentration and REDD50 of wild-type but did not alter those values in knockout mice. The wild-type mice, when given L-NAME for a week, showed a minimum alveolar concentration and REDD50 identical to that of untreated wild-type mice.

Conclusions: Although acute nonselective inhibition of nitric oxide synthase reduces the anesthetic requirements of wild-type mice, a chronic congenital deficiency of neuronal nitric oxide synthase or a week of L-NAME treatment of wild-type mice does not produce a state of greater sensitivity to the effects of isoflurane anesthesia. (Key words: Anesthetics, volatile; isoflurane. Anesthetic potency; minimum alveolar concentration. Animal: mouse. Genetics: nitric oxide synthase. Neurotransmitter: nitric oxide. Pharmacology: nitric oxide synthase inhibitor.)

INITIALLY identified as a potent vasodilator, nitric oxide has been implicated in synaptic transmission in the central and peripheral nervous system. Nitric oxide is produced enzymatically by nitric oxide synthase (NOS) from L-arginine and exerts many of its effects by increasing intracellular concentrations of cyclic guanosine monophosphate (cGMP) in target cells through activation of soluble guanylate cyclase. A number of NOS isoforms have been characterized. Endothelial and neuronal NOS (nNOS) enzymes are produced constitutively and are Ca2+/calmodulin-dependent, whereas macrophage NOS can be induced by lipopolysaccharide and interferon and does not depend on free intracellular calcium for activity. NOS is localized to neurons throughout the peripheral and central nervous system, including the cerebellum, hypothalamus, midbrain, striatum, and hippocampus. It has been shown that nitric oxide mediates the cerebellar increase of cGMP content in response to stimulation by N-methyl-D-aspartate (NMDA), glutamate, and kainate. On the other hand, halothane and enfurane decrease the cGMP content of selected brain regions, including the cerebellum.

It remains uncertain whether nitric oxide plays an important role in nociception or maintaining consciousness. In the rat, administration of a chemical NOS inhibitor, nitro-G-L-arginine methylester (L-NAME), has...
been reported to either reduce or not change the halothane minimum alveolar concentration.\cite{12,13} Analogs of N\textsuperscript{\text{-}}-substituted arginine, such as t-NAME, nonselectively block all of the known NOS isoforms including the endothelial, neuronal, and inducible isoforms. Furthermore, arginine analogs may interact with other iron-containing biologic systems\cite{14,15} and antagonize muscarinic receptors.\cite{16} Therefore, the effects of systemically administered arginine analogs may not necessarily be limited to nNOS inhibition.

Huang et al. generated mice in which the nNOS gene is disrupted.\cite{17} We believed that a study of this nNOS knockout mouse (knockout) would provide information about the state of chronic neuronal nitric oxide deficiency on anesthetic susceptibility and might illuminate the role of nitric oxide in mediating nociception or consciousness. Accordingly, we conducted this study to try to examine the following questions: (1) Is the chronic congenital deficiency of nNOS associated with decreased general anesthetic requirements? (2) Does acute t-NAME treatment affect general anesthetic requirements of wild-type or knockout mice? (3) Does week-long t-NAME treatment in wild-type animals mimic the level of susceptibility to general anesthesia of the nNOS knockout mouse? To explore these questions, we measured the minimum alveolar concentration and righting reflex ED\textsubscript{50} (RRED\textsubscript{50}) for isoflurane in wild-type and nNOS knockout mice. The determinations of minimum alveolar concentration and RRED\textsubscript{50} were repeated after acute and week-long t-NAME administration.

### Materials and Methods

These investigations were approved by the Subcommittee for Research Animal Care of the Massachusetts General Hospital (Boston, MA).

Fifty knockout mice and 80 wild-type mice (SV129, Taconic, MA) with approximately the same body weight (18–26 g) of both sexes were used in this study. Knockout mice were generated by homologous recombination, as described previously.\cite{17} These homozygous knockout mice showed no evidence of nNOS gene expression when DNA, mRNA, and proteins from brain extracts were analyzed.\cite{17} Residual brain NOS catalytic activity was less than 5% over background activity as detected by the enzymatic conversion of radiolabeled arginine to citrulline in brain homogenates.\cite{17} This residual activity is likely to be attributable to endothelial NOS.\cite{17-19

### Protocol

**Determination of Baseline Values of Minimum Alveolar Concentration and the RRED\textsubscript{50} in Wild-type and Knockout Mice.** Baseline values of minimum alveolar concentration and RRED\textsubscript{50} of isoflurane were established according to the methods described by Eger et al.\cite{20-22} Mice were placed in individual Plexiglas chambers (25 cm long and 5.0 cm in diameter) for the determination of minimum alveolar concentration values. Each chamber was fitted with a rubber stopper at one end through which the mouse’s tail and a rectal temperature probe protruded. Groups of eight mice were given isoflurane in oxygen (4 l/min total gas flow). A gas sample was continuously drawn from the expiratory limb of the circuit, and the anesthetic concentration was measured using an infrared analyzer (Datest Ultima, Helsinki, Finland). A rectal temperature probe was inserted under light general anesthesia, and temperature was monitored in each mouse. The rectal temperature was kept between 36.5°C and 38.0°C with heat lamps. Mice initially breathed approximately 1.5 vol% isoflurane for 60 min. A clamp (alligator clip) was applied to the tail for 1 min, and the mice were observed for movement in response to the stimulation. In every case, the tail was stimulated proximal to the previous test site. Only the middle third of the tail was used for tail-clamping. The concentration of the anesthetic agent at which the mouse exhibited motor activity (gross movements of the head, extremities, and/or body) was considered one that permitted a positive motor response. The anesthetic concentration was increased (or decreased) in steps of 0.12–0.15% until the positive response disappeared (or vice versa), with 20 min for equilibration allowed after each change of anesthetic concentration. Minimum alveolar concentration is defined as the concentration midway between the lowest concentration that permitted movement in response to the stimulus and the lowest concentration that prevented movement. A typical minimum alveolar concentration study of a group of eight mice took approximately 4–5 h, including the initial equilibration period.

For the determination of baseline RRED\textsubscript{50}, unrestrained mice were placed in individual wire-mesh cages rotated at 4 rpm in a 20-l plastic chamber.\cite{20-22} In one additional restrained mouse in the same chamber, rectal temperature was monitored and maintained as above. Controlled amounts of volatile anesthetic were delivered by temperature-compensated vaporizers in a flow of oxygen (4 l/min) and allowed through the chamber during the equilibration period. After 60 min of equilibration, they were gently rolled over two or more times to prevent hypothermia. The concentration of isoflurane was increased or decreased in 0.12–0.15% steps. An initial concentration that would prevent movement was selected and kept constant for 15–20 min. Mice were then reexamined for their ability to right. A 30-min equilibration period was given if a sufficiently high anesthetic concentration, as described in the previous study, was achieved. A sufficient concentration was selected and just prevented the righting reflex study of a group of eight mice approximately 3–4 h, including the equilibration period.

**Influence of Acute t-NAME on Minimum Alveolar Concentration and RRED\textsubscript{50} in Wild-type and Knockout Mice.** We administered 50, 75, or 100 mg/kg as an intraperitoneal injection to wild-type and knockout mice via a tail vein injection at least 1 h prior to the maximum of the RRED\textsubscript{50} of isoflurane. Seven to nine mice were used in each test. t-NAME dose. We chose a number of mice based upon prior experience with 1 mg/kg. The effect of t-NAME on the minimum alveolar concentration was assessed using the same methods and conditions as previously described. After minimum alveolar concentrations were determined, isoflurane was given to determine RRED\textsubscript{50} in each group of mice as described above. After the concentration effect of t-NAME had been established, minimum alveolar concentrations were determined in a group of eight mice with the most potent dose of isoflurane (600 mg/kg) to determine the RRED\textsubscript{50} in each group of mice as described above. After the concentration effect of t-NAME had been established, minimum alveolar concentrations were determined in a group of eight mice.
in a flow of oxygen (4 l/min) and passed continuously through the chamber during the entire experimental period. After 60 min of equilibration, the mice were subjected to five complete turns of the rotator. Animals that rolled over two or more times were considered anesthetized. The concentration of the anesthetic agent was increased or decreased in 0.12–0.15% steps from the initial concentration that we tested, and the mice were reexamined for their ability to right themselves after a 50-min equilibration period. These changes and subsequent equilibration periods were continued until a sufficiently high anesthetic concentration was provided in which all animals failed the righting-reflex test and then until a sufficiently low concentration was provided in which all the animals righted themselves. RRED₅₀ was calculated for each mouse as the mean value of the anesthetic concentrations that just permitted and just prevented the righting-reflex. A typical righting-reflex study of a group of seven mice took approximately 3–4 h, including the initial equilibration period.

**Influence of Acute l-NAME on Minimum Alveolar Concentration and RRED₅₀ in Wild-type and Knockout Mice.** We administered l-NAME at 10, 25, 50, 75, or 100 mg/kg as an intraperitoneal injection to wild-type and knockout mice to study the influence of l-NAME on the minimum alveolar concentration and RRED₅₀ of isoflurane. Seven to nine mice were studied at each l-NAME dose. We chose these doses and the number of mice based upon prior studies.²³–²⁵ l-NAME was dissolved in 0.2 ml of normal saline. l-NAME or placebo (0.2 ml of normal saline) was injected 15 min before beginning each study, and minimum alveolar concentration and RRED₅₀ were determined in each group of mice as described above. As a separate set of experiments, we examined stereospecificity of the effects of l-NAME by administering l-arginine or D-arginine. After minimum alveolar concentration and RRED₅₀ were determined in a group of mice pretreated with the most potent dose of l-NAME (50 mg/kg), either l-arginine (600 mg/kg) or D-arginine (600 mg/kg) were dissolved in 0.2 ml of normal saline and injected intraperitoneally. Minimum alveolar concentration and RRED₅₀ were reetermined starting 15 min later. As an additional control experiment, the effects of ketamine on isoflurane minimum alveolar concentration in knockout mice were examined. Ketamine (50 mg/kg) was given as an intraperitoneal injection to eight knockout mice, and isoflurane minimum alveolar concentration was determined as above.

**Influence of Week-long l-NAME Administration on Minimum Alveolar Concentration and Righting Reflex in Wild-type Mice.** l-NAME (50 mg/kg in 0.2 ml of normal saline) was given to 20 wild-type mice by oral gavage every 12 h for 7 days followed on the 8th day by intraperitoneal l-NAME (50 mg/kg).²⁶ We determined RRED₅₀ and minimum alveolar concentration before and after giving intraperitoneal l-NAME on the 8th day. We gave the same volume of normal saline (placebo) by gavage to another group of 20 wild-type mice, and their minimum alveolar concentration and RRED₅₀ were determined. We chose the gavage route of administration because it has been used previously to deliver l-NAME on a chronic basis.²⁷ This portion of the study was done without the investigators knowing the treatment status of the animals.

**Blood Gas Analysis.** To rule out the presence of hypoxia, hypercapnia, and acidosis during these experiments, we sampled arterial blood from four mice in each group (wild-type mice, wild-type mice treated with l-NAME (50 mg/kg), and nNOS knockout mice) by percutaneous left ventricular puncture at the end of the minimum alveolar concentration experiments.

**Statistical Analysis.** All data are presented as mean ± SE. The data were analyzed using an analysis of variance followed by Dunnnett’s multiple comparison tests or an unpaired t-test. P < 0.05 was the criterion for statistical significance.

**Results**

The baseline values of isoflurane minimum alveolar concentration and RRED₅₀ of nNOS knockout mice did not differ from those of wild-type mice (minimum alveolar concentration (vol %): knockout 1.24 ± 0.05, wild-type 1.24 ± 0.05; RRED₅₀ (vol %): knockout 0.56 ± 0.01, wild-type 0.58 ± 0.01; table 1). The administration of 10, 25, 50, and 100 mg/kg intraperi-

**Table 1. Baseline Values of Isoflurane MAC and RRED₅₀ of Wild-type and Knockout Mice**

<table>
<thead>
<tr>
<th>Isoflurane</th>
<th>Wild-type</th>
<th>nNOS Knockout</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC (vol %)</td>
<td>1.24 ± 0.05 (n = 15)</td>
<td>1.24 ± 0.05 (n = 16)</td>
</tr>
<tr>
<td>RRED₅₀ (vol %)</td>
<td>0.56 ± 0.01 (n = 28)</td>
<td>0.58 ± 0.01 (n = 24)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE. No significant differences were found between wild-type and knockout mice.

MAC = minimum alveolar concentration; RRED₅₀ = righting reflex ED₅₀.
ICHINOSE, HUANG, AND ZAPOL

Table 2. Effects of Intraperitoneal l-NAME on Isoflurane MAC and RREDso of Wild-type and nNOS Knockout Mice

<table>
<thead>
<tr>
<th></th>
<th>l-NAME 10 mg·kg⁻¹</th>
<th>25 mg·kg⁻¹</th>
<th>50 mg·kg⁻¹</th>
<th>75 mg·kg⁻¹</th>
<th>100 mg·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC (vol %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type (n = 8-10)</td>
<td>1.17 ± 0.07</td>
<td>0.89 ± 0.04*</td>
<td>0.94 ± 0.05*</td>
<td>1.00 ± 0.05*</td>
<td>1.02 ± 0.07*</td>
</tr>
<tr>
<td>Knockout (n = 9-12)</td>
<td>1.21 ± 0.08</td>
<td>1.15 ± 0.05</td>
<td>1.22 ± 0.05</td>
<td>1.29 ± 0.06</td>
<td>1.23 ± 0.06*</td>
</tr>
<tr>
<td>RREDso (vol %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type (n = 7-9)</td>
<td>0.58 ± 0.03</td>
<td>0.45 ± 0.04</td>
<td>0.35 ± 0.03*</td>
<td>0.37 ± 0.03*</td>
<td>0.40 ± 0.02*</td>
</tr>
<tr>
<td>Knockout (n = 7-9)</td>
<td>0.69 ± 0.01</td>
<td>0.57 ± 0.04</td>
<td>0.59 ± 0.02</td>
<td>0.62 ± 0.01</td>
<td>0.61 ± 0.03</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.
* Value differs significantly from baseline values without l-NAME (P < 0.05).

Intraperitoneal l-NAME caused a decrease from isoflurane baseline minimum alveolar concentration of wild-type mice of 5.81 ± 5.3%, 28.3 ± 3.1% (P < 0.01), 24.0 ± 4.3% (P < 0.01), 19.4 ± 3.8% (P < 0.05), and 17.7 ± 6.0% (P < 0.05), respectively (table 2). The isoflurane RREDso of wild-type mice also was reduced by 10.25, 50, 75, and 100 mg/kg intraperitoneal l-NAME from its baseline value by -3.82 ± 5.2% (increased), 19.6 ± 7.1% (P < 0.01), 37.3 ± 5.4% (P < 0.01), 32.3 ± 5.1% (P < 0.01), and 29.2 ± 6.1% (P < 0.01), respectively (table 2). However, the responses of knockout mice to both tests were not altered by l-NAME injections at any of the l-NAME doses we examined (table 2). In contrast, the administration of intraperitoneal ketamine (50 mg/kg) caused a significant reduction of the isoflurane minimum alveolar concentration value of knockout mice from its baseline value of 1.24 ± 0.05 to 0.96 ± 0.04 (vol%). No apparent increase of morbidity or mortality was noted in mice treated with intraperitoneal l-NAME at any dose. The reduction of minimum alveolar concentration and RREDso in wild-type mice treated with 50 mg/kg l-NAME was completely reversed by an injection of 600 mg/kg intraperitoneal l-arginine, but not by administering d-arginine (table 3).

After 7 days of either l-NAME or placebo (normal saline) gavage-feeding, minimum alveolar concentration and RREDso of wild-type mice did not differ from their baseline values (table 4). When placebo-gavaged mice were given l-NAME (50 mg/kg) intraperitoneal injections on the 8th day, there was a significant reduction of minimum alveolar concentration and RREDso by 24.0 ± 3.2% (P < 0.001) and 26.2 ± 7.4% (P < 0.01), respectively, but not in the week-long l-NAME-gavaged mice (table 4). During week-long l-NAME gavage-feedings, 4 of 20 mice died in the l-NAME-gavaged group of undetermined causes. No untoward effects were observed in the placebo-gavaged group.

The results of blood gas analyses are shown in table 5. No significant hypoxia, hypocapnia, or acidosis was present in any of the mice. No significant differences were noted among groups of these measured indices.

Discussion

Our study revealed that targeted disruption and inactivation of the NOS gene in mice did not alter their sensitivity to isoflurane anesthesia. Mice that were congenitally deficient in nNOS had an isoflurane minimum alveolar concentration and RREDso identical to that of wild-type mice. Our study confirms the results of Johns et al. and demonstrates a reduction of the minimum alveolar concentration for isoflurane by acute l-NAME treatment in wild-type mice. In addition, we demonstrated that the RREDso for isoflurane was decreased by acute l-NAME administration. The l-NAME-induced reduction of minimum alveolar concentration and RREDso in wild-type mice was completely reversible by subsequent l-arginine administration but not by d-arginine, suggesting the specific site of action of l-NAME is NOS.

The precise mechanisms of general anesthesia remain largely unknown. A number of studies suggest that the

Table 3. Influence of L-Arginine and D-Arginine on l-NAME-Induced Changes in Isoflurane MAC and RREDso in Wild-type Mice

<table>
<thead>
<tr>
<th></th>
<th>MAC (vol %)</th>
<th>RREDso (vol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.24 ± 0.05 (n = 15)</td>
<td>0.56 ± 0.01 (n = 28)</td>
</tr>
<tr>
<td>l-Arginine 50 mg/kg</td>
<td>0.94 ± 0.03* (n = 16)</td>
<td>0.33 ± 0.02* (n = 14)</td>
</tr>
<tr>
<td>l-Arginine 600 mg/kg</td>
<td>1.27 ± 0.06 (n = 8)</td>
<td>0.52 ± 0.02 (n = 7)</td>
</tr>
<tr>
<td>D-Arginine 600 mg/kg</td>
<td>0.85 ± 0.04* (n = 8)</td>
<td>0.30 ± 0.03* (n = 7)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.
* Value differs significantly from untreated baseline (P < 0.05).


Anesthesiology, V 83, No 1, Jul 1995
ISOFLURANE MAC OF NEURONAL NOS-DEFICIENT MICE

Table 4. Effect of Week-long Gavage Feeding (Either l-NAME or Placebo) and Thereafter Acute l-NAME Challenge on the Isoflurane MAC and RRED_{50} of Wild-type Mice

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Before Intrapitoneal l-NAME</th>
<th>After Intrapitoneal l-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC (vol %)</td>
<td>1.24 ± 0.05 (n = 15)</td>
<td>1.27 ± 0.05</td>
<td>1.25 ± 0.09</td>
</tr>
<tr>
<td>l-NAME gavaged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo gavaged</td>
<td>1.38 ± 0.05 (n = 8)</td>
<td>1.05 ± 0.02*</td>
<td></td>
</tr>
<tr>
<td>RRED_{50} (vol %)</td>
<td>0.56 ± 0.01 (n = 28)</td>
<td>0.50 ± 0.01</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>l-NAME gavaged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo gavaged</td>
<td>0.54 ± 0.03 (n = 7)</td>
<td>0.39 ± 0.02*</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

* Value differs significantly from baseline without l-NAME (P < 0.05).

anesthetic actions of general anesthetics are, at least in part, due to inhibition of brain NMDA receptors. The NMDA receptor is the best characterized of the various excitatory glutamate-activated receptors in mammalian brain. Both competitive and noncompetitive antagonism of the NMDA receptor have been shown to reduce the minimum alveolar concentration of volatile anesthetics. Ketamine is believed to produce anesthesia by noncompetitive antagonism of the NMDA receptor, and we demonstrated that ketamine reduced the minimum alveolar concentration of isoflurane in nNOS knockout mice. Thus it is likely that the NMDA receptor mechanism is intact in nNOS knockout mice. Activation of the NMDA receptor has been shown to result in a Ca^{2+}-dependent increase in cGMP via the production of nitric oxide in the CNS. Current evidence also indicates that NMDA receptor activation and the subsequent release of nitric oxide play a pivotal role in spinal nociceptive processing in chronic pain models and long-term potentiation as a persistent increase of synaptic strength implicated in certain forms of learning and memory. Moore et al. reported antinociceptive action of NOS inhibitors given by various routes (intraperitoneal, intracerebroventricular, and oral) evaluated by three noiception paradigms in mice. Although most of the evidence is accumulated from studies of chronic pain models and long-term potentiation, the weight of prior observations suggests that nitric oxide plays a major role as a neurotransmitter in the central nervous system.

Because many of the effects of NMDA receptor activation appear to be mediated ultimately via production of nitric oxide, our findings, along with the report by Johns et al., of an l-NAME-induced reduction of halothane minimum alveolar concentration appear consistent with the above hypothesis. In contrast, our discovery that nNOS knockout mice had the same minimal anesthetic requirements as wild-type mice was surprising. Studies of this nNOS knockout mouse demonstrated that long-term potentiation and formalin-induced pain behavior (a test of spinally mediated central sensitization) were intact in these animals. Because these knockout mice are completely congenitally deficient in the nNOS enzyme, we considered the possibilities that other isoforms of NOS may serve to generate nitric oxide in the CNS or the existence of other unknown compensatory mechanisms.

As a control experiment, we examined the effects of l-NAME in the knockout mice. Interestingly, the isoflurane minimum alveolar concentration or RRED_{50} of knockout mice were not decreased by bolus intraperitoneal l-NAME injection at doses between 10 and 100 mg/kg. Because non-nNOS enzymes, such as cNOS, would be inhibited by l-NAME administration, our ob-
servation strongly suggest that, in knockout mice, mechanisms independent of nitric oxide compensate for the chronic congenital deficiency of nitric oxide and preserve the pathways mediating the responses to tail-clamping and the righting reflex against anesthetic depression. In contrast, long-term potentiation was blocked by NOS inhibitors in the mutant mice just as it was in wild-type mice, suggesting the importance of alternative nitric oxide isoforms in this particular form of synaptic plasticity. Immuno staining demonstrated the endothelial form of NOS (eNOS) in the CA1 region of hippocampus from which long-term potentiation was induced.

We examined the effects of ketamine on isoflurane minimum alveolar concentration to learn whether the inability of L-NAME to reduce minimum alveolar concentration in these knockout mice was a generalized phenomenon. An intraperitoneal injection of 50 mg/kg ketamine decreased the isoflurane minimum alveolar concentration of knockout mice by more than 20%. This suggests that the failure of L-NAME to reduce anesthetic requirements in knockout mice is quite specific and not a generalized phenomenon in these mutant knockout animals.

Our observation that nNOS knockout mice have an anesthetic requirement identical to that of wild-type mice provides evidence that nitric oxide is not the sole messenger of pain perception or maintaining consciousness. Because of the vital importance of these processes for protecting all life forms, it is likely that multiple mechanisms coexist to mediate these responses. The dose-response curves of L-NAME effects on isoflurane minimum alveolar concentration and RRED_{50} of our current study, as well as the study of Johns et al., provide evidence that there may be a plateau of antinociceptive effect at high L-NAME doses. In addition, the largest reduction of anesthetic requirements by L-NAME was approximately 30–50% in both studies. Our observations suggest the coexistence of multiple pathways for maintaining pain perception and consciousness. Another possible explanation for the existence of the dose plateau effect is the incompleteness of inhibition of nNOS by L-NAME. In the rat, bolus intravenous administration of 10–40 mg/kg L-NAME attenuated nNOS catalytic activity by approximately 50%. On the other hand, nNOS activity is inhibited by 90% after a single intravenous injection of L-NAME (10–40 mg/kg) in cats. Therefore, the degree of nNOS inhibition after a single systemic dose of L-NAME varies widely among species. No data are available for mice. It is conceivable that there was a plateau effect of L-NAME on minimum alveolar concentration reduction in our study because nNOS was only partially inhibited by the route and dose of L-NAME that we selected. We do not know why our study showed less dose-dependency of L-NAME on minimum alveolar concentration than that of Johns et al. This difference may be related to differences of route of administration and species.

To better understand the marked difference in the minimum alveolar concentration and RRED_{50} response to an acute L-NAME injection of wild-type and knockout mice, we examined the effects of week-long L-NAME treatment in wild-type mice. A previous investigation demonstrated that L-NAME gavage at doses larger than 10 mg/kg resulted in a tenfold reduction of the arterial wall cGMP content, suggesting inactivation of the nitric oxide-cGMP pathway. It has been reported that another potent NOS inhibitor, N-nitro-L-arginine, when given at 50 mg/kg intraperitoneally twice a day for 4 days, inhibits NOS activity by 95% in rats, and NOS activity did not return to normal for at least 5 days. We noted that wild-type mice given an L-NAME gavage every 12 h for a week demonstrated a minimum alveolar concentration and RRED_{50} identical to that of untreated wild-type mice as well as saline-gavaged mice. Further challenges with intraperitoneal L-NAME on the 8th day decreased the isoflurane minimum alveolar concentration and RRED_{50} of saline-gavaged mice but not of L-NAME-gavaged mice. These results demonstrate neural tolerance to L-NAME administration and suggest the existence of compensatory alternative pathways when the brain’s nitric oxide-cGMP pathways are inactivated by either congenital gene disruption or pharmacologic inhibition.

Although the isoflurane minimum alveolar concentration and RRED_{50} values were measured as inspired gas concentrations in the current study, our baseline values are similar to previously reported values (minimum alveolar concentration 1.35–1.41 vol%, RRED_{50} 0.57–0.66 vol%). Ideally, volatile anesthetic gas concentrations used for the determination of anesthetic potency should be measured as alveolar concentrations because of the moderate solubility of these gases. However, it has been suggested that the inspired-to-alveolar difference of isoflurane concentration might be negligible in the mouse (or other small animals), in which the ventilation per gram of body tissue is high and blood uptake will have less influence on the rate of alveolar concentra-

Anesthesiology. V 83, No 1, Jul 1995
ISOFLURANE MAC OF NEURONAL NOS-DEFICIENT MICE

There was no apparent hypoxia, hypercapnia, or acidosis in the mice immediately after minimum alveolar concentration studies, ruling out the possibility that hypoxia or hypercapnia impaired minimum alveolar concentration reduction (table 5). There were no differences among wild-type mice, wild-type mice treated with bolus l-NAME, or nNOS knockout mice of these respiratory indexes, suggesting the difference in anesthetic requirements is not due to differing respiratory status.

We did not measure hemodynamic parameters because of the logistics of the apparatus and the size of the mouse. Although arterial hypertension due to l-NAME administration per se should not affect minimum alveolar concentration,\(^6\) we cannot rule out the possibility that any differences between knockout and wild-type mice are due to differing hemodynamic responses to isoflurane and/or l-NAME. We have not observed significant differences in mean systemic arterial blood pressure values or the ability to autoregulate cerebral blood flow over a wide range of systemic arterial blood pressure between wild-type and nNOS knockout mice.\(^58\)

In summary, mice that lack nNOS have a minimum alveolar concentration and RRE\(_{50}\) identical to that of wild-type mice. Treatment with NOS inhibitors reduces the minimum alveolar concentration and RRE\(_{50}\) of wild-type mice, but not knockout mice, suggesting that other non-nitric oxide nociceptive mechanisms exist in the knockout mice to compensate for this mutation. Our studies confirm that acute NOS inhibition has analgesic or anesthetic effects in the wild-type mouse, whereas week-long NOS inhibition by l-NAME induces compensatory adaptations of the nervous system, making wild-type mice once again sensitive to normal levels of inhalational anesthesia. Week-long administration of l-NAME appeared to mimic the normal anesthetic sensitivity of the nNOS knockout mouse, and similar adaptive nervous processes may have occurred. We speculate that the nitric oxide-cGMP pathway plays an important role in pain perception and maintaining consciousness, but nNOS is not an essential nociceptive pathway and can be compensated for by alternative pathways when congenitally inactivated by mutation or chemically inactivated during development for a week. Further studies using isoform-specific NOS inhibitors or other congenital NOS isoform knockouts (e.g., the endothelial NOS knockout mouse) will provide additional information about the role of the nitric oxide-cGMP pathway in modulating pain perception and consciousness, as well as possible alternative nociceptive pathways in the central nervous system.

References


Anesthesiology, V 83, No 1, Jul 1995
ICHINOSE, HUANG, AND ZAPOL


