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Myocardial Depression by Nitrous Oxide and Its Reversal by Ca^{++}

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The effects of 50 per cent nitrous oxide on isometric contractile force of electrically stimulated cat papillary muscle suspended in a Tris-buffered solution at five concentrations of Ca^{++} ranging from 1 to 15 mM were studied. Compared with an equal concentration of nitrogen, or with pure oxygen, nitrous oxide caused a highly significant reduction in contractile force, averaging 22 per cent at 2.5 mM Ca^{++}. This reduction in force, like that caused by halothane, could be antagonized by increasing $[Ca^{++}]$ in the bathing medium. However, the reductions in force caused by equinarcotic concentrations of halothane and nitrous oxide are significantly different in magnitude, suggesting that the mechanisms of anesthetic action in the central nervous system and in the myocardium may not be the same. (Key words: Anesthetics, gases, nitrous oxide; Heart, myocardium, nitrous oxide; Ions, calcium.)

IT HAS BEEN APPRECIATED since the middle of the last century that nitrous oxide can produce general anesthesia. It is also widely recognized that drugs capable of producing general anesthesia depress myocardial contractility in direct proportion to concentration and anesthetic potency.[1,2] For these reasons, the recent statement by Goldberg *et al.*[3] that "nitrous oxide does not possess any direct myocardial depressant . . . properties" deserves further investigation. This finding, although widely disseminated among clinicians in the past, appears to fly in the face of any unitary hypothesis of anesthetic action, and so deserves examination on this point alone. In addition, the possibility that there exists an anesthetic completely devoid of myocardial depressant properties requires further documentation in order to support the choice of an anesthetic for patients with heart disease, where such a lack of action could be beneficial, if not life-saving.

Methods

Papillary muscles were obtained from kittens by methods previously described.[4] They were excised, suspended from muscle hooks, bathed with a Tris-buffered solution† in a muscle chamber, and exposed to oxygen by continuous bubbling. All experiments were performed at 25 C. The muscles were stimulated electrically by 1-cm square platinum field electrodes supplied from an AEL stimulator delivering 8–12-v, 5-msec pulses at a frequency of 12/min. Contractile force was measured by a Statham universal force transducer and transcribed by a Grass model 7 polygraph.

Nitrous oxide was administered to the preparation in a concentration of 50 per cent in oxygen. Control measurements were made using 50 per cent oxygen in nitrogen. Muscles in which the administration of 50 per cent nitrogen caused function to deteriorate (compared with 100 per cent oxygen) were not used in the experiments reported.

In order to distinguish the effects of 50 per cent nitrous oxide from those of 50 per cent nitrogen, the temporal course of contractile force in relation to substitution of nitrous oxide for nitrogen was closely followed. In general, 8–10 minutes sufficed to equilibrate the muscle with a new gas tension, or to wash out the added gas, and the results reported are the means of the initial and final control values versus those observed during exposure to nitrous oxide (see fig. 1). The data were analyzed statistically by means of Student's t test for paired data.

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† NaCl 130 mM; KCl 4.0 mM; $CaCl_2$ 1–15 mM; $MgCl_2$ 1.0 mM; Tris 20 mM; glucose 100 mg/100 ml; *p*H adjusted to 7.40 with HCl.

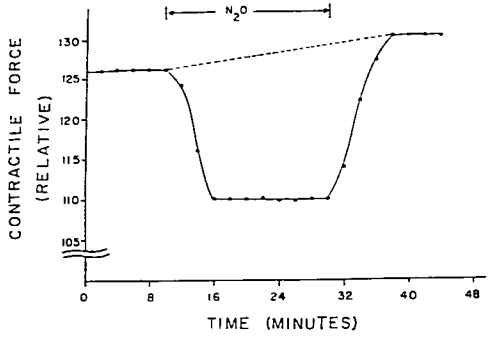

FIG. 1. Isometric contractile forces (in arbitrary units) before, during and after exposure to 50 per cent N_2O. Arrow shows duration of nitrous oxide administration. The control level utilized is the mean of observations made before and after exposure (*dotted line*). [Ca^{++}] = 10 mmol.

Ionized calcium (Ca^{++}) was measured by an Orion research model 92-20 calcium electrode with a Corning model 12 research meter, and found to range from 80 to 90 per cent of total [Ca^{++}]. The following data are given as total [Ca^{++}].

TABLE 1. Percentage Reductions in Isometric Contractile Force Caused by 50 Per Cent N_2O at Various [Ca^{++}]'s*

	Calcium Concentration [mM l]				
	1	2.5	5	10	15
Date					
5/31	—	26	26	16	—
6/1	27	32	26	14	—
6/6	27	27	21	24	22
6/8	34	27	22	11	13
6/15	28	25	14	10	9
6/22	33	24	22	14	11
6/27	24	20	13	10	13
7/18	26	17	16	6	5
7/19	26	17	12	12	14
7/26	19	15	11	10	8
8/1	28	22	17	10	—
8/2	25	17	12	6	—
8/3	37	26	11	8	—
8/6	21	15	12	—	—
8/10	32	22	17	10	—
MEAN	27.6	22.1	16.8	11.5	11.8
± SE	± 1.3	± 1.3	± 1.3	± 1.2	± 1.8
t	4.97	5.53	4.71	0.31	
P	<0.001	<0.001	<0.001	N.S.	

* Dates identify individual muscles. Control force levels are averages of observations made before addition of N_2O and after its removal (see Fig. 1).

Results

In two of 17 muscles, substitution of 50 per cent oxygen and 50 per cent nitrogen for pure oxygen caused function to decrease. These were not further studied.

In the other muscles, substitution of N_2O for N_2 caused function to decrease in all cases, the mean decrease amounting to 22.1 ± 1.3 (SE) per cent at 2.5 mM Ca^{++} at 8–10 minutes, which was the time of maximal change. These changes were rapidly reversible once N_2O was removed, and the "control" levels from which the changes caused by N_2O were calculated therefore include the effects (if any) of time itself. Figure 1 shows an example of the data obtained.

In all studies, the effects of altering [Ca^{++}] were ascertained. It was found that increasing [Ca^{++}] reduced the effect of substituting 50 per cent N_2O for 50 per cent N_2. Data from 15 studies are shown in table 1. In summary of these results, the percentage depressions of isometric contractile force at 1, 2.5, 5, and 10 mM Ca^{++} were 27.6, 22.1, 16.8, and 11.5 per cent, respectively. Each of these results was significantly different from any of the others, $P < 0.001$. Plotting the percentage reduction in contractile force on the y axis and the concentration of ionized Ca^{++} on the x axis (fig. 2) produced a curvilinear array of data points asymptotic to the x axis, suggesting that the depression caused by N_2O could be entirely eliminated if the [Ca^{++}] could be increased

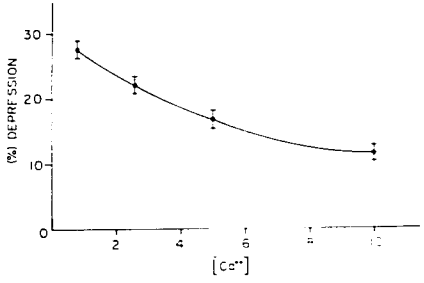

FIG. 2. Percentage depressions of isometric contractile force caused by 50 per cent N_2O at various calcium concentrations. Paired data from 15 muscles. Bars show ±2 SE.

sufficiently. Unfortunately, the highest [Ca^{++}] used in these studies (15 mM) was apparently toxic to the muscle (significant depression of function occurred in three of eight preparations), so the hypothesis could not be tested directly.

An alternative method consists of reciprocal plotting of contractile force and [Ca^{++}] with back-extrapolation to the y axis, the method of Lineweaver and Burk. Figure 3 shows an example of this method, using the data of July 19. It can be seen that the lower (N_2) and upper (N_2O) curves nearly intersect at the y axis, where 1/[Ca^{++}] equals zero. Since 1/[Ca^{++}] can equal zero only when [Ca^{++}] becomes infinite, intersection of the lines at the y axis is identical to the statement that at infinite [Ca^{++}] N_2O does not affect contractile force. Of the 15 muscles studied, 12 gave data useful for analysis by this method. (The requirements are simply three sets of data points that result in two straight lines when back-extrapolated to the y axis: data from three of the muscles studied failed to produce straight lines.) Data from the 12 acceptable studies have been analyzed and entered in table 2, which shows that the maximal force attainable (*i.e.*, when [Ca^{++}] becomes infinite) was insignificantly different whether the muscle was exposed to 50 per cent N_2O or to 50 per cent N_2, despite the fact that N_2O was clearly depressant to contractile force at physiologic concentrations of Ca^{++}.

Discussion

These results are of interest from several aspects. In the first place, the myocardial depressant action of nitrous oxide is quantitatively different from those of certain other anesthetics when compared at equipotent anesthetic concentrations. We found a 60 per

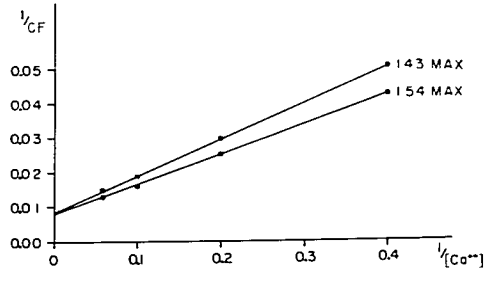

FIG. 3. Lineweaver-Burk plot of data from 7/19. Contractile force is shown in millimeters, [Ca^{++}] in mM. MAX = maximal contractile forces attainable in the presence and absence of 50 per cent N_2O. For explanation see text.

TABLE 2. Maximal Contractile Force

	Control	Experimental	Difference
Date			
6/1	313	333	−20
6/6	166	142	+24
6/8	167	164	+3
6/15	125	123	+2
6/22	201	190	+11
7/18	111	113	−2
7/19	154	143	+11
7/26	106	103	+3
8/1	182	200	−18
8/2	132	133	+4
8/3	95	124	−29
8/6	250	260	−10
MEAN	166.8	169.0	−1.8
± SE	± 18.4	± 19.6	± 4.3
t		−0.50	
Significance		None	

cent depression in contractile force for halothane at 0.45 per cent concentration and 25 C when $[Ca^{++}]$ was 2.5 mM.[4] Considering the MAC ratio for halothane and nitrous oxide, the levels of ionized Ca^{++} in these and previous[4] studies, and Ostwald coefficients at 25 versus 37 C,[5,6] this indicates that, at equipotent anesthetic concentrations, halothane is roughly twice as potent as is nitrous oxide in depressing the myocardium. However, the myocardial depressant effect of nitrous oxide was not negligible, and was easily demonstrated. Interestingly, the extent of depression was not significantly different from that determined in 1955 in the dog heart–lung preparation,[1] assuming normal $[Ca^{++}]$ in the animals studied. Intermediate failures to demonstrate such an action[3] apparently result from methodologic differences.

In particular, Goldberg compared at 37 C the effects on contractile force of 25, 50, and 75 per cent N_2 and N_2O over a period of several hours. Each concentration was administered for 15 minutes and was followed by a complete bath change. Both gas mixtures depressed contractile force, but there was no statistically significant difference between the effects of N_2O and N_2. Obviously, therefore, the muscles were hypoxic under conditions of reduced oxygen tension, and it was necessary to separate statistically the effects of hypoxia from those of N_2O. Since a paired method of analysis apparently was not used, some statistical power must have been lost. In addition, the long temporal separation between the various observations in the study introduced the possibility that the spontaneous variability of the preparation over long periods (as much as 15 per cent—see figure 1) could have interfered with the ability to determine small changes caused by a weakly depressant substance such as N_2O.

In contrast, we worked at 25 C and studied only muscles that were not hypoxic. We made only paired observations at a single concentration of N_2O or N_2. Each observation of the effect of N_2O was complete within 20 minutes (fig. 1). The bath was not emptied between observations except when $[Ca^{++}]$ was changed; actually this maneuver was followed by large variations in contractile force in our experience. Finally, exposure to 50 per cent N_2O at 25 C is equivalent to equilibration with 66 per cent N_2O at 37 C.[5] We assume that the foregoing methodologic differences account for the differences between Goldberg's results and ours. Interestingly, Goldberg did find more depression by N_2O than by N_2 (his figure 1); it was simply that he could not show a statistically significant difference between the two effects.

Second, the reversal of myocardial depression by Ca^{++} is similar to that observed with halothane and ether,[4] and this suggests a common mode of action of these anesthetics on the heart. Recent studies have shown that halothane acts both by inhibiting access of extracellular Ca^{++} to the cytoplasmic interior and by diminishing the ability of Ca^{++} to effect contraction following arrival at the troponin–tropomyosin complex.[7] Nayler's recent experiments[8] suggest that digitalis acts by increasing the amount of Ca^{++} bound to the surface of myocardial cells, and available to effect contraction during systolic depolarization. Our present findings, therefore, suggest that digitalization could prevent or reduce myocardial depression caused by nitrous oxide and other anesthetics that act similarly, a conclusion already documented in animals in the case of halothane.[9]

The clinical implications of our findings stem from the fact that nitrous oxide, although it resembles other general anesthetics in depressing the myocardium, has substantially

less effect *in vitro* than does halothane. Moreover, there is evidence from animal studies *in vivo*[10,11] that nitrous oxide stimulates sympathetic nervous activity, an action that would tend to antagonize the myocardial depression caused by its direct action on the heart. Clinical studies in normal man have indicated that nitrous oxide increases arterial blood pressure, total peripheral resistance, and pupil size when added to an anesthetic mixture of halothane and oxygen; cardiac output remains unchanged.[12] Similarly, cardiovascular depression does not result when nitrous oxide is added to low concentrations of halothane in patients undergoing operations for valvular heart disease.[13] Although an earlier study by the same author suggested slight cardiovascular depression when nitrous oxide was added to morphine anesthesia in patients undergoing cardiac operations,[14] these results could stem from production of increased analgesia. The foregoing considerations suggest that the use of nitrous oxide for patients with heart disease may have practical as well as theoretical advantages over other anesthetic regimens.

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