Pressor Response to Nitric Oxide Synthase
Inhibition during Halothane Anesthesia in Rats Is Altered by Inspired Oxygen Concentration

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THE purpose of these studies was to determine whether methodologic differences could explain a conflict in the literature regarding the role of nitric oxide (NO) on hemodynamics during halothane anesthesia. Some investigators1,2 have reported that NO synthase (NOS) inhibitors failed to increase blood pressure during halothane anesthesia in rats breathing room air and that halothane may interfere with NO-dependent circulatory control in vitro. However, we3 and others4,5 found that halothane anesthesia did not impair the expected increase in blood pressure after NOS inhibition in rats breathing air supplemented with oxygen. We hypothesized that blood oxygen tension was an important factor in the different responses to NOS inhibition because the only significant difference between the two groups of studies was the concentration of inspired oxygen.

Methods

Male Wistar rats (300-400 g) were used in these studies with approval from the Institutional Animal Care and Use Committee. Inhalation anesthesia was induced as previously described3 and maintained with halothane (1.2%, Halocarbon Laboratories, Riveredge, NJ) or isoflurane (1.4%, Ohmeda Caribe Inc., Guayama, Puerto Rico) in room air. Alternatively, rats were anesthetized with pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL) using an initial intraperitoneal dose of 60 mg/kg. All rats in the pentobarbital group required intravenous anesthesia supplementation (8-10 mg/kg) at least once during the protocol based on blood pressure responses to a tail clamp. Rats were mechanically ventilated and paralyzed with intravenous pancuronium bromide (1 mg/kg, Abbott Laboratories) to facilitate ventilation.3 Inspired O₂ concentration was constantly monitored (Critikon Inc., Tampa, FL). Catheters were placed in the femoral artery and vein for direct blood pressure monitoring and drug administration, respectively. Body temperature was maintained at 37-38°C by a heating pad. Arterial blood (200 μl) was sampled periodically and analyzed for pH, PaO₂, and PaCO₂ (Corning 168, Medfield, MA). PaCO₂ was maintained at normal values (approximately 35-40 mmHg) by changing ventilation rate. All animals received a saline infusion (1 ml/h) to maintain intravascular volume.

During a 60-min equilibration period, animals were ventilated with room air (pentobarbital group) or volatile anesthetic in room air. After equilibration, mean arterial blood pressure (MAP, determined every 5 min) was monitored for an additional 15 min, and arterial blood gases were measured to determine baseline values. At t = 0, saline (0.5 ml) or the NOS inhibitor N⁶-monomethyl-L-arginine (L-NMMA, 100 mg/kg in 0.5 ml over 30 min, CYCLOPSS Biochem, Salt Lake City, UT) was infused, as previously described.5,6 At t = 15 min, additional oxygen was added to the inspired gas mixture to obtain a concentration of 30%. Some rats did not receive supplemental oxygen. The L-NMMA or control saline infusion was completed at t = 30 min, and another blood gas sample was obtained. The inspired gas was changed to room air at t = 45 or left at 30% O₂. A final blood gas sample was obtained at t = 60. Data were ex-
pressed as mean ± SEM. Comparisons among groups and treatment periods were made using analysis of variance (ANOVA; two-way with repeated measures) and Student-Newman-Keuls method. Differences among groups were judged significant if $P < 0.05$.

**Results**

The effect of oxygen supplementation on the pressor response to L-NMMA during anesthesia with pentobarbital, halothane, or isoflurane is shown in figure 1. L-NMMA significantly increased MAP in all groups. Increasing the inspired oxygen concentration from 21% (room air) to 30% did not influence MAP significantly in any group. When the added oxygen in the inspired gas was replaced with room air at $t = 45$, MAP returned toward baseline in the rats anesthetized with halothane. In contrast, there were no significant changes in MAP from $t = 15$ to $t = 60$ in the isoflurane or pentobarbital anesthesia groups, as determined by 2-way repeated measures ANOVA (i.e., blood pressures remained elevated).

As shown in figure 2, the effects of NOS inhibition and O$_2$ supplementation on MAP during halothane anesthesia were investigated by (1) changing the inspired concentration of O$_2$ alone, (2) administration of L-NMMA alone (with room-air breathing), and (3) leaving the inspired O$_2$ at 30% from $t = 15$ until the end of the experiment. Increasing the inspired O$_2$ from 21% (room air) to 30% in the absence of L-NMMA did not significantly increase MAP. L-NMMA alone transiently increased MAP (69 ± 3 to 108 ± 6 mmHg, $t = 15$). In contrast, MAP was sustained in the presence of L-NMMA by leaving the inspired O$_2$ at 30% after $t = 15$.

Increasing the inspired oxygen concentration from 21% to 30% increased PaO$_2$ in all groups, as shown in figure 3. Replacing the oxygen enriched gas with room air restored PaO$_2$ to initial values in all groups. PaCO$_2$ values remained constant.

**Discussion**

These data demonstrate that inhibition of NOS with L-NMMA increased MAP during anesthesia with halothane, isoflurane, or pentobarbital, although during halothane anesthesia, MAP remained increased after NOS inhibition only when the inspired gas was enriched with 30% O$_2$. Our data suggest that the reason that some investigators may not have observed an increase in MAP after NOS inhibition *in vivo* during halothane anesthesia was that the animals were hypoxic. In studies by Wang *et al.* and Simon *et al.*, spontaneously breathing rats were anesthetized with halothane in room air, a situation known to produce low PaO$_2$ values in the 50-

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60 mmHg range. Combined with data suggesting that microvascular oxygen tension would be low under these conditions (<25 mmHg) and that NOS activity is inhibited when PaO_2 values are less than 40 mmHg, room air breathing during anesthesia could result in relative hypoxia in the tissues and a reduction in NOS activity. For example, in the pentobarbital anesthetized spontaneously breathing hamster, Dulung and Berne showed that while femoral artery PaO_2 was 69 ± 4 mmHg, PaO_2 in the cheek pouch microcirculation was 24 ± 3 mmHg in small arterioles and 8 ± 2 mmHg in the tissue.

The mechanism for the reduced vasopressor response to NOS inhibition during halothane anesthesia is not clear. Our data clearly show that a low PaO_2 was not the only reason for the decrease in blood pressure after NOS inhibition during halothane anesthesia because blood gas values were similar in all groups at similar time points in the protocol (see fig. 3). A specific halothane-induced change in the activity of NOS was also unlikely because NOS activity is unaffected by these volatile anesthetics. In addition, isoflurane and halothane do not alter guanylyl cyclase activity, the first step in NO stimulated relaxation of vascular smooth muscle.

One could argue that the different initial blood pressures among groups may have contributed to the results, although the relative pressor responses to L-NMMA were not different (MAP increased by 142% [halothane]; 162% [isoflurane]; or 151% [pentobarbital]). The critical difference between groups in our experiments appeared to be the concentration of O_2 in the inspired gas. When inspired O_2 was maintained at 30% during halothane anesthesia, the MAP response to L-NMMA administration was sustained. However, when breathing room air, the pressor response to NOS inhibition was transient, and MAP returned to control values rapidly, as shown in figure 2. These findings in rats breathing room air were similar to others using different NOS inhibitors (L-NNA, L-NAME) who did not supplement the inspired gas with O_2 during halothane anesthesia, indicating that the response was not specific to the NOS inhibitor used.

These experiments confirm that methodologic differences could explain why some investigators reported that administration of nitric oxide synthase (NOS) inhibitors failed to increase MAP during halothane anesthesia. Further, they suggest that the NO pathway is more sensitive to the effects of moderate hypoxia produced by room air breathing during halothane anesthesia compared with pentobarbital or isoflurane anesthesia.

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References


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