Nitrous Oxide Inhibition of Sodium Transport

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Sacs made of inverted frog skin were filled with an electrolyte solution and exposed in a pressure chamber to nitrogen or nitrous oxide with a small concentration of oxygen. Changes in sodium ion concentration were measured after 20 to 22 hours. With increasing concentrations of nitrous oxide in the range of 100 to 200 psig. inhibition of sodium transport increased. Inhibition of sodium transport was shown not to be due to pressure *per se* since 175 psig. nitrogen did not result in inhibition. Inhibition of sodium transport paralleled previous reports of nitrous oxide depression of nerve excitability.

MOLECULAR or cellular theories of anesthesia should consider alterations in ion transport. A small but impressive literature exists concerning the effectiveness of local anesthetics in inhibiting active sodium transport.[1-3] A review of the literature shows that relatively little has been written regarding the effects of gaseous anesthetics on ion transport mechanisms. Alterations of ion transport may be of fundamental importance in the development of the anesthetic state. Investigations were therefore undertaken to determine the effects of nitrous oxide on sodium transport.

Methods

The inverted skin bag technique of Huf *et al.*[4] employing the skin of *Rana pipiens* was used as the sodium ion transport system. The skin was stripped from the hind legs of the frogs and the distal ends tied off. The "inside-out" bags were filled with 2.5–3.0 ml. of a standard oxygenated solution having the following composition in mEq./ml.: NaCl 48; KCl 1.0; $NaHCO_3$ 2.0; *p*H 8.3–8.5. To facilitate gas transport into and out of the bags so as to avoid compression and decompression problems, they were tied at their tops to glass tubing. The bags were then suspended in separate flasks each containing a sufficient volume, approximately 30 ml., of the oxygenated solution to eliminate effects of hydrostatic pressure. The flasks containing the skin bags were placed in a pressure chamber and exposed to pressures (6.8, 10.2, or 14.2 atmospheres) of nitrous oxide for 20 to 22 hours. The nitrous oxide was added to the air already in the chamber, which was not exhausted. Similar experiments were performed using 98 per cent nitrogen–2 per cent oxygen at 11.9 atmospheres; prior to pressurization the chamber was flushed with this gas mixture for five minutes at a rate of 5 liters/minute. Increase in pressure within the chamber occurred at a rate of 1.8 atmospheres per minute; decompression was carried out at a rate of 0.5 liters per minute. All experiments were conducted within a range of 20–23° C. In each instance a bag made from the other leg of each frog served as the control, kept at 1 atmosphere. The sodium concentration of the initial Ringer's solution and the final solutions inside and outside the bags were analyzed by means of flame photometry.

Results

Sodium transport was inhibited when the pressure of nitrous oxide was greater than 6.8 atmospheres. At 14.2 atmospheres (200 psig.) of nitrous oxide, transport of sodium ceased completely (table 1). The inhibition was due to N_2O and not to pressure, for in separate experiments 11.9 atmospheres (175 psig.) of a 98 per cent N_2–2 per cent O_2 mixture did not measurably inhibit sodium transport: under these conditions the P_{O_2} was essentially equal to that of 1 atmosphere and thereby equivalent to the P_{O_2} in the nitrous oxide experiments. The data on nitrogen essentially confirm the

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TABLE 1. Effects of Nitrous Oxide and Nitrogen on Sodium Transport Through Frog Skin

Treatment		Number of Frogs	Average Transport Micro Moles/cm.²/Hr. ± St'd Deviation	% Inhibition	Probability
Gas	Atmospheres				
Air	1	8	0.124 ± 0.034	4.8	.45
N_2O	6.8	8	0.118 ± 0.026		
Air	1	6	0.174 ± 0.048	63.2	.005
N_2O	10.2	6	0.064 ± 0.032		
Air	1	8	0.146 ± 0.061	93.1	.001
N_2O	14.2	8	0.010 ± 0.0079		
Air	1	4	0.112 ± 0.027	0	
98% N_2 2% O_2	11.9	4	0.112 ± 0.028		

results of Falsetti [5] who found that 8 to 10 atmospheres of nitrogen do not inhibit sodium transport across frog skin under identical conditions as reported here. The average net sodium transport per square centimeter of membrane in contact with solution per hour reported herein is within the range reported by others.[5, 6]

Discussion

Increasing concentrations of nitrous oxide in the range of 100–200 psig. (6.8–14.2 atmospheres) was shown to inhibit sodium transport across frog skin. It has also been shown [7, 8] that ether and chloroform inhibit the trans-skin potential of Obelia and frog skin. Recently, Andersen [9] using the short circuit current technique, demonstrated that haothane and ethyl ether reversibility inhibit active sodium transport across the toad bladder.

Carpenter [10] reported that 10–13 atmospheres of nitrous oxide are required for conduction blockade to electrical stimulation in rat sciatic nerve. Gottlieb and Weatherly [11] also reported that increases in nitrous oxide pressure from 6.8 to 11.9 atmospheres resulted in corresponding decrease in excitability of frog sciatic nerve to electrical stimulation. Figure 1 depicts the correlation between decreased nerve excitability and inhibition of sodium transport. The almost identical slopes of the two curves depicting these phenomena, in essentially the same pressure range, suggests a causal relationship between the decrease in nerve excitability induced by nitrous oxide, and sodium ion transport.

Andersen failed to discover inhibition of sodium transport in the toad bladder with 30 or 80 per cent nitrous oxide at 1 atmosphere. In view of the fact that high tensions of nitrous oxide are required to decrease excitability of nerve and if anesthesia is brought about by an interference with sodium transport, or at least

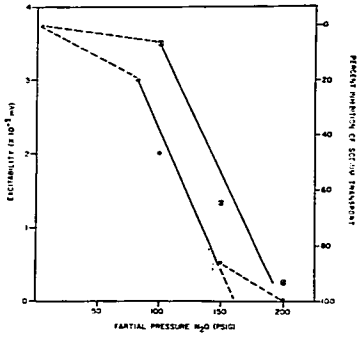

FIG. 1. Effect of N_2O on sodium transport. Correlation of N_2O-induced inhibition of sodium transport and N_2O-induced decrease of nerve excitability. The nerve excitability data of Gottlieb and Weatherly [8] are reproduced by permission of the *American Journal of Physiology*. • Nerve excitability. X Sodium transport: at 100 psig., datum average of 8 frogs: $P_{(t)} < 0.45$; at 150 psig., datum average of 6 frogs: $P_{(t)} < 0.005$; at 200 psig., datum average of 8 frogs: $P_{(t)} < 0.001$.

correlated with or coincident with decreased sodium transport, it can be postulated that high pressures of nitrous oxide are required to inhibit active sodium transport across these model systems. The difference between Andersen's observations and the data reported here is most likely the result of the differences in pressure of nitrous oxide employed.

Other than indicating an inhibition of active sodium transport on the cellular level of biological organization, these data do not indicate, on the molecular level, the specific enzyme system that is being affected by the nitrous oxide. In view of the involvement of sodium ions in membrane depolarization and nerve impulses conduction, it is possible that inhibition of sodium ion transport by nitrous oxide indicates a cellular explanation for nitrous oxide-induced anesthesia. To bring about a closer link of nitrous oxide-induced inhibition of sodium transport to the clinical phenomenon requires further study of the degree of inhibition and the kinetics of inhibition of sodium ion transport using time as the variable and pressure as the constant. Along this line of reasoning, preliminary results obtained with the short circuit current technique support the conclusions from the experiments reported here that high tensions of nitrous oxide inhibit active sodium transport, and in addition indicate that this inhibition is reversible.

Probably more is involved in the production of anesthesia than just an inhibition of sodium transport. There are probably other functional as well as structural changes occurring in the membrane during the induction of the anesthetic state. Inhibition of sodium transport may be one of the many changes that occur. On the other hand it is conceivable that inhibition of sodium transport may be the result of and not the cause of anesthesia. The study herein reported provides a model system for studying cellular changes induced by gaseous anesthetics.

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