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Nitrous Oxide Neurotoxicity Studies in Man and Rat

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To assess the effects of chronic exposure to low levels of nitrous oxide on neural function of man, the authors evaluated the neurologic condition, motor and sensory nerve conduction, and computerized tests of sensation of approximately half of the dentists in Rochester, Minnesota. Results of scored tests of neural function were not significantly different for dentists who used nitrous oxide extensively in their practices and dentists who did not. To assess the effects of chronic exposure to high levels of nitrous oxide on neural function and structure of experimental animals, groups of rats were exposed to 70 per cent N_2O in 30 per cent oxygen for four hours, five days a week, for six months. Rats exposed to N_2O and control rats showed no difference in well-being, in caudal nerve conduction, in axonal content and transport of acetylcholinesterase and dopamine-β-hydroxylase, or in number and size distribution and pathologic abnormality of teased myelinated fibers. Although these results indicate a lack of peripheral nerve neurotoxicity of N_2O in the rat, one cannot assume a similar lack of neurotoxicity in man with heavy exposures. (Key words: Anesthesia, dental. Anesthetics, gases: nitrous oxide. Nerve: function; toxicity. Toxicity: neurotoxicity.)

NITROUS OXIDE (N_2O) enjoys a record of considerable safety when used with proper safeguards during anesthesia. However, peripheral nerve and spinal cord symptoms have been reported to occur among pleasure-seeking chronic users,[1–3] and among a few dental practitioners exposed to N_2O in their surgical practices. Nitrous oxide was suspected of being the cause of this neurologic syndrome because the syndrome was considered to be unique, was not explained by another cause, and was alleviated during cessation of N_2O use. On the other hand, we have examined dental practitioners with recurring myeloradiculoneuropathic syndromes that we could not attribute to N_2O. Their neurologic syndrome was not attributed to N_2O because they had either insufficient or no exposure, or because other factors had been judged to be the more likely cause (alcoholism or an inflammatory–demyelinating disorder). Establishing a role for N_2O in the development of neurologic symptoms is also complicated by the possibility that neurotoxins may be present in N_2O dispensers used by pleasure seekers.[4]

To test whether N_2O, as used in dental practice, might affect neural function, we evaluated 19 of the 36 dentists practicing in Rochester, Minnesota, by scored neurologic examination, by evaluating conduction in motor and sensory fibers of peripheral nerve using clinical electromyographic tests, and by measuring detection thresholds of touch-pressure sensation of the great toe.

To test whether chronic exposure to high concentrations of N_2O might cause pathologic changes in nerves, we exposed rats to 70 per cent N_2O in 30 per cent oxygen for four hours, five days a week, for six months, and compared the numbers and sizes of myelinated fibers (MFs) of nerves and the frequency of graded teased fiber abnormalities with those of a control group.

Materials and Methods

Nineteen dentists (of the 36 dentists in private practice in Rochester, Minnesota) willing to be neurologically evaluated were questioned regarding motor, sensory, and autonomic symptoms. The neurologic examination was scored by using a scale of 0 for normality, 1 for minimal, 2 for moderate, 3 for severe, and 4 for complete neurologic deficit. All deficits for both sides of the body, on a standardized neurologic sheet (as used in the Peripheral Neuropathy Clinical Center at Mayo Clinic), were then summated to give the neurologic disability score (NDS).[5] A person without deficit would have a score of 0, and a person with a generalized symmetric severe peripheral neuropathy would have a score greater than 100. The other 17 dentists, who did not undergo the neurologic evaluation, were interviewed by telephone. All of these 17 used N_2O sparingly or not at all in their practices. None had neurologic symptoms.

Touch-pressure sensation was evaluated at nine grid points on the dorsum of the great toe with use of a computerized system recently described.[6] Abnormality was recognized by finding insensitive points or by finding an increased threshold of sensitive points (or both). Percentile values for healthy per-

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TABLE 1. Values for Age, Nitrous Oxide Usage, Neurologic Disability Score (NDS), and Touch-pressure Sensation of Toe in Two Groups of Dental Practitioners Using No or Small Amounts of N_2O (Group I) and Large Amounts of N_2O (Group II) in Their Practices

	Age (Years)	N_2O Use		NDS	Touch-pressure	
		l-h/wk	Years		Points Sensitive	Threshold (ln mg)*
Group I (n = 9)						
Mean	49.2	0.7	5.3	1.2	9	6.11
SD	± 12.2	± 1.1	± 13.2	± 2.4	0	± 0.62
Range	28–65	0–2.5	0–40	0–6	0	5.40–7.40
Group II (n = 10)						
Mean	40.8†	30.9‡	6.4†	0†	9†	5.73†
SD	± 7.2	± 27.0	± 1.8	0	0	± 0.44
Range	31–53	5–90.0	3–10	0	0	4.96–6.44

* Natural log of pressure in mg.
† $P > 0.05$; ‡ $0.001 < P < 0.005$; levels of statistical significance for differences between mean values in Group I and Group II, using Student's two-sample t test.

sons for this site for age and sex have been obtained previously, but have not been published.

Conventional electromyographic methods were used to study conduction in peripheral nerves. Measurements included the following: conduction velocity of the "fastest" motor fibers in the elbow to wrist (ulnar and median nerves) and knee to ankle (peroneal nerve) segments of mixed nerves, distal latency (DL) of the muscle response after stimulation at the wrist or ankle, amplitude of the compound muscle action potential in response to a maximal stimulus, and latency of the F response (ulnar) after stimulation at the wrist. Studies of cutaneous nerves included conduction velocity of the "fastest" sensory fibers between elbow and wrist (digital nerves of index finger) and between calf and ankle (sural nerve), amplitude and duration of the compound nerve action potential evoked by a maximal stimulus, and latency of the response in the distal segment (DL).

Sprague-Dawley rats from the Mayo Clinic colony (n = 36) weighing approximately 250 g were ear-tagged and randomized into a test group (18 rats) and a control group (18 rats). Rats had not been sprayed with pesticide, and were housed in cages with solid floors covered by untreated wood shavings. The test group was exposed to a 70 per cent N_2O–30 per cent oxygen environment for four hours, five days a week, for six months. The control group was given 70 per cent nitrogen–30 per cent oxygen for the same periods. Only hospital-grade gases were used. Groups of rats were exposed to these environments in specially prepared plexiglass chambers with floors that were covered with a heavy layer of wood shavings. Flow rates of gases were adjusted to ensure adequate inspired oxygen and low carbon dioxide concentrations, tested by gas chromatography. The sural nerve and a defined tibial nerve branch to calf muscles were fixed *in situ* with 4 per cent buffered glutaraldehyde for 10 min and subsequently with 2.5 per cent buffered glutaraldehyde by immersion for four hours. After additional fixation in buffered osmium tetroxide, portions of nerves were embedded in epoxy and other portions were teased so that at least 100 fibers could be graded by criteria previously reported.[7] The number and size distribution of MFs and median diameters of nerves were obtained by our previously described morphometric method, which uses photographic enlargements, a particle size analyzer, and programmed calculation and plotting.[7] A circle of light was approximated to the area of the transverse profile of the MF.

In rats anesthetized with pentobarbital sodium, the caudal nerve was stimulated distally in the tail and its action potential was recorded with subcutaneous needle electrodes at sites 2.5, 6.5, and 12.5 cm proximal to the stimulating cathodes. Tail temperature, measured subcutaneously with a thermistor needle, was maintained at 35.0 to 35.5 C in a mineral-oil bath.[8] Conduction time of the nerve action potential was measured from shock artifact to onset of the nerve action potential. Conduction velocity was calculated over a distance of 10 cm.

The methods used to measure axonal flow of dopamine-β-hydroxylase (DBH) and acetylcholinesterase (AChE) were the same as those described previously.[9]

Results

On the basis of their own estimates of average N_2O use during the past two years, dental practitioners were divided into two groups—Group I, a low-use group (nonuse and <5 liter-hours/week [l-h/wk] use calculated as 100 per cent N_2O), and Group II, a high-use group (≥5 l-h/wk use) (table 1). On the average, Group I used only 0.7 l-h/wk N_2O, whereas Group II used 30.9 l-h/wk ($0.001 < P < 0.005$). Most com-

monly, N_2O analgesia was provided by the nasal route (by mask) using 50 per cent N_2O and 50 per cent O_2 at a total flow rate of 6 l/min. Only one of the anesthesia machines employed had a scavenging system. Ambient N_2O concentrations measured at the mouth level (sampled 10 cm behind the dentist's head) during typical dental procedures by three dentists studied ranged from 300 to 1,200-plus ppm (V/V). Dentists from Group II, therefore, were repeatedly exposed to ambient N_2O levels higher than those recommended for operating rooms (25 ppm)[10] for an average of approximately ten hours per week. None of the surgical rooms save one had an exhaust fan. Most of these rooms had forced-air heating and cooling systems, which recirculate air to various extents. The mean age of practitioners was somewhat younger for the high-use group, but the difference did not reach statistical significance (table 1).

One person in each group had symptoms, as well as slow conduction in the median nerve at the wrist, consistent with a carpal tunnel syndrome. One person in Group I had an abnormal form of compound muscle action potential in one ulnar nerve, suggesting an ancient focal nerve lesion, but conduction velocity and DL measurements were normal and there were no symptoms. Apart from these isolated instances, neurologic symptoms were not elicited. No significant difference in the NDSs or in touch-pressure sensation thresholds (every person tested was sensitive for all nine grid points) was found (table 1). Mean values of amplitude and DL of the maximal compound muscle action potentials and the conduction velocities of motor fibers of the ulnar, median, and peroneal nerves were calculated. Overall, values for Groups I and II were not significantly different (table 2). In addition, mean values for the amplitude, conduction velocity, and DL of the compound action potentials of afferent fibers of the median and sural nerves in Group I and Group II were not significantly different (table 2). The amplitude of the muscle action potential was higher in Group II, but this did not reach statistical significance ($P > 0.05$). The amplitude of the afferent nerve action potentials was slightly higher in Group II ($P > 0.05$). Because dentists in Group II had been more heavily exposed to N_2O, and this is not expected to cause an increase in amplitude, we assume that the higher amplitude was due to the slightly younger age of this group (table 1). Thus, we have not been able to detect a relevant difference in neurologic symptoms or signs, in touch-pressure thresholds of the great toe, or in nerve conduction indices between dental practitioners using N_2O extensively in their practices and those using it sparingly or not at all.

Rats chronically exposed to high levels of N_2O for six months did not show any clinical neuromuscular or neurologic abnormality. Mean weights of N_2O-exposed and control rats before sacrifice were essentially the same. We did not evaluate hematologic or other tissue abnormalities other than that of peripheral nerve. The mean conduction velocities of the compound action potentials of caudal nerve fibers were 53.8 m/s (SD ± 2.14, n = 6) for control rats and 54.4 m/s (SD ± 2.7, n = 8) for N_2O-exposed rats—not significantly different. The basal enzymatic activities in sciatic nerves of the N_2O-treated rats (AChE—mean 27.4, SD ± 2.0 nmol/h/mm length of nerve; DBH—mean 374.8, SD ± 34.9 pmol/h/mm) were

TABLE 2. Mean Values and Standard Deviations of Amplitude, Conduction Velocity, and Distal Latency Measurements in Studies of Conduction in Motor and Sensory Fibers of Ulnar, Median, Peroneal, and Sural Nerves of Dental Practitioners Using Small (Group I) and Large (Group II) Amounts of N_2O in Their Practices

	Motor										Sensory				
	Ulnar				Median			Peroneal			Median			Sural	
	Amp (mV)	CV (m/s)	DL (ms)	F (ms)	Amp (mV)	CV (m/s)	DL (ms)	Amp (mV)	CV (m/s)	DL (ms)	Amp (μV)	CV (m/s)	DL (ms)	Amp (μV)	DI (ms)
Group I (n = 9)															
Mean	10.5	59.9	2.5	27.6	9.4	57.3	3.1	5.5	49.7	4.1	27.5	60.7	3.0	12.3	3.5
SD	± 1.0	± 4.6	± 0.3	± 1.8	± 3.0	± 2.7	± 0.4	± 1.5	± 1.6	± 0.3	± 10.8	± 4.0	± 0.3	± 4.3	± 0.4
Group II (n = 10)															
Mean	10.8	58.6	2.5	27.8	11.2	60.2	3.3	6.9	49.9	4.0	28.0	62.6	3.0	14.8	3.5
SD	± 1.6	± 5.4	± 0.3	± 2.3	± 2.1	± 3.3	± 0.4	± 2.7	± 3.3	± 0.7	± 7.1	± 5.8	± 0.3	± 6.0	± 0.4

Under "Motor," Amp is amplitude of compound action potential of muscle (negative phase). CV is conduction velocity of nerve between elbow and wrist or knee and ankle. Under "Sensory," Amp is amplitude of the compound nerve action potential (peak to peak). The digital nerve of the index finger was tested as the median sensory nerve. DL is distal latency; mean distances were *7.0* and *7.1* cm (ulnar nerve), *7.2* and *7.3* cm (median motor nerve), *7.7* and *7.9* cm (peroneal nerve), *13.6* and *13.7* cm (digital nerve), and *14.0* and *14.1* cm (sural nerve) in groups I and II, respectively. F is latency of the F wave after a maximal stimulus to the ulnar nerve at the wrist. None of the mean values were significantly different at the 0.05 level using Student's two-sample *t* test. All values are in the midpart of the normal range.

TABLE 3. Myelinated Fiber (MF) Numbers, Median Diameters, and Graded Teased Conditions (Per Cent) of Sural Nerves of Rats Breathing 70 Per Cent N_2O in 30 Per Cent Oxygen for Four Hours, Five Days per Week, for Six Months, and of Control Rats

	No. MFs/ mm²	No. MFs/ Nerve	Median Diameter (μm)	Graded A + B*	Teased Fibers (Per Cent)	
					C,D,F,G†	E‡
Control (n = 15)						
Mean	16,282	782	5.80	99.27	0.44	0.29
SD	± 2,467	± 295	± 0.63	± 0.92	± 0.54	± 0.52
Experimental (n = 16)						
Mean	14,718	768	5.89	99.45	0.44	0.11
SD	± 1,993	± 135	± 0.38	± 0.92	± 0.65	± 0.41

* Conditions A and B are teased fibers having a normal appearance and fibers with excessive irregularity of myelin, respectively.
† Conditions C, D, F, and G are teased fibers showing various morphologic stages of the process of segmental demyelination and remyelination.
‡ Condition E is teased fibers undergoing axonal degeneration.
None of the mean or median values were significantly different at the 0.05 level using Student's two-sample *t* test.

TABLE 4. Myelinated Fiber (MF) Numbers, Median Diameters, and Graded Teased Conditions (Per Cent) of Nerve to Tibial Muscle of Rats Breathing 70 Per Cent N_2O in Thirty Per Cent Oxygen for Four Hours, Five Days per Week, for Six Months, and of Control Rats

	No. MFs/ mm²	No. MFs/ Nerve	Median Diameter (μm)	Graded A + B	Teased Fibers (Per Cent)*	
					C,D,F,G	E
Control (n = 4)						
Mean	9,643	77.3	7.898	99.17	0.83	0
SD	± 2,381	± 38.6	± 1.521	± 1.42	± 1.42	0
Experimental (n = 8)						
Mean	9,617	73.1	8.178	98.57	0.66	0.77
SD	± 2,195	± 35.2	± 0.971	± 2.44	± 1.53	± 1.27

* For description of teased fiber conditions, see footnote to table 3.
None of the mean or median values were significantly different at the 0.05 level using the Student two-sample *t* test.

similar to those of control rats (AChE—mean 28.8, SD ± 2.4 nmol, h/mm; DBH—mean 387.8, SD ± 29.5 pmol/h/mm). Transport velocities in nerves of treated animals for DBH (mean 1.28, SD ± 0.29 mm/h) and AChE (mean 0.50, SD ± 0.14 mm/h) did not differ significantly from control values (mean 1.38, SD ± 0.20 mm/h; mean 0.56, SD ± 1.10 mm/h, respectively).

The mean numbers and the median diameters of MFs, and the mean percentages of teased fiber abnormalities, were not different in N_2O-exposed rats and control rats (tables 3 and 4). Likewise, a qualitative light and electron microscopic survey of transverse sections did not reveal structural differences between groups.

Despite exposure of rats to 70 per cent N_2O for four hours a day, five days a week, for six months, we did not find disturbances of conduction velocity or axonal flow, or morphologic abnormalities.

Discussion

Our failure to demonstrate an abnormality of neural function or structure resulting from N_2O exposure in dentists and in rats might be explained by lack of sensitivity in methods used to detect neural abnormality, by too-small a sample size, by insufficient exposure, or by lack of a neuropathic effect of N_2O. The methods used to detect and grade neuropathy are sensitive, inasmuch as we used scored assessment of neurologic symptoms and signs, nerve conduction, electromyographic abnormalities, and morphometric and teased fiber abnormalities. Because of the sensitivity of methods employed, it seems unlikely that a dose-related neuropathic deficit of clinical magnitude was missed because of the small sample size. On the other hand, an idiosyncratic neuropathic effect might easily be missed using such small sample sizes. It is our opinion, therefore, that repeated exposures to low concentrations of N_2O ($\leq$300 ppm) probably does not constitute a neuropathic health hazard, unless one postulates an idiosyncratic rather than a direct neurotoxic action. Moreover, the lack of a functional or morphologic abnormality in nerves of rats heavily exposed to nitrous oxide for six months provides evidence against the view that N_2O is a peripheral nerve neurotoxin, at least in rats.

Our findings differ from the expectations raised by reports linking N_2O exposure to myelopathic and neuropathic symptoms and thereby suggesting

that N_2O may be a neurotoxin even with prudent clinical use. In those reports, because dentists self-administered N_2O, the possibilities arise of uncertainty of dose, of possible contaminants, and of the occurrence of hypoxia. It is further conceivable that some of the myelopathic or neuropathic symptoms reported in earlier series and attributed to N_2O may, in fact, have been due to coincident disease (*e.g.*, in the patient whose symptoms subsided for a time despite continuous use of N_2O).[1]

We cannot say that N_2O, especially when used to excess, is without neuropathic toxicity, because minimal deficits or pathologic effects may have been unrecognized in our study, and species differences may exist between rat and man. Furthermore, a much larger dose than that used by dentists in our study might result in neurotoxicity in man. Credence that frequent exposures to high doses of N_2O in man may cause myelopathic symptoms comes not only from the clinical experiences of people who have self-administered N_2O for pleasure but from some animal studies. Dinn reported typical hematologic, neurologic, biochemical, and pathologic changes of subacute combined degeneration of the spinal cord in a monkey exposed to 50 per cent nitrous oxide with 50 per cent oxygen for two months.[11] Recent articles have reported that nitrous oxide interferes strikingly with vitamin B_{12} metabolism.[12,13] Conceivably, therefore, very heavy chronic N_2O use might result in vitamin B_{12} deficiency and subacute combined degeneration of the cord. The myelopathic effects associated with N_2O exposure in man and monkey, if confirmed, relate to chronic heavy exposure. Although it would be desirable to study a larger group of dentists, it is reassuring that prudent use of N_2O in the clinical situation was unassociated with any symptom or finding suggesting neurotoxicity.

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