Anesth Prog 38:57–64 1991

Macro- and Microvascular Effects of Nitrous Oxide in the Rat

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The aims of this study were: (1) to determine the macro- and microvascular actions of nitrous oxide (N_2O) in the rat, and (2) to determine whether the vascular actions of N_2O involved specific interactions with α-adrenergic receptors or opioid receptors. Systolic blood pressure, heart rate, total tail blood flow, blood cell velocity in subepidermal capillaries of the tail, and percentage of capillaries exhibiting flow were monitored in conscious rats during the administration of N_2O before and after administration of clonidine (an α_2-adrenergic agonist), prazosin (an α_1-adrenergic antagonist) or naloxone (an opioid antagonist). Total tail blood flow increased significantly in a dose-dependent manner with N_2O at 20% and 40% with oxygen. This action of N_2O was not blocked by clonidine, prazosin, or naloxone. Capillary flow velocity increased during 20% and 40% N_2O compared to 100% O_2, but the changes were not statistically significant nor did they correlate with the changes in tail blood flow. These data suggest that the peripheral vascular action of N_2O does not involve specific actions at α-adrenergic receptors or opioid receptors and may be the result of direct actions on the peripheral vasculature.

Nitrous oxide (N_2O) is frequently employed as an inhalation/anxiolytic/analgesic agent in dentistry.[1–3] It is administered either alone or in combination with local anesthetics and other sedative, analgesic, anxiolytic, or general anesthetic agents. Thus, it is important to understand all aspects of the pharmacology of this agent, including its cardiovascular actions.

There is controversy in the literature concerning the peripheral vascular effects of N_2O and the mechanism(s) through which they occur. Some investigators have proposed that inhalation of N_2O results in an increase in sympathetic nervous system activity, indicated by an increased systemic vascular resistance, increased serum and urinary catecholamine concentrations, and maintenance of systemic arterial pressure.[4,5] These peripheral effects, which are accompanied by a small depression of myocardial function, were considered to be a reflection of increased central sympathetic outflow. Studies in both humans and animals suggest that N_2O may produce an intermittent sympathetic discharge.[6]

Other, contradictory findings include those from a study[7] reporting that the vasodilation following N_2O was caused by a redistribution of forearm and hand blood flow resulting from simultaneous cutaneous vasodilation and constriction of skeletal muscle vasculature. Another recent investigation showed that N_2O induced a dose-dependent decrease in both systolic blood pressure and heart rate in spontaneously hypertensive and control rats,[8] indicating that N_2O is probably not contraindicated in cardiovascularly compromised dental outpatients, as had been previously suggested.[9,10] Additionally, another recent study[11] showed that N_2O produced a cerebrovasodilation in rats that was not related to a change in metabolic demand. Plasma catecholamines did not change, indicating that the increase in blood flow was not due to a general stress response.

Deutsch and Samra[12] demonstrated increased cortical blood flow in humans receiving N_2O. The N_2O-induced blood flow changes differed from those due to simple vasodilatory agents, such as carbon dioxide, and were thought to reflect differential effects on cerebral metabolism. The authors stated that their results contradicted some frequently cited clinical studies[13,14] but were consistent with most animal studies and some human data. They

Received April 24, 1991; accepted for publication June 17, 1991.

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ISSN 0003-3006/91/$3.50

proposed two possible explanations for the differences in results among studies of the mechanism of action of N_2O: (1) their highest N_2O concentration was 50%, whereas others used 70%, and (2) the concomitant use of other drugs in some previous studies masked the true effect of N_2O.

In previous human experiments using strain-gauge plethysmography, videomicroscopy, and ultrasound Doppler flowmetry, we found that: (1) inhalation of a sedative concentration (33%) of N_2O resulted in an increase in radial artery and digit blood flow in young subjects; (2) this effect was due to the N_2O and not the coadministered oxygen (O_2); and (3) changes in arterial blood pressure and heart rate were small and could not account for the increased peripheral blood flow.[15,16] Interestingly, the increase in macrovascular flow we observed was not reflected in increased flow in the nailfold capillaries.

We previously investigated the effects of N_2O on cutaneous capillary blood flow in an abdominal skin flap in rats.[17] Capillary blood flow velocity and vascular conductance were both significantly increased by 40% N_2O administered with O_2 as the carrier gas.

Experimental evidence indicates that some actions of N_2O, particularly analgesia, may involve interactions with the endogenous brain opioid peptide system. Two areas of investigation support this interaction. First, several studies[18–20] implicate N_2O in the synthesis and release of endogenous opioid peptides. Second, reversal or blockade of analgesia from N_2O has been demonstrated with opioid antagonists.[21–29]

The contradictory findings regarding the cardiovascular effects of N_2O are difficult to resolve because of methodological differences in the studies reported in the literature. Confounding factors include multiple drugs in anesthetic gas mixtures, administration of preanesthetic drugs, the sympathetic tone of the subject at the time of N_2O inhalation, and the concentration and depth of sedation/anesthesia.

In an attempt to clarify the contradictory results of previous studies, we designed the present study to determine the macro- and microvascular effects of sedative concentrations of N_2O alone in the unanesthetized rat and to investigate the mechanism(s) through which they occur. The protocol for N_2O administration was designed to mimic the clinical use of N_2O in dentistry, except that no local anesthesia of the oral cavity was involved. N_2O was administered with O_2 in sedative doses, and no other anesthetics or anesthetic adjuvants were administered. The cardiovascular effects of N_2O were assessed before and after the administration of drugs that alter pathways through which N_2O has been proposed to act. These agents were clonidine, a central α_2-adrenergic agonist that decreases central adrenergic outflow, prazosin, an α_1-adrenergic antagonist that blocks direct peripheral action on α_1-receptors, and naloxone, a pure opioid antagonist that blocks any actions N_2O might have at central opioid receptors.

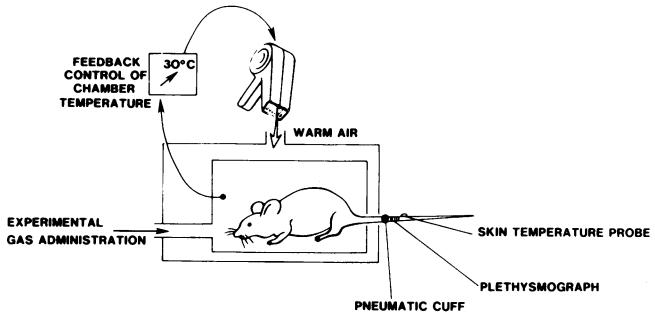

Figure 1. Schematic diagram of the experimental setup. Experimental gases were administered to rats enclosed in a double-walled, temperature-controlled chamber using a flow-through technique. Environmental chamber temperature was maintained at 30°C and tail temperature was monitored but left at room temperature (approximately 25°C). Also illustrated is the placement of the pneumatic cuff and plethysmograph used to determine systolic blood pressure and total tail blood flow.

METHODS

Animals

Adult (age 5–15 mo) male Sprague-Dawley rats were used in this study. This particular strain of rats has been used by us in previous cutaneous microcirculatory studies.[30] The animals were housed two per cage and were allowed free access to water and food. Animals were weighed prior to each experiment so that proper dosage for drugs could be calculated. The tails of all rats were carefully shaved to allow better visualization of the microvasculature. This was done 2 days prior to the experimental procedures to ensure recovery from any local trauma resulting from the shaving.

All experiments were approved by the Institutional Animal Care and Use Committee in accordance with federal regulations.

Experimental Setup

Unanesthetized animals were placed in an adjustable restraining cage and then into a larger Plexiglas chamber so that environmental temperature could be controlled and N_2O and O_2 could be administered by a flow-through technique (Figure 1). Temperature in the body chamber was monitored by a sensor probe (YSI Model 401) and maintained at 30°C using a feedback controlled heater. The tail was left exposed to room temperature. A colonic Probe (YSI Model 402) and a surface probe (YSI Model

Table 1. Cardiovascular Effects of Nitrous Oxide in the Rat

Parameter	*Nitrous Oxide Concentration*[a]		
	0%	*20%*	*40%*
Systolic Blood Pressure (mm Hg)	202 ± 7	203 ± 9	196 ± 7
Heart Rate (beats/min)	342 ± 8	337 ± 7	341 ± 8
Tail Blood Flow (mL/min/100 g tissue)	3.00 ± 0.38	3.50 ± 0.36	5.06 ± 0.79[b]
Capillary Flow Velocity (mm/sec)	0.088 ± 0.009	0.091 ± 0.008	0.093 ± 0.007
% Vessels Flowing (% in field)	50.3 ± 5.0	52.7 ± 5.6	57.0 ± 5.4

[a] Mean values are shown ± SE; $n = 18$.
[b] Significantly different ($P \leq 0.05$) from 0% nitrous oxide (100% oxygen).

427) were placed to monitor the animals' core and tail surface temperatures, which remained at 24.3 ± 1.32 and 38.7 ± 0.53°C, respectively (mean ± SE). N_2O and O_2 were administered directly into the small chamber within the larger, temperature-controlled chamber. The experimental chamber was designed such that the experimental gases could be changed rapidly, thereby allowing random administration of various N_2O concentrations.

Blood Flow, Heart Rate, and Blood Pressure

A pneumatic digit cuff (D.E. Hokanson, Inc., Issaquah, WA) was placed around the most proximal segment of the rat's tail. A 25-cm mercury-in-silastic strain gauge (Parks Electronics Lab., Beaverton, OR) was wrapped around the rat's tail just distal to the digit cuff. The number of circumferential wraps was recorded along with tail diameter and length to permit calculation of tail blood flow.[15,31] To allow calculation of total tail blood flow (TBF) the output from the strain gauge was recorded on a Grass Model 7 polygraph during a 5-sec occlusion of venous blood flow from the tail, as achieved by rapid inflation of the digit cuff to 60 mm Hg. The initial slope of the strain gauge output was used to calculate TBF by accepted methods.[31] Near the end of each experimental period, a series of five to eight measurements of TBF were so obtained. Heart rate was determined from the same tracing by counting the pulses per unit time from the recorded blood pressure tracing.

Capillary Erythrocyte Velocity

To obtain measures of capillary flow velocity (CFV), the rat's tail was stabilized on the stage of a Leitz microscope, and the microvasculature within the subepidermal plexus was visualized using a 20X oil immersion epiillumination objective. A Newvicon 67M TV camera was mounted on the Leitz microscope and coupled through a time-code generator (Instrumentation for Physiology and Medicine Model 710) to a television monitor for viewing of microvascular fields and finally to a videocassette recorder (Panasonic NV8410) for recording and subsequent analysis of microvascular dynamics. Descriptions of the subepider-

Figure 2. Effects of N_2O administered with O_2 on total tail blood flow (TBF) and capillary blood velocity (CFV) in the rat both before and after clonidine administration. Brackets indicate ± SE ($n = 8$).

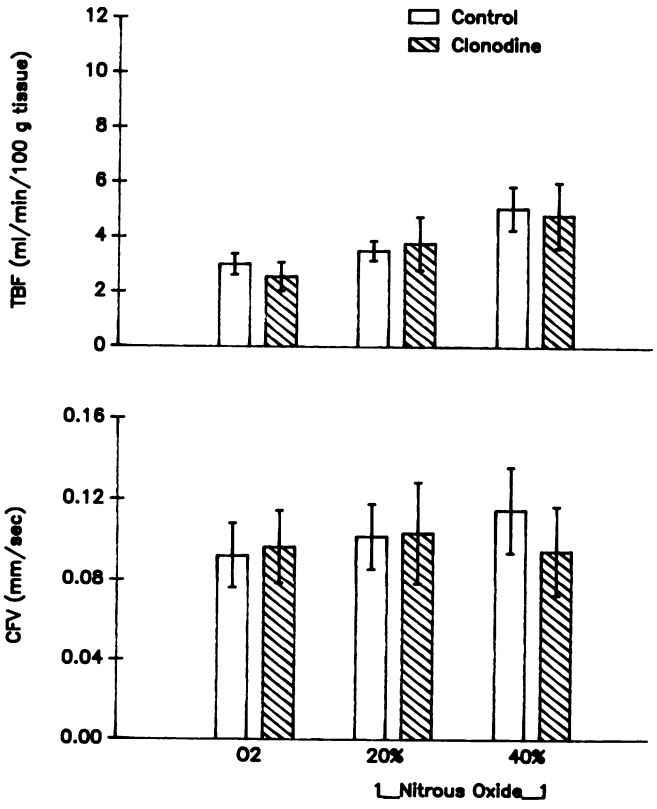

Table 2. Cardiovascular Effects of Nitrous Oxide After Clonidine Pretreatment

	Nitrous Oxide Concentration[a]		
Parameter	*0%*	*20%*	*40%*
Systolic Blood Pressure (mm Hg)	210 ± 9	204 ± 10	216 ± 10
Heart Rate (beats/min)	282 ± 15	288 ± 15	272 ± 9
Tail Blood Flow (mL/min/100 g tissue)	2.55 ± 0.52	3.77 ± 0.97	4.81 ± 1.19[b]
Capillary Flow Velocity (mm/sec)	0.100 ± 0.016	0.096 ± 0.012	0.104 ± 0.016
% Vessels Flowing (% in field)	50.8 ± 7.4	58.9 ± 5.8	57.3 ± 7.9

[a] Mean values are shown ± SE; $n = 8$.
[b] Significantly different ($P \leq 0.05$) from 0% nitrous oxide (100% oxygen).

mal vascular plexus of the rat tail and the videomicroscopy recording and analysis system used are given elsewhere.[30]

The same vascular field was monitored throughout each experimental session, so that changes in individual vessels could be compared. In addition, capillary recruitment data, ie, the changes in number of capillaries exhibiting active flow during a given treatment period were determined by simply counting the total number of vessels per field and the number of "active" vessels (vessels exhibiting blood flow) from the video images of that period.

Experimental Protocol

Animals were placed in the temperature-controlled chamber and instrumented as described above, and a subepidermal vascular field was brought into view on the television monitor. After bringing the vascular field into view, the animal was allowed at least 15 min to stabilize before any data were recorded. Blood flow was visually evident in several individual vessels in any given microscopic field. Videoimages of blood flow in microvessels were recorded for at least 3 min during each treatment period for later analysis. Following videotaping for CFV analysis, TBF, systolic arterial blood pressure (SBP), and heart rate (HR) were determined.

In each experimental run, 100% O_2 was first administered followed by 20% and 40% N_2O in O_2. Concentrations of N_2O were administered in random order in individual experiments, ie, the order of presentation was varied such that 20% N_2O was given before the 40% concentration in half the experiments. Test gases were administered using a dental analgesia unit (Quantiflex M.D.M.). Total gas flow was set initially at 5 L/min for 1 min to flush the chamber and then decreased to 2 L/min for 10 min to allow equilibration before any data were recorded. Data are presented as changes seen with N_2O compared to O_2, the carrier gas.

To explore the mechanism involved in changes seen with N_2O in TBF, peripheral vascular responses to N_2O were recorded before and after the administration of the antagonists naloxone and clonidine. The same microvascular field was observed throughout each experiment.

Table 3. Cardiovascular Effects of Nitrous Oxide After Prazosin Pretreatment

	Nitrous Oxide Concentration[a]		
Parameter	*0%*	*20%*	*40%*
Systolic Blood Pressure (mm Hg)	156 ± 12	149 ± 10	162 ± 11
Heart Rate (beats/min)	374 ± 19	367 ± 33	398 ± 29
Tail Blood Flow (mL/min/100 g tissue)	1.55 ± 0.35	1.98 ± 0.46[b]	2.18 ± 0.51[b]
Capillary Flow Velocity (mm/sec)	0.054 ± 0.011	0.023 ± 0.003	0.065 ± 0.014
% Vessels Flowing (% in field)	26.8 ± 7.8	30.3 ± 8.5	20.5 ± 5.7

[a] Mean values are shown ± SE; $n = 8$.
[b] Significantly different ($P \leq 0.05$) from 0% nitrous oxide (100% oxygen).

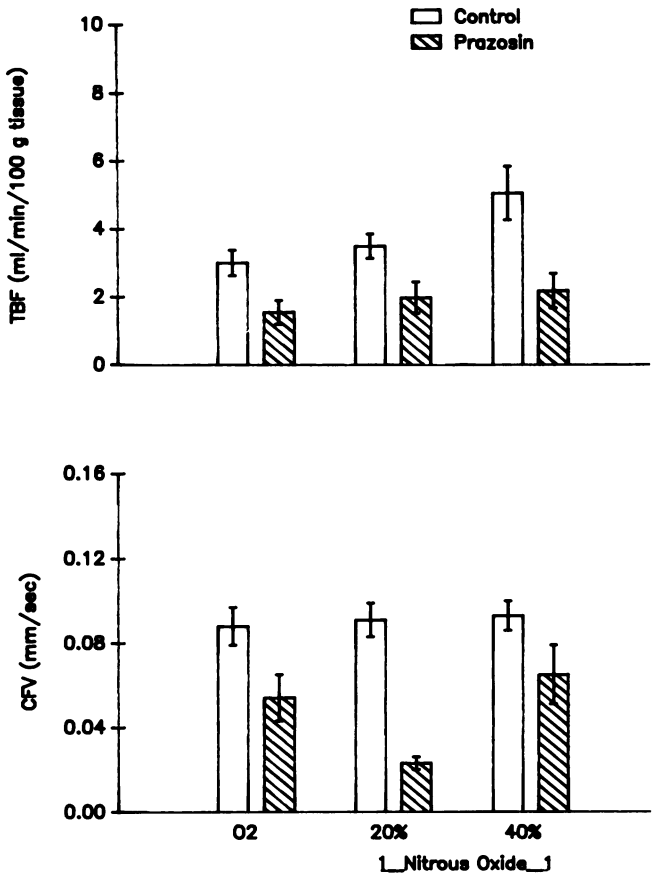

Figure 3. Effects of N_2O administration with O_2 on total blood flow (TBF) and capillary flow velocity (CFV) in control (n = 18) and prazosin-treated (n = 8) rats. Brackets indicate ± SE.

These agents were administered at least 15 min prior to the second administration of the O_2 and N_2O. All interactive drugs were prepared fresh for each experiment in normal saline. Naloxone and clonidine were prepared in normal saline and administered subcutaneously (SC) at 20 mg/kg and 50 μg/kg, respectively. Prazosin was administered to naive animals (ie, animals not exposed to a control test period). The drug was prepared by suspension and sonication in saline prior to injection SC. The drug doses were chosen based on previously published reports.[25,32,33] Preliminary experiments showed little effect of naloxone or clonidine alone. However, during preliminary experiments with 1 mg/kg prazosin, capillary flow completely stopped in some animals; thus, the dose was reduced to 0.5 mg/kg for the final studies. The dramatic decrease in capillary blood flow was likely the result of decreased systemic blood pressure.

Statistical Analysis of Data

A one-way analysis of variance (ANOVA) with repeated measures was used to evaluate the effects of N_2O alone, whereas a two-way ANOVA with repeated measures was used to evaluate the interactions between N_2O and the antagonist drugs. A significant difference was accepted at $P \leq 0.05$. Correlation analysis (Pearson r) was used to compare the relationship of the effects of N_2O on TBF and CBV.

RESULTS

The micro- and macrovascular effects of N_2O alone are presented first, followed by the results obtained during the administration of N_2O after pretreatment with clonidine, prazosin, and naloxone. Basal cardiovascular parameters were within normal limits previously reported for awake, restrained rats.[34–36]

Effects of N_2O with O_2

SBP and the percentage of microvessels exhibiting active flow (%VF) remained statistically unchanged through all N_2O inhalation periods, though %VF did increase slightly in a dose-dependent manner during N_2O administration (Table 1). TBF increased significantly in a dose-dependent

Figure 4. Effects of N_2O administration with O_2 on total tail blood flow (TBF) and capillary flow velocity (CFV) in the rat both before and after naloxone administration. Brackets indicate ± SE (n = 10).

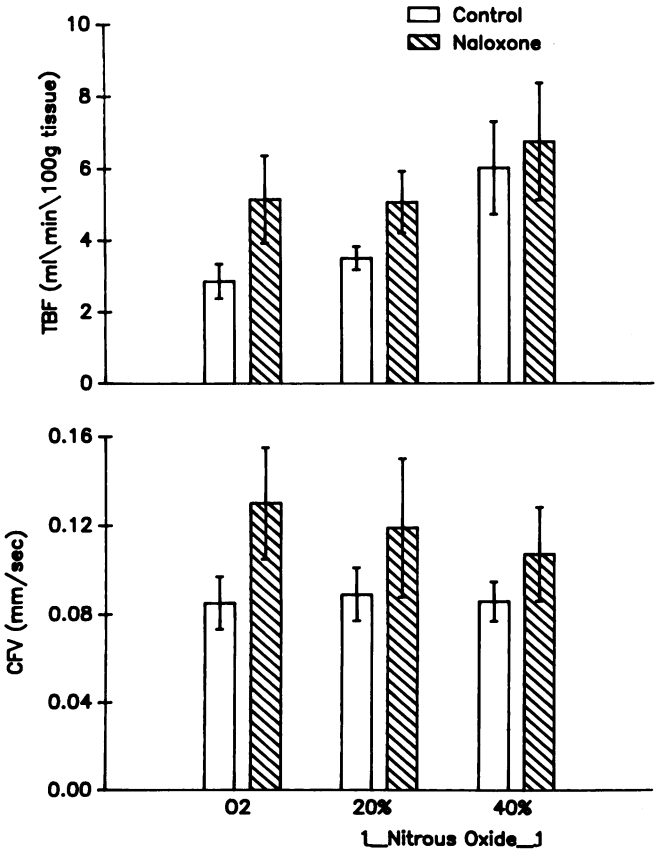

Table 4. Cardiovascular Effects of Nitrous Oxide After Naloxone Pretreatment

	Nitrous Oxide Concentration[a]		
Parameter	*0%*	*20%*	*40%*
Systolic Blood Pressure (mm Hg)	192 ± 8	189 ± 7	187 ± 8
Heart Rate (beats/min)	319 ± 9	327 ± 10	335 ± 10
Tail Blood Flow (mL/min/100 g tissue)	5.2 ± 1.2	5.1 ± 0.8	6.8 ± 1.6[b]
Capillary Flow Velocity (mm/sec)	0.130 ± 0.024	0.119 ± 0.031	0.107 ± 0.022
% Vessels Flowing (% in field)	51.5 ± 6.8	53.6 ± 6.8	46.6 ± 4.9

[a] *Mean values are shown ± SE; $n = 10$.
[b] Significantly different ($P < 0.05$) from 0% nitrous oxide (100% oxygen).

manner with N_2O. Capillary flow velocity (CFV) also increased during 20% and 40% N_2O compared with 100% O_2, but the changes were quite small and not statistically significant. The increases in TBF and CFV did not appear to be related ($r = 0.11$).

Effect of Clonidine on Response to N_2O

Clonidine decreased TBF, HR, and SBP compared with its own pretreatment control. N_2O at 20% and 40% after clonidine pretreatment caused similar significant increases in TBF to those seen before clonidine (Figure 2). CFV was not altered by N_2O following clonidine (Table 2). No significant differences were seen in %VF within control or clonidine periods or between control and clonidine treatment periods.

Effect of Prazosin on Response to N_2O

Prazosin alone caused significant decreases in CFV, SBP, TBF, and %VF when compared with the pooled control data of the clonidine and naloxone groups; concurrently, HR increased significantly. N_2O after prazosin pretreatment again showed little effect on SBP, %VF, and HR (Table 3) but increased TBF in a dose-related fashion (Figure 3).

Effect of Naloxone on Response to N_2O

HR was significantly decreased by naloxone, CFV was significantly increased, and SBP was unchanged. TBF increased significantly during N_2O at 40% N_2O compared with 100% O_2 both before and after naloxone (Figure 4). No significant differences in %VF were noted within or between the control or post-naloxone (Table 4) series of gas administration.

DISCUSSION

As previously mentioned, studies of humans in our laboratory and of both humans and animals by others have yielded conflicting results with respect to the cardiovascular actions of N_2O. In an attempt to clarify the above controversy we used the rat model so that the vascular actions of N_2O could be assessed in detail. We also used N_2O alone, ie, without other anesthetic or sedative agents.

N_2O increased macrovascular (tail) blood flow in rats (Table 1). The increase was dose-dependent and statistically significant. There was no related significant increase in numbers of vessels exhibiting flow under the conditions employed in this study. Moreover, increases observed in TBF and CFV were not correlated. This lack of relationship between regional cutaneous blood flow and flow in individual capillaries is consistent with what has been observed in human skin.[37]

To explore the notion that N_2O acts through central or peripheral alterations in sympathetic nervous system activity, the effects of N_2O were evaluated following pretreatment of rats with clonidine to block central sympathetic outflow or prazosin to block peripheral stimulation of vasoconstrictive α_1-adrenergic receptors. Though pretreatment with the antihypertensive agent clonidine alone caused expected decreases in TBF, HR, and SBP, subsequent administration of N_2O induced dose-dependent increases in TBF as before clonidine. These data indicate that N_2O does not increase peripheral vascular flow by decreasing central sympathetic outflow. Prazosin alone caused significant decreases in CFV, SBP, TBF, and %VF. It is proposed that these changes are secondary to intense peripheral vasodilation, which shifts blood from the extremities (including the tail) to the trunk. Again, N_2O administered following pretreatment with prazosin caused dose-dependent increases in TBF as in control animals, indicating that N_2O does not act through peripheral α_1-adrenergic receptors.

Previous studies by others involving the analgesic actions of N_2O indicated that the drug may be acting at central opioid receptors.[18–20,23–28] To test whether the vascular actions that we demonstrated following N_2O involved opioid receptors, we recorded the responses to N_2O both before and after naloxone, a pure opioid antagonist. Despite some alteration of TBF by naloxone alone, the response to N_2O remained intact after naloxone administration, indicating that this response did not involve an action of N_2O at specific opioid receptors. A recent study supports this conclusion in that cerebral blood flow was increased by N_2O but this increase was not altered by prior administration of morphine.[38]

CONCLUSIONS

Taken together, the present findings indicate that N_2O does not act directly at central α_2-adrenergic, peripheral α_1-adrenergic, or opioid receptors. Its actions may involve some other specific receptor but more likely involves a direct, nonspecific action on the vasculature.

ACKNOWLEDGMENTS

The authors thank Cynthia Davis for assistance in data analysis and preparation of this manuscript. This research was supported by the University of Kentucky Research Foundation.

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