Mechanism of Age-related and Nitrous Oxide-associated Anesthetic Sensitivity: The Role of Brain Catecholamines

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To provide a neurochemical basis for differences in their anesthetic requirements, the authors examined mice selectivity bred for resistance (HI) and susceptibility (LO) to nitrous oxide anesthesia for brain levels of catecholamines. Concentrations of norepinephrine and dopamine in whole brain were 26% and 13% higher (P < 0.001), respectively, in HI mice than in LO mice. Whole-brain levels of 3,4-dihydroxyphenylacetic acid, a major metabolite of dopamine, were the same for both HI and LO groups of mice. The authors then analyzed portions of the HI and LO mice brains for concentrations of norepinephrine and dopamine. A significant correlation was found between norepinephrine content in the medulla and nitrous oxide requirement. In other regions of the brain (cerebellum, cerebral cortex, hippocampus, pons, midbrain, hypothalamus), no significant differences in norepinephrine or dopamine levels could be detected. Differences in anesthetic requirements between resistant and susceptible mice decrease from 0.99 to 0.53 atm as they aged from 100 days to 600 days old, paralleling the decrease in differences in norepinephrine levels in medulla oblongata between HI and LO mice from 1.6 to 0.73 ng/mg protein. Thus, the difference in anesthetic requirement between HI and LO mice may arise from alterations in catecholamine content in specific regions of the brain. (Key words: Age factors. Anesthetics, gases: nitrous oxide. Genetic factors. Potency: ED₅₀; minimum anesthetic concentration; righting reflex. Theories of anesthesia: catecholamines.)

We found that mice from a normal population could be divided into two groups that possessed either a high or low nitrous oxide requirement. By mating animals with a consistently high requirement with each other and those with a consistently low nitrous oxide requirement with each other, we developed two lines of mice that were resistant (HI mice) or susceptible (LO mice) to anesthesia.¹² By the tenth generation of selective breeding, the nitrous oxide ED₅₀ (the partial pressure of nitrous oxide required to abolish the righting reflex in half the mice) for these two lines were separated by more than 0.7 atm.²

In an attempt to provide a basis for these differences in anesthetic requirement, we examined the lipid composition of the synaptic membrane in these two lines of mice. Because of the excellent correlation between lipid solubility and anesthetic potency, and the ability of inhaled anesthetics to alter the physical state of membrane lipids,¹ we believed that our breeding process might have selected mice with an altered membrane lipid composition; the HI mice may have possessed a membrane lipid composition that resists the perturbing properties of anesthetics. However, no differences in phospholipid, fatty acid, or cholesterol composition of the synaptic membrane could be detected between the two lines.¹ We therefore investigated other biochemical alterations in the central nervous system (CNS) that might explain the differences in anesthetic potency.

The present experiments investigated whether these differences in anesthetic requirement were related to levels of catecholamines in the whole brain or in specific brain regions. We tested this possibility because an alteration in central catecholamine availability significantly influences anesthetic requirement. For example, drugs (e.g., reserpine) that decrease CNS levels of norepinephrine and/or dopamine produce a dose-related decrease in halothane MAC,⁵—⁶ whereas drugs that elevate central norepinephrine levels increase MAC (the minimal alveolar concentration of an anesthetic that just prevents movement in 50% of animals or humans exposed to a noxious stimulus).³—⁵ Furthermore, the ablation of certain norepinephrine-enriched areas of the brain stem decreases MAC in rats, as compared with littermate control rats that undergo sham operations.⁷ If catecholamine levels are an important factor in determining the differences in anesthetic potency between HI and LO mice, we would expect to find higher levels of these neurotransmitters in the brains of HI mice. Secondly, we would expect to find a decline in the catecholamine levels in the brains of HI and LO mice as anesthetic requirements decrease with aging. Furthermore, we would expect to find the differ-

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ence in norepinephrine levels between HI and LO mice to narrow as anesthetic requirement differences narrow with age.

**Materials and Methods**

The procedure for selectively breeding mice resistant and susceptible to nitrous oxide anesthesia has been described in earlier reports.\(^1\,^2\) In the present experiments, we studied male mice from the sixth, eighth, ninth, and eleventh generations. Mice were fed with Purina\(^\text{®}\) lab chow *ad libitum* and kept in a 12-h light-, 12-h dark-cycled area (light 6 A.M. to 6 P.M.) for the duration of the study. Nitrous oxide ED\(_{50}\) was measured when the animals were 7–10 weeks of age. For experiments comparing the changes in norepinephrine levels and anesthetic requirements with aging, we studied male mice from the twelfth and thirteenth generations. Nitrous oxide ED\(_{50}\) was measured when they were approximately 100, 188, and 600 days of age. In both sets of experiments the mice were returned to normal housing conditions for at least 1 week before being examined for brain levels of catecholamines.

Mice were placed in a 20-l stainless steel hyperbaric chamber. For each MAC determination, eight unrestrained mice were placed in individual wire mesh cages that could be rotated at 4 revolutions/min. Rectal temperatures were monitored in two additional restrained mice and maintained between 36.5 and 38.0°C by adjusting the chamber temperature through circulating water-heat exchangers. The chamber gases were circulated through a soda-lime container to remove carbon dioxide. The pressure chamber was flooded with 100% oxygen for 10 min, and a subanesthetic dose of nitrous oxide was added. After a ½-h equilibration period, animals rolling over twice or more during five complete turns of the rotator were considered anesthetized. Further additions of nitrous oxide (usually in increments of 0.11 atm) were made, and the righting reflex was redetermined after a 15-min equilibration period. After the highest anesthetic dose was tested, one or two of the lower doses were repeated to ensure that measurements were independent of time and dose sequence. Oxygen content did not decrease below 0.6 atm during testing. Nitrous oxide concentrations were determined by gas chromatography with the use of a thermal conductivity detector. The mean values of nitrous oxide ED\(_{50}\) (the partial pressure of nitrous oxide required to abolish the righting reflex in half the mice) and standard errors were calculated from the nitrous oxide requirements for individual mice.\(^1\,^8\) Anesthetic requirement was calculated for each mouse by averaging the partial pressures of nitrous oxide that just abolished and just allowed the righting reflex.

For analysis of catecholamine levels in whole brain, mice were decapitated; each brain was quickly removed, weighed, placed in a 17 × 100 mm plastic test tube, and frozen at −80°C. Nine volumes of cold 0.1 N perchloric acid were added to each brain, which was then homogenized with a Brinkman Polytron. For analysis of catecholamines in different regions of the brain, the whole brain was removed and dissected with a modification of the procedure used for rat brain.\(^7\) Brain sections were homogenized in 2 ml of 0.1 N perchloric acid.

Neurotransmitters were analyzed with two methods. For whole brains, we determined concentrations of norepinephrine, dopamine, and 3,4-dihydroxyphenylacetic acid (DOPAC), a major metabolite of dopamine, using high-pressure liquid chromatography with electrochemical detection and a method similar to that of Mefford *et al.*\(^9\) By use of an internal standard dihydroxybenzylamine (DHBA), concentrations in the homogenates were calculated by correcting for incomplete recovery (varying between 65 and 75% for norepinephrine, dopamine, and DHBA, and 15% less for DOPAC). Recovery and detector response was linear beyond the extremes of the quantities assayed. The assay had sensitivities of 50–70 pg of norepinephrine, 60–90 pg of dopamine, and 50–100 pg for DHBA and DOPAC with coefficients of variation between 8 and 15% for the assays in this study. Catechol levels in whole brain were expressed as nanograms per gram of brain.

For the analysis of neurotransmitters in regions of the brain, norepinephrine and dopamine concentrations were determined with the use of radioenzymatic analysis.\(^1^1,^1^2\) This second method was used because at the time it was more sensitive—having a sensitivity of 6–10 pg of norepinephrine, 6–12 pg of epinephrine, and 10–20 pg of dopamine with coefficients of variation between 6 and 10% for the assays in this study. Catecholamine levels in brain sections were expressed as nanograms per milligram of protein. Protein was quantitated with the method of Lowry *et al.*\(^1^3\) All assays were performed in duplicate. Statistical significance was calculated with the use of an unpaired *t* test, analysis of variance, and linear regression.

**Results**

Norepinephrine and dopamine concentrations were 26% and 15% greater, respectively, in the whole brains of HI mice than of LO mice (table 1). However, concentrations of DOPAC were not significantly different between the two lines. For this experiment, the nitrous oxide requirements of HI and LO mice were separated by 0.73 atm (table 1).

To determine whether these differences in catecholamine content of whole brain were localized in specific regions, we examined the content of norepinephrine and dopamine in various brain sections. In the first series of experiments in which HI and LO mice had a 0.65 atm separation in nitrous oxide requirement (table 2), we
found a twofold higher \((P \leq 0.001)\) norepinephrine content and a greater \((P \leq 0.05)\) dopamine content in the medulla of the HI mice (dopamine may only be a precursor of norepinephrine in this area). However, no significant differences in norepinephrine or dopamine content could be detected in the cerebellum, cerebral cortex, hippocampus, pons, midbrain, or hypothalamus (table 2). To compare the medullary norepinephrine and dopamine levels in HI/LO animals, we analyzed the brains of an additional 42 HI and 50 LO animals. These data are combined with the data in table 2 and displayed in figure 1. If the medullary norepinephrine levels from these experiments are plotted against nitrous oxide requirement for each HI and LO animal tested, a weak \((r = 0.443)\) but significant \((P \leq 0.01)\) correlation is found between nitrous oxide requirement and medullary norepinephrine content (fig. 1).

As in our previous studies,\(^1\) we found that anesthetic requirements of both HI and LO mice and the difference between their anesthetic requirements decreased with increasing age \((P \leq 0.01)\). Similarly, the norepinephrine concentration in the medulla of both HI and LO mice and the difference in that concentration decreased with age \((P \leq 0.01)\) (table 3). The decrease with age in difference of nitrous oxide required for HI and LO animals paralleled the decrease in medullary norepinephrine concentration \((r^2 = 0.956; P \leq 0.001;\) fig. 2).

### Table 1. Concentrations of Norepinephrine, Dopamine, and 3,4-Dihydroxyphenylacetic Acid (DOPAC) in Whole Brains of Mice Resistant (HI) and Susceptible (LO) to Nitrous Oxide Anesthesia\(^*\)

<table>
<thead>
<tr>
<th></th>
<th>HI Mice</th>
<th>LO Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Norepinephrine (ng/g)</td>
<td>474 ± 20</td>
<td>377 ± 11†</td>
</tr>
<tr>
<td>Dopamine (ng/g)</td>
<td>1352 ± 17</td>
<td>1201 ± 21†</td>
</tr>
<tr>
<td>DOPAC (ng/g)</td>
<td>174 ± 6</td>
<td>171 ± 12</td>
</tr>
<tr>
<td>Nitrous oxide ED(_{50}) (atm)</td>
<td>1.80 ± 0.05</td>
<td>1.07 ± 0.04†</td>
</tr>
</tbody>
</table>

\(^*\) Mean values ± SE.

† HI and LO mice differ from each other at a significance level of \(P \leq 0.001\).

### Discussion

The greater anesthetic requirement in mice selectively bred for resistance to nitrous oxide anesthesia is associated with an increase in catecholamine content in the brain. Similarly, other investigators have found altered levels of catecholamines in the CNS of animals bred for resistance and susceptibility to another depressant, alcohol. For example, dopamine concentrations are 15–25% greater in brains of rats preferring ethanol compared with those preferring water.\(^1\) Mice selectively bred for resistance to a hypnotic dose of alcohol have significantly higher levels of norepinephrine and dopamine in whole brain than mice bred for susceptibility.\(^1\)

Although we found significant differences in norepinephrine and dopamine levels in whole brains of HI and LO mice (table 1), these differences were relatively small, and we wondered whether these changes in neurotransmitters were localized to specific brain regions. That catecholamine content in discrete areas of the brain may be an important determinant of anesthetic requirement is suggested by the decrease in anesthetic requirement that occurs when selected areas of the brain are destroyed. Destruction of the locus coeruleus or of the ventral bundle, which supplies a large fraction of norepinephrine to the central catecholamine gray area, decreases halothane and cyclopropane MAC in rats.\(^7\) Furthermore, cyclopropane, or halothane anesthesia increases norepinephrine and dopamine concentrations only in the locus coeruleus and central gray catecholamine area without changing total brain norepinephrine levels.\(^1\) This suggests that depression of norepinephrine release at specific sites in the brain is associated with anesthesia. Alternatively, anesthetics may desensitize the postsynaptic membrane; the increased transmitter levels may indicate the brain’s attempt to compensate for this desensitization. Turnover studies would have to be performed to determine which mechanism is operative.

We found higher levels of norepinephrine in the medulla of HI than of LO mice (tables 2 and 3) and a cor-

### Table 2. Norepinephrine and Dopamine Levels in Brain Regions of Mice Resistant (HI) and Susceptible (LO) to Nitrous Oxide Anesthesia\(^*\)

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>HI Mice ((n = 13))</th>
<th>LO Mice ((n = 11))</th>
<th>HI Mice ((n = 13))</th>
<th>LO Mice ((n = 11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>0.056 ± 0.027</td>
<td>0.712 ± 0.034</td>
<td>0.101 ± 0.012</td>
<td>0.114 ± 0.014</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.598 ± 0.055</td>
<td>0.447 ± 0.066</td>
<td>2.42 ± 0.67</td>
<td>1.44 ± 0.25</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.55 ± 0.19</td>
<td>1.35 ± 0.27</td>
<td>2.20 ± 0.69</td>
<td>2.21 ± 0.94</td>
</tr>
<tr>
<td>Pons</td>
<td>6.28 ± 1.24</td>
<td>5.98 ± 2.88</td>
<td>1.00 ± 0.17</td>
<td>1.46 ± 0.51</td>
</tr>
<tr>
<td>Medulla</td>
<td>5.54 ± 0.67</td>
<td>3.21 ± 0.41†</td>
<td>1.16 ± 0.25</td>
<td>0.530 ± 0.103‡</td>
</tr>
<tr>
<td>Midbrain</td>
<td>10.4 ± 1.04</td>
<td>9.02 ± 0.49</td>
<td>4.19 ± 0.42</td>
<td>4.72 ± 0.49</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>3.91 ± 0.45</td>
<td>6.08 ± 1.14</td>
<td>2.45 ± 0.69</td>
<td>2.49 ± 0.62</td>
</tr>
</tbody>
</table>

\(^*\) Mean values ± SE. Thirteen HI and 11 LO mice were examined in this series of experiments. Nitrous oxide ED\(_{50}\) for these HI mice was 1.81 ± 0.03 and for these low mice was 1.18 ± 0.03 atm. HI and LO mice differ from each other at a significance level of \(P < 0.001\) and \(P < 0.05\).
relation between nitrous oxide requirement in both HI and LO mice and medullary norepinephrine content (fig. 1). However, a large degree of scatter occurred in these values. Although we are uncertain of the reason for this variability, four explanations seem possible. First, "hot spots" of norepinephrine may exist at certain nuclei that border the areas of dissection, and slight inconsistencies in dissecting the mouse brain could give rise to large deviations in catecholamine content in the brain sections. Second, neurotransmitters may have been destroyed during the dissection of brain regions. Although we kept the specimens as cold as possible during the procedure, each brain required approximately 4 min to dissect, and the levels of neurotransmitters, especially dopamine, may have decayed during this period. Third, and we think most likely, differences between these two groups of mice could be localized to specific brain nuclei, and these differences may have been "swamped" by the variable levels in other brain stem nuclei, as seen in the experiments with cyclopropane or halothane anesthesia. This would suggest examining specific nuclei in the brains of additional colonies of these two groups of mice for differences in neurotransmitter content or turnover. A fourth possibility is suggested by the correlation coefficient of 0.443 (fig. 1). This coefficient suggests that only 20% of the difference in anesthetic requirements is accounted for by difference in norepinephrine content. That is, other unknown factors may be far more important.

These initial findings, however, require more extensive study. For example, if alterations in catecholamine effect are indeed important in explaining the separation in nitrous oxide requirement between HI and LO mice, a maximal drug-induced depletion of central catecholamines in selectively bred mice should remove this separation. Furthermore, processes that alter anesthetic requirement in these animals should be accompanied by a corresponding change in catecholamine content. For instance, we found that cross-mating HI and LO mice (i.e., mating one HI mouse with one LO mouse) produces offspring having nitrous oxide requirements that approximate the average nitrous oxide requirement of the parents. Compared with their parents, these offspring would be expected to have intermediate levels of catecholamines. Also, we found that nitrous oxide requirement decreased with age in HI mice but less so in LO mice. That is, with age, HI and LO mice tend to become equally sensitive to nitrous oxide. Thus, we would expect catecholamine levels to decrease with age in HI mice and to remain relatively unchanged in LO mice. We examined this hypothesis and did indeed find a rather strong correlation between the change in the difference in anesthetic requirements and in norepinephrine content in medullas of HI versus LO animals as they age (r = 0.996, see table 3 and fig. 2). These findings further strengthen the argument that central catecholamines play an important role in modulating or causing anesthesia. In addition, classic pharmacologic studies show that drugs that increase central catecholamine (alpha-1-adrenergic or dopaminergic release or availability) increase anesthetic require-

![Graph showing correlation of nitrous oxide requirement with medullary norepinephrine content]

**Table 3. Effect of Age on Anesthetic Requirements and Medullary Norepinephrine Concentration for Mice Resistant (HI Mice) and Susceptible (LO Mice) to Nitrous Oxide Anesthesia**

<table>
<thead>
<tr>
<th>Age (Day)</th>
<th>Nitrous Oxide ED₅₀ (atm)</th>
<th>Medullary Norepinephrine Concentration (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI Mice</td>
<td>LO Mice</td>
</tr>
<tr>
<td>100 (n=8, 8)</td>
<td>2.10 ± 0.02†</td>
<td>1.11 ± 0.02†</td>
</tr>
<tr>
<td>180 (n=7, 7)</td>
<td>1.90 ± 0.02†</td>
<td>1.07 ± 0.03†</td>
</tr>
<tr>
<td>600 (n=8, 8)</td>
<td>1.21 ± 0.06§</td>
<td>0.68 ± 0.05§</td>
</tr>
</tbody>
</table>

* Mean values ± 1 SE.
† n = number of HI and LO mice of each age.
‡ HI and LO mice of same age significantly different from each other at the P ≤ 0.001 level.
§ HI and LO mice of same age significantly different from each other at the P < 0.05 level.
¶ Mice different from animals of 100 days at the P < 0.05 level.
** Differences with age between HI and LO mice are significant at the P < 0.01 level.
ments, and drugs or lesions that decrease such availability decrease anesthetic requirements.5-7 Both our studies and those of others have shown the importance of sympathetic pathways to inflammatory injury and analgesia. Sympathetic, either central or peripheral, decreases inflammatory injury in an experimental model of arthritis,19 whereas alpha-2-agonists, which decrease central alpha-1-adrenergic and dopaminergic neurotransmission, increase analgesia and decrease anesthetic requirement.20-23 The site of these effects remains in question.

Finally, our data suggest another set of experiments to determine the site of such effects. Analogous lines of rats that are resistant and susceptible to cyclopropane anesthesia could be developed. Using these strains of rats, we could examine catecholamine content in specific brain nuclei; this cannot be done with mice because of the small size of their brains. Such experiments may help to define the principal areas of the brain that are important in producing anesthesia.

References
2. Kobrin DD, Deady JE, Eger EI II: Potencies of inhaled anesthetics and alcohol in mice selectively bred for resistance and susceptibility to nitrous oxide anesthesia. ANESTHESIOLOGY 56:18-24, 1982
5. Mueller RA, Smith RD, Spruill WA, Breese GR: Central monoaminergic neuronal effects on minimum alveolar concentrations (MAC) of halothane and cyclopropane in rats. ANESTHESIOLOGY 42:143-152, 1975

Fig. 2. Correlation of difference of anesthetic requirement with difference in concentration of norepinephrine in medulla. The line represents the best fit to the data using a linear regression analysis (r² = 0.956). The highest anesthetic requirements and content of norepinephrine (NE) were in the youngest mice, and the differences between susceptible and resistant mice in both anesthetic requirements and medullary NE content declined with age (see table 3, and text).