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Differential Effects of Nitrous Oxide on Baroreflex Control of Heart Rate and Peripheral Sympathetic Nerve Activity in Humans

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Acute regulation of blood pressure in humans is mediated by arterial baroreflex regulation of heart rate, cardiac contractility, and peripheral sympathetic outflow. Brief pharmacologic reductions of blood pressure were employed in 11 healthy volunteers to determine the effects of N_2O on baroreflex-mediated increases in heart rate and efferent muscle sympathetic nerve activity. R-R intervals (ECG), blood pressure (radial artery), central venous pressure, respiratory rate (abdominal bellows), and end-tidal gas concentrations (mass spectrometer) were monitored. Efferent sympathetic nerve activity directed to skeletal muscle blood vessels (MSNA) was recorded from an epoxy-coated tungsten needle placed into the peroneal nerve. Data were obtained from six subjects before and during iv bolus administration of sodium nitroprusside (100 μg), during control while breathing 40% N_2/60% O_2, during administration of N_2O (40% N_2O/60% O_2), and during recovery (40% N_2/60% O_2). Five subjects served as time controls and breathed 40% N_2 in O_2 throughout the protocol. Nitrous oxide produced a 59 ± 18% ($P < 0.05$) increase in baseline MSNA but did not alter the reflex augmentations in MSNA produced by nitroprusside. In contrast, there was a 39 ± 14% decrease in the slope of the relationship between systolic pressure and R-R interval ($P < 0.05$) in subjects breathing N_2O. N_2O thus produces activation of the sympathetic nerves directed to skeletal muscle blood vessels, and it decreases baroreflex-mediated tachycardia without diminishing baroreflex-mediated augmentations in sympathetic outflow. (Key words: Anesthetics, gases: nitrous oxide. Blood pressure. Sympathetic nervous system: baroreceptors.)

SEVERAL STUDIES indicate that enflurane, halothane, and isoflurane diminish the baroreceptor reflex.[1–4] Compared with the effect of these agents, N_2O may exert only subtle effects on baroreflex function.[5] Most, if not all, previous studies in humans have examined only the effects of the inhaled anesthetics on arterial baroreceptor control of heart rate.[1–5] Changes in heart rate that are mediated predominantly by cardiac vagal activity[5–7] reflect only one limb of the baroreflex response to blood pressure perturbations. Because ventricular diastolic filling time and stroke volume are inversely proportional to baroreflex-mediated heart rate changes,[8,9] the net baroreflex effect on cardiac output to maintain blood pressure homeostasis may be relatively small. In contrast, baroreflex-mediated changes in peripheral sympathetic outflow that alter vascular resistances may have a major role in buffering blood pressure perturbations in humans. Relatively little information exists on how anesthetics influence peripheral sympathetic outflow in humans. Moreover, there are few published data describing baroreflex-mediated responses to decreasing blood pressure. This is because most previous studies of baroreceptor reflexes have only examined heart rate responses to increasing blood pressure (by injection of either phenylephrine or angiotensin).[1–3,5] For example, an earlier report suggests that baroreflex-mediated slowing of heart rate in humans is reduced by N_2O administration, but this reduction is less than that produced by halothane.[1] In this study the effects of N_2O on baseline and reflex control of peripheral sympathetic nerve responses directed to skeletal muscle blood vessels were determined with microneurographic recordings from the peroneal nerve of human volunteers. We present data demonstrating that N_2O produces a differential effect on heart rate and efferent sympathetic nerve responses during sodium nitroprusside-induced hypotension.

Materials and Methods

With approval of the Institution's Human Research Review Committee, informed consent was obtained from eleven ASA Physical Status 1 volunteers. All subjects were NPO and unmedicated. A peripheral 18-G iv catheter was inserted for drug infusions. A 20-G catheter was inserted into the radial artery for direct measurement of blood pressure. An 18-G catheter was placed into the external jugular vein and advanced to an intrathoracic location for measurement of central venous pressure. A small bellows was strapped to the abdomen to record excursions produced by respiration. R-R intervals were calculated from lead II of the ECG. Multiunit sympathetic recordings were obtained from the common peroneal nerve. The location of this nerve was identified below the bony prominence at the head of the fibula on the lateral aspect of the right leg by application of brief (0.1 s) electric pulses (40 V, 1 Hz) to a probe moved about the skin surface to elicit muscle contractions distal to the probe. The skin was then cleansed and two epoxy-coated tungsten microelectrodes with 5 μm diameter exposed tips, and 0.2-mm diameter shafts (Tektronics Medical Instruments, Iowa City, Iowa) were inserted in close proximity over

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the peroneal nerve. One microelectrode was advanced below the fibular head while impulses of 0.5 V (0.1 s, 1 Hz) were applied through its tip. A nerve fascicle within the peroneal nerve innervating muscle was identified by observing twitches of the lower leg or foot. Stimulation was then halted and the reference electrode was advanced until its tip was near the recording electrode but not within the nerve. Signals from both microelectrodes were directed to a custom-made, differential preamplifier in which signals common to both electrodes were canceled (*e.g.*, 60 cycle and electrostatic noise). The remaining unique signal from the nerve was amplified 1,000-fold, filtered below 400 and above 2,000 Hz, and amplified again 100-fold. The signal was then integrated in 100 ms intervals and displayed on an oscilloscope. Signal discrimination was employed for the audio output.

Characteristic integrated muscle sympathetic bursts were sought by fine manipulations of the microelectrode within the muscle fascicle. These characteristics have been previously described and validated.[10,11] Briefly, stretching muscles or tapping tendons of muscles innervated by the nerve elicits afferent bursts of neural activity from mechanoreceptors, whereas light stroking of the skin in the innervated area does not. An increase in spontaneous efferent bursts of sympathetic activity can be demonstrated during the hypotension that occurs in Phase II and III of Valsalva's maneuver and during hypoxia and hypercarbia that occurs during prolonged voluntary apnea. Efferent bursts frequently occur in pulse-synchronous groups and are often phase-locked to late expiration and early inspiration. Once an acceptable site for recording is located, subjects remain still and at ease to avoid altering the microelectrode tip location.

A comfortable-fitting face mask was applied and all subjects breathed a mixture of 40% N_2 and 60% O_2 delivered at 6 l/min into a semiclosed circle system for a 15-min control period. End-tidal gas concentrations were continuously monitored by mass spectrometry. ECG, arterial blood pressure, respiration, and integrated muscle sympathetic nerve activity were monitored in real-time by a computer. Three-minute epochs of data were rapidly transferred to disk throughout the study, and these were analyzed off-line. Five minutes after application of the mask, when heart rate, blood pressure, and end-tidal nitrogen and CO_2 concentrations were stable, two consecutive 3-min epochs of data were recorded. A 100-μg iv bolus of sodium nitroprusside was then given to transiently decrease blood pressure while a third 3-min data sample was obtained. Following a 5-min recovery, five subjects continued to breathe the O_2/N_2 mixture for an additional 20 min (time-control) while the other six subjects received N_2O. In these six subjects N_2O in O_2 was administered gradually over the first 10 min to achieve a steady state end-tidal concentration of 40%. Repeat data were then obtained during quiet breathing and followed by a 100-μg bolus of sodium nitroprusside. N_2O was then discontinued and replaced with 40% N_2 (and 60% O_2). Recovery data, which included those collected during and following a third bolus of sodium nitroprusside, were collected 8–10 min later.

Computer analyses of sympathetic recordings enabled derivation of burst frequency and burst amplitude. The mathematical product of frequency times amplitude can be used as a quantitative index of "total" sympathetic activity if the position of the microneedle within the peroneal nerve remains fixed throughout the testing protocol. Reflex responses to reductions in blood pressure were quantitated by applying stepwise, least-squares regression analyses to the linear portion of the relationship between systolic or mean pressure and R-R interval. Similar analyses were applied to the linear portion of the relationship between diastolic pressure and sympathetic nerve activity. Prior to applying statistics, variations in R-R intervals and muscle sympathetic nerve activity that may be related to respiration or fluctuating descending influences from higher CNS centers were averaged. All the R-R intervals or MSNA that occurred in each 2–3 mmHg increment of blood pressure were averaged and plotted prior to application of regression statistics. Absolute changes from baseline and changes between groups were compared with Student's paired and unpaired *t* test, respectively, and the null hypothesis was rejected if *P* values were less than 0.05.

Results

There were no differences in age (19–25 years), weight (68–84 kg), height (172–187 cm), or baseline R-R intervals (fig. 1) between N_2 or N_2O groups. The average control blood pressure was slightly higher and central venous pressure slightly lower in the N_2O group (fig. 1); however, this difference was not statistically significant. Baseline muscle sympathetic nerve activity (MSNA) expressed as burst frequency per 100 cardiac cycles or burst frequency multiplied by the average burst amplitude (in millivolts) per 100 cardiac cycles did not differ between groups (fig. 2). Inhalation of N_2O, however, resulted in a significant 59 ± 18% increase in MSNA from baseline (fig. 2) compared with a 17 ± 8% nonsignificant reduction in MSNA from baseline in the N_2 group. N_2O did not produce significant alterations in other measured parameters (fig. 1). A representative recording showing responses to sodium nitroprusside from one subject is shown in figure 3. Administration of sodium nitroprusside reduced blood pressure and produced marked increases in MSNA and heart rate. The average reduction in systolic and diastolic blood pressure produced by sodium nitroprusside in the control situation in each group (N_2 group, $\Delta SP = 14.7 \pm 4.8$,

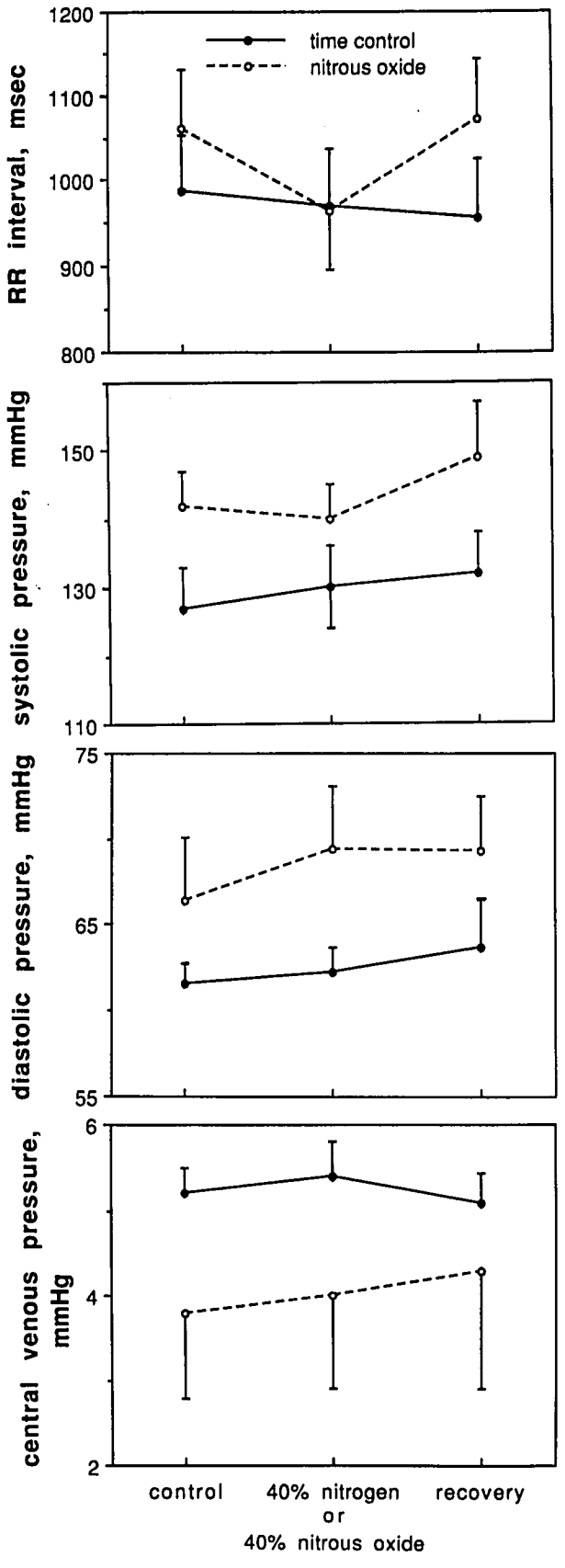

FIG. 1. Average cardiovascular responses (±SEM) produced by the administration of 40% nitrous oxide to six volunteers and by the administration of 40% nitrogen to five time control subjects. There were no significant differences noted between groups.

ΔDP = 10.5 ± 2.7; N_2O group, ΔSP = 18 ± 2.7, ΔDP = 12.1 ± 1.2) was not significantly altered by the administration of placebo (ΔSP = 14.2 ± 5, ΔDP = 9.5 ± 3) or 40% N_2O (ΔSP = 18.3 ± 2.1, ΔDP = 13.6 ± 1.5).

Representative averaged data from one volunteer during sodium nitroprusside-induced hypotension before and during administration of N_2O are shown in figure 4. Reductions in blood pressure produced linear increases in MSNA and decreases in the R-R interval. Average baroreflex slopes are shown in figure 5. N_2O produced a 39 ± 14% reduction in R-R interval slopes ($P < 0.05$) but no significant change in the MSNA slopes (figs. 4 and 5).

Discussion

To our knowledge, the recording of peripheral sympathetic outflow during baroreceptor pertubations in humans during administration of an inhaled anesthetic has not been previously reported. The major findings of this research are that brief exposure to 40% N_2O results in: 1) activation of efferent sympathetic traffic directed to the vascular smooth muscle in skeletal muscle, 2) attenuation of baroreflex-mediated tachycardia, and 3) a maintained ability of the baroreflex to augment peripheral MSNA during hypotension.

Earlier studies have provided indirect evidence that brief exposure to N_2O enhances sympathetic nervous system activity. Urinary catecholamines, plasma norepinephrine and peripheral resistance have been shown to increase during administration of N_2O.[12–14] It has also been shown that N_2O inhibits uptake of norepinephrine in the lung.[15] Thus, it is conceivable that the peripheral vasoconstrictor effects observed with N_2O are simply due to a delayed clearance of norepinephrine from the circulation. In a recent publication,[16] however, we have provided direct evidence of sympathetic activation produced solely by N_2O in human volunteers. We found striking augmentation of muscle sympathetic outflow with increasing concentrations (25–40%) of N_2O. This coincided with a large increase in forearm vascular resistance and a more subtle increase of plasma norepinephrine concentration. In the present study, 15–20 min of exposure to 40% N_2O resulted in a 59% increase in MSNA (in contrast to a 17% nonsignificant reduction in nerve activity recorded in the time control subjects).

Previous human studies suggest that sympathetic nervous system excitation is most prevalent during the first 15–30 min of exposure to N_2O.[14] Our experimental pro-

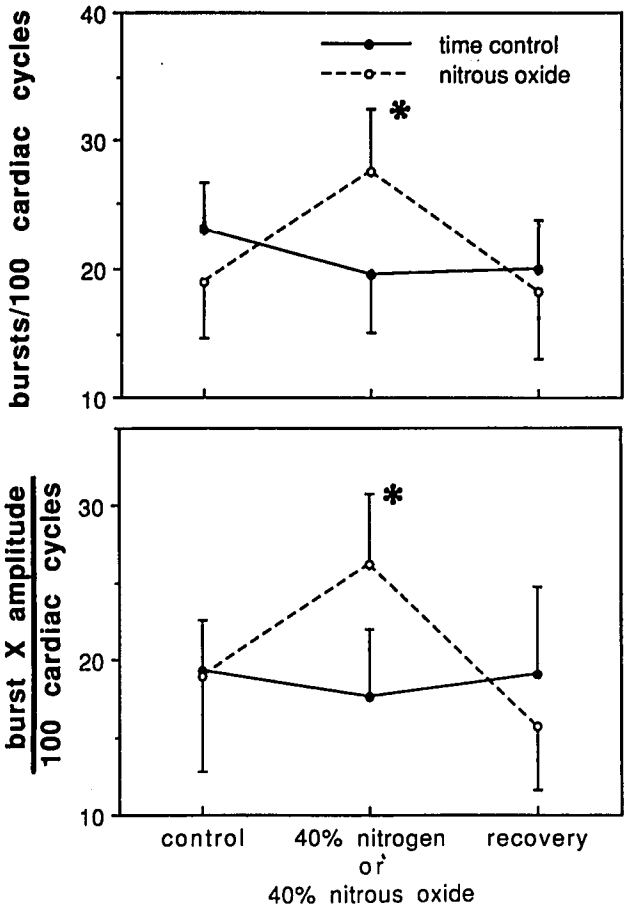

FIG. 2. Muscle sympathetic nerve activity (quantified in several ways) was increased during the administration of nitrous oxide but unchanged in time control subjects who breathed 40% nitrogen. Data are mean ± SEM.

tocol was kept brief so that subjects remained completely immobile (which is essential for stable, quantifiable nerve recordings). It is difficult to remain at ease and quiet during longer testing protocols. A maximum of 40% N_2O was chosen because preliminary studies with higher concentrations (50%) resulted in subject anxiety and restlessness.

The acute regulation of blood pressure in humans is primarily dependent on arterial baroreceptor reflexes. The effector mechanisms of this reflex include cardiac parasympathetic (vagal) outflow, which regulates heart rate, and peripheral sympathetic activity, which regulates arteriolar and venous compliance. The effect of N_2O on the heart rate slowing component of the baroreceptor reflex in response to increases in blood pressure produced by vasopressor agents has been previously reported.[1-3,5] Bristow *et al.*[1] studied four subjects who were premedicated with meperidine, anesthetized with sodium thiopental, and administered 70% N_2O. They noted that 70% N_2O produced a subtle attenuation (from awake values) of the baroreflex-mediated slowing of heart rate. The effect of 70% N_2O during a background halothane anesthetic in humans has also been reported.[2,5] There was less attenuation of baroreceptor reflex-mediated cardiac slowing with the combination of halothane and N_2O than that noted with an equivalent MAC multiple of halothane administered without N_2O. However, upon closer examination of these two consecutive studies, it appears that the awake control baroreflex slopes in the report that examined the effects of N_2O on reflex function were greater than those in the report that examined only halothane's influence on baroreflex function. Thus, the net change of baroreceptor slopes from awake to one MAC equivalent concentrations in each study appears to be essentially the same. Our interpretation of these results agrees well with another human study in which the baroreflex-mediated bradycardia at equipotent levels (1 MAC) of enflurane or enflurane with 70% N_2O were compared.[3] The absolute reductions in baroreflex function in each group were not different.

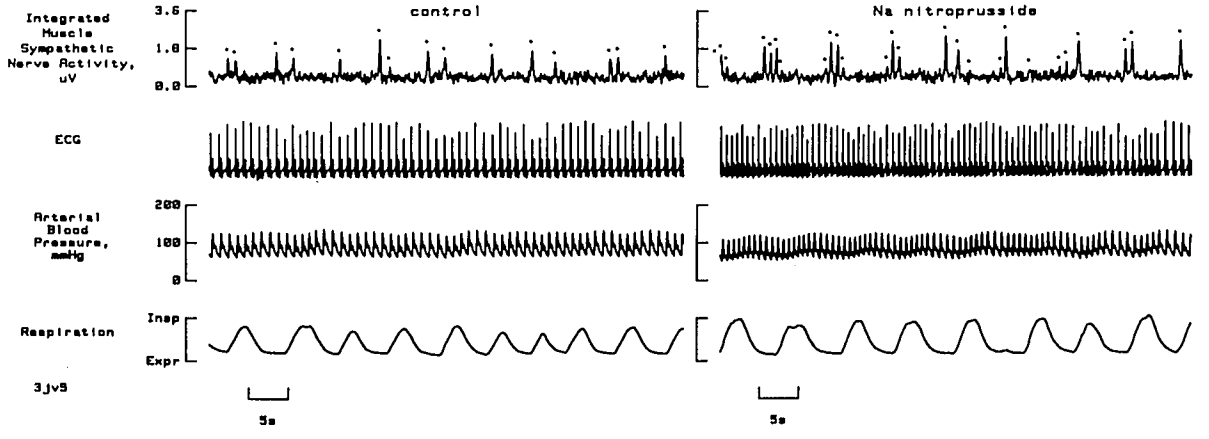

FIG. 3. Typical tracings from one subject during control (40% N_2/60% O_2) while breathing quietly and during sodium nitroprusside-induced hypotension.

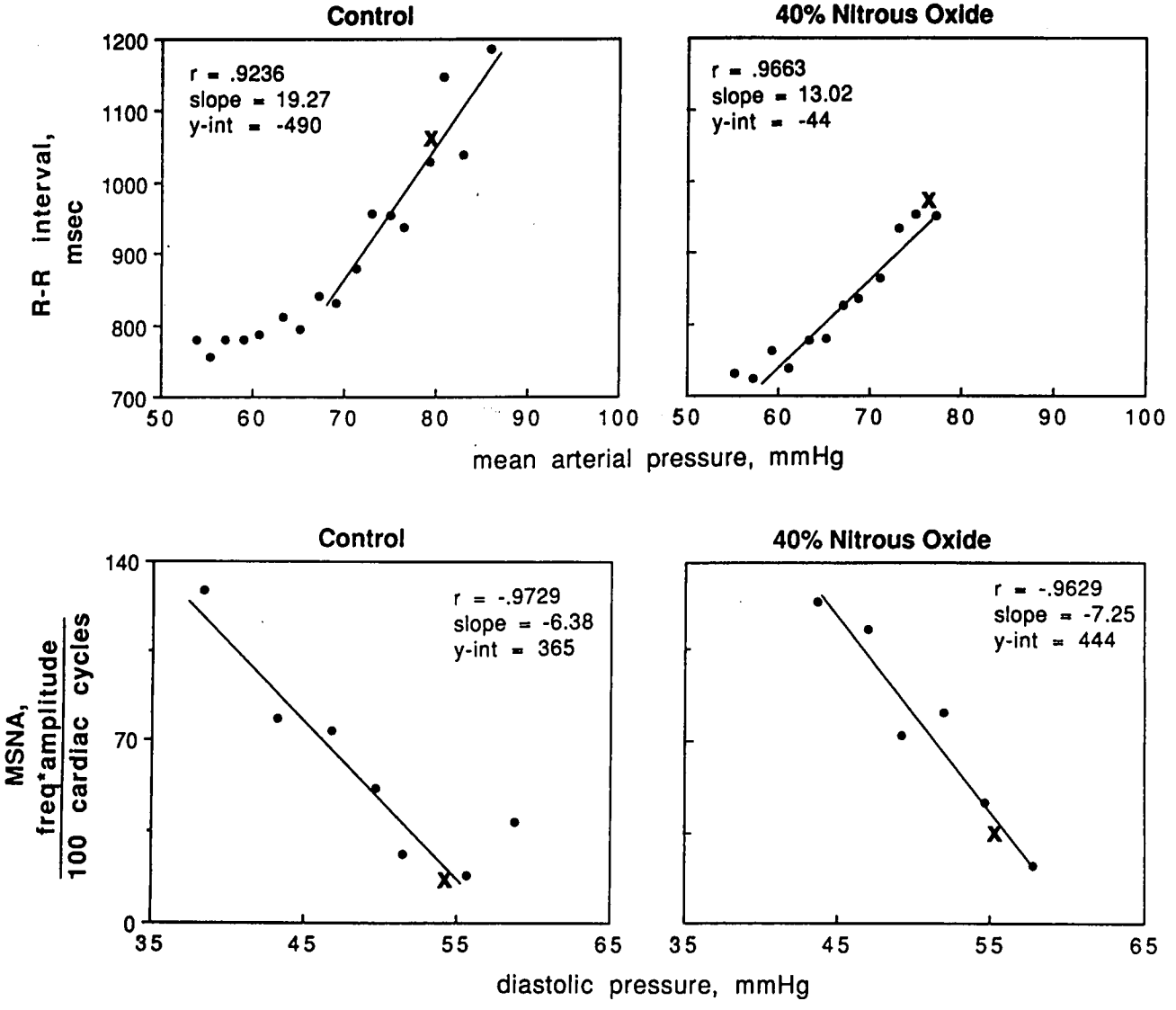

FIG. 4. Representative data from one volunteer which demonstrates typical responses to acute reductions in blood pressure. Independent variables were averaged in 2–3 mmHg intervals, and regression analyses were applied to the linear portion of each response curve. Nitrous oxide administration reduced the slope of the R-R interval response but produced a subtle increase in the slope of the MSNA response to hypotension. The X on each graph is located at the operating point for each variable determined from the average of ten cardiac cycles prior to injection of sodium nitroprusside.

Relatively little information is available concerning reflex regulation of sympathetic outflow in humans. However, this regulation may serve as an important controller of blood pressure. For example, baroreceptor reflex-mediated changes in heart rate lead to reciprocal alterations of both ventricular diastolic filling time and stroke volume in humans.[8,9] Thus, the net effect of a change in heart rate on cardiac output may be relatively small. In contrast, peripheral sympathetic vasoconstrictor impulses decrease the radius of blood vessels and result in exponential increases in peripheral resistance (as described by Pouseille's law). An example of the failure of cardiac output to maintain blood pressure in the clinical setting can be derived by recalling the typical hemodynamics of a patient in septic shock. In such a situation peripheral vasoconstrictor mechanisms fail because of potent vasodilation due to endotoxins. Despite extremely high heart rates, the elevation in cardiac output is often insufficient to maintain a normal blood pressure. In contrast, patients in heart failure with reduced cardiac output often maintain a normal blood pressure because of large increases in peripheral sympathetic outflow.[17] Thus, sympathetic vasoconstrictor mechanisms may play a major role in blood pressure homeostasis in humans.

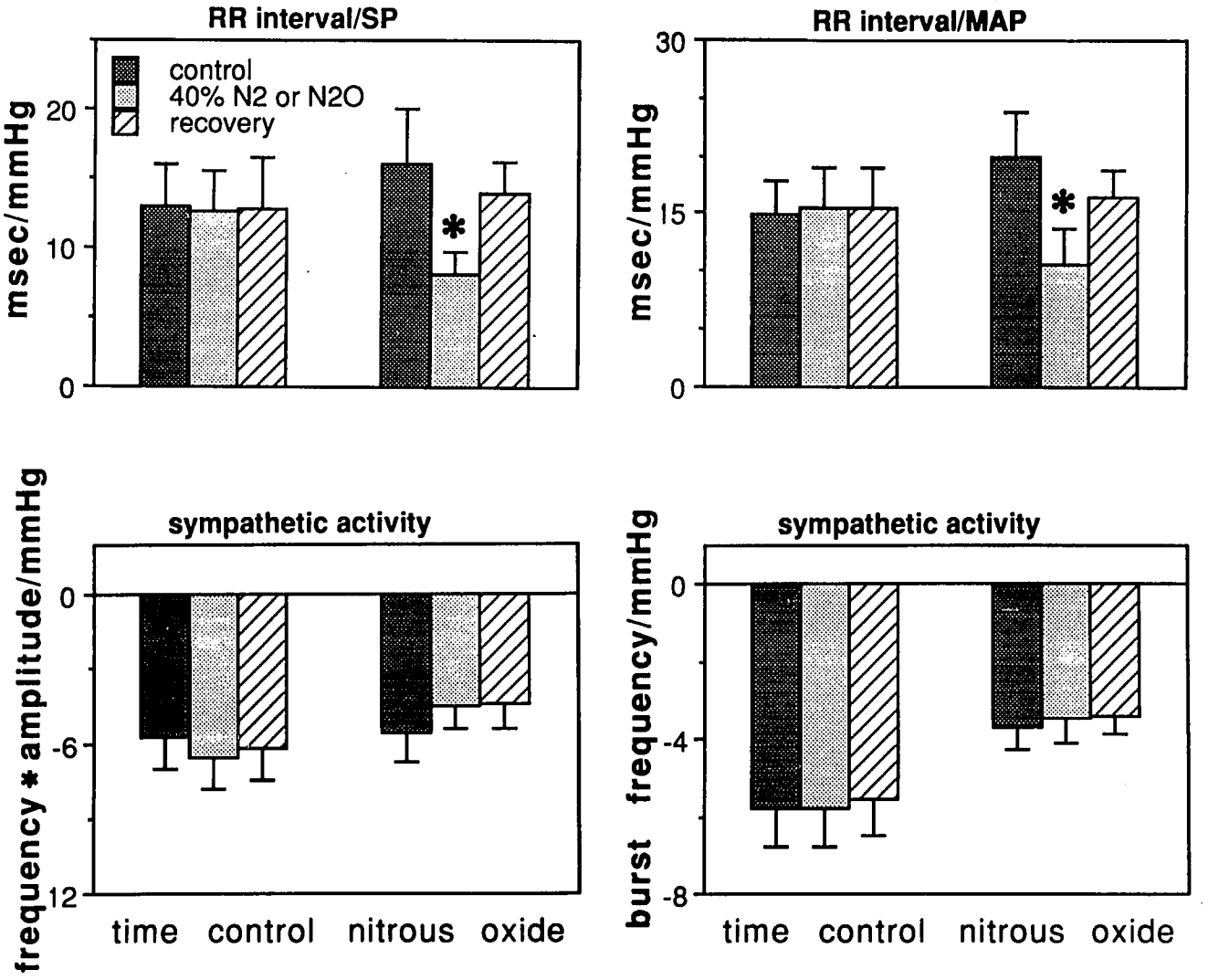

FIG. 5. Average (±SEM) baroreflex responses (slopes) determined in time control (40% N_2/60% O_2) and nitrous oxide (40% N_2O/60% O_2) groups. The upper two graphs show a consistent significant ($P < 0.05$) depression of R-R interval slopes in the N_2O group regardless of the selected independent variable (SP or MAP). The lower two graphs demonstrate that reflex increases of muscle sympathetic nerve activity (quantified by two methods) during hypotension were similar in the nitrogen and nitrous oxide groups.

Anesthetic-induced alterations in the ability of the baroreflex to respond to intraoperative hypotension have not been carefully examined in humans. Thus, we examined the changes in baroreceptor reflex-mediated responses to acute reductions rather than acute increases in blood pressure. We found that baroreflex-mediated tachycardia was reduced by about 40% during brief exposure to N_2O. However, reflex augmentations of muscle sympathetic outflow were well maintained during N_2O. The preservation of reflex sympathetic responses during N_2O administration has also been indirectly shown in several previous animal studies. In chronically instrumented dogs with prior vagal afferent denervation, the substitution of 67% N_2O for N_2 during 0.92% halothane anesthesia did not alter carotid sinus reflex regulation of blood pressure despite the increase in anesthetic depth.[18] Moreover, in cats anesthetized with halothane, the substitution of N_2O for chloralose substantially reduced the depression of baroreflex control of sympathetic nerve activity produced by the combination of halothane and chloralose.[19]

Although our discussion has focused on arterial baroreceptor reflexes, it is known that bolus injection of sodium nitroprusside reduces both systemic and central venous pressures. Therefore, it is most likely that sodium nitroprusside resulted in simultaneous unloading of both arterial and low pressure, cardiopulmonary baroreceptors. Because cardiopulmonary baroreceptor reflexes also regulate sympathetic outflow in humans,[11] we cannot exclude

their role in preserving MSNA responses during N_2O administration.

In summary, brief exposure to 40% N_2O in human volunteers produces an augmentation in baseline sympathetic nerve activity directed to blood vessels that supply skeletal muscles and reduces baroreflex-mediated cardioacceleration. However, baroreflex-mediated augmentation in muscle sympathetic outflow is well maintained during the administration of N_2O. This maintained ability of baroreceptor reflexes to augment sympathetic nerve activity may be partially responsible for the relatively stable cardiovascular effects noted when N_2O is administered in conjunction with other inhalational agents.

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References

1. Bristow JD, Prys-Roberts C, Fisher A, Pickering TG, Sleight P: Effects of anesthesia on baroreflex control of heart rate in man. ANESTHESIOLOGY 31:422–428, 1969
2. Duke PC, Fownes D, Wade JG: Halothane depresses baroreflex control of heart rate in man. ANESTHESIOLOGY 46:184–187, 1977
3. Morton M, Duke PC, Ong B: Baroreflex control of heart rate in man awake and during enflurane and enflurane-nitrous oxide anesthesia. ANESTHESIOLOGY 52:221–223, 1980
4. Kotrly KJ, Ebert TJ, Vucins E, Igler FO, Barney JA, Kampine JP: Baroreceptor reflex control of heart rate during isoflurane anesthesia in humans. ANESTHESIOLOGY 60:173–179, 1984
5. Duke PC, Trosky S: The effect of halothane with nitrous oxide on baroreflex control of heart rate in man. Can Anaesth Soc J 27:531–534, 1980
6. Leon DF, Shaver JA, Leonard JJ: Reflex heart rate control in man. Am Heart J 80:729–739, 1970
7. Raczkowska M, Eckberg DL, Ebert TJ: Muscarinic cholinergic receptors modulate vagal cardiac responses in man. J Auton Nerv Syst 7:271–278, 1983
8. Ebert TJ, Kotrly KJ, Kampine JP: Carotid baroreflex regulation of cardiac inotropic state in conscious man. Proc Int Union Physiol Sci 30th Congress, 16:169, 1986
9. Stein E, Damato AN, Kosowsky BD, Lau SH, Lister JW: The relation of heart rate to cardiovascular dynamics. Circulation 33:925–932, 1966
10. Vallbo AB, Hagbarth HE, Turebjork HE, Wallin BG: Somatosensory, proprioceptive and sympathetic activity in human peripheral nerves. Physiol Rev 59:919–957, 1979
11. Ebert TJ: Reflex activation of sympathetic nervous system by ANF in humans. Am J Physiol 255:H685–H689, 1988
12. Eisele JH, Smith NT: Cardiovascular effects of 40 percent nitrous oxide in man. Anesth Analg 51:956–963, 1972
13. Hill GE, English JE, Lunn J, Stanley TH, Sentker CR, Loeser E, Liu W-S, Kawamura R, Bidwai AV, Hodges M: Cardiovascular responses to nitrous oxide during light, moderate, and deep halothane anesthesia in man. Anesth Analg 57:84–94, 1978
14. Kawamura R, Stanley TH, English JB, Hill GE, Liu W-S, Webster LR: Cardiovascular responses to nitrous oxide exposure for two hours in man. Anesth Analg 59:93–99, 1980
15. Naito H, Gillis CN: Effects of halothane and nitrous oxide on removal of norepinephrine from the pulmonary circulation. ANESTHESIOLOGY 39:575–580, 1973
16. Ebert TJ, Kampine JP: Nitrous oxide augments sympathetic outflow: Direct evidence from human peroneal nerve recordings. Anesth Analg 69:444–449, 1989
17. Leimbach WN, Wallin BG, Victor RG, Aylward PE, Sundlof G, Mark AL: Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. Circulation 73:913–919, 1986
18. Bagshaw RJ, Cox RH: Nitrous oxide and the baroreceptor reflexes in the dog. Acta Anaesthesiol Scand 26:31–38, 1982
19. Skovsted P, Price ML, Price HL: Effects of basal anesthesia on the response of sympathetic nervous activity and barostatic reflexes to a subsequent administration of halothane. J Pharmacol Exp Ther 175:183–188, 1970