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Nitrous Oxide Effects on Isolated Myocardium: A Reexamination In Vitro

Dan Lawson, M.D., Martha J. Frazer, B.S.,† Carl Lynch III, M.D., Ph.D.‡*

This study examined *in vitro* myocardial depression by 50% N_2O. Maximal isometric contractions of guinea pig right ventricular papillary muscles were studied in Tyrode's superfusate at 37° C within a gas-tight chamber. Superfusate (*p*H at 7.45) and chamber were equilibrated with 95% O_2/5% CO_2. After control measurements in 95% O_2, muscles were studied with 50% N_2 and 50% N_2O (45% O_2/5% CO_2) in random order with an intervening and final recovery in oxygen. Muscles were field stimulated after rest and at 0.1–3 Hz. At 37° C, muscle performance deteriorated over time with exposure to reduced oxygen; therefore, identical experiments were performed at 30° C in which no systematic deterioration occurred. Peak tension and maximum rate of tension development (dT/dt_{max}) were compared for each stimulation rate. At both temperatures, N_2O caused a 10–15% depression of contractility as compared to that observed with nitrogen. In a second protocol, muscles were studied at 37° C in 26 mM K^+ Tyrode's solution with 0.10 μM isoproterenol to study enhanced contractions mediated by slow (Ca^{2+}-channel-dependent) action potentials. Rested-state double stimulations were used (stimulus interval, 250–600 ms) resulting in a first rested-state contraction followed by a second contraction (C2) with rapid initial tension development. The muscles were exposed to nitrogen and N_2O as in the force–frequency experiments and did not deteriorate over time. In this setting, N_2O also caused a 10–15% depression of C2 contractility as compared with nitrogen. Another set of muscles was studied in 95% O_2 to which 0.5% halothane or 1% isoflurane was added before exposure to nitrogen and N_2O. The combined depressant action of N_2O with either halothane or isoflurane did not differ from that predicted by the simple addition of independent effects; there was no evidence of synergism. Furthermore, N_2O (50%) alone depressed dT/dt_{max} in a manner similar to that of 0.5% halothane and different from that of 1.0% isoflurane. Experiments conducted in iso-osmolar 40 mM Na^+ Tyrode's solution, in which activator Ca^{2+} arose from the sarcoplasmic reticulum Ca^{2+}, also showed greater depression by N_2O than nitrogen. N_2O (50%) is a myocardial depressant independent of concurrent hypoxic effects with a pattern and magnitude of contractile depression similar to that of 0.5% halothane. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: halothane; isoflurane. Animal: guinea pig. Heart: contractility; force–frequency relation; papillary muscle.)

* Assistant Professor of Anesthesiology.

† Advanced Laboratory Specialist.

‡ Associate Professor of Anesthesiology.

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Address reprint requests to Dr. Lawson: Department of Anesthesiology, Box 238, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908.

EXPERIMENTAL TECHNIQUES devised to study myocardial contractile physiology and pharmacology have significantly advanced our understanding of the mechanisms by which anesthetics exert their effects.[1] Data accumulated thus far suggest that the volatile anesthetics interfere with calcium (Ca^{2+}) availability to myofibrils, thereby reducing contractility, while the agents also modify myofibrillar Ca^{2+} release, thus altering relaxation.[2–7] Conspicuously absent from recent experimental scrutiny is N_2O. In use for over 130 years and the most ubiquitous of the inhaled anesthetics, N_2O effects on myocardium remain unresolved. Goldberg *et al.*,[8] using rat trabeculae carneae bathed at 37° C, demonstrated similar degrees of contractile depression with N_2O and nitrogen; they suggested that reduced contractility was not attributable to N_2O. Price[9] used cat papillary muscles suspended in a 25° C bath to examine N_2O-induced depression. On exposure to 50% N_2, no effect was seen; however, significant depression was demonstrable with 50% N_2O, which was reversed by increasing the Ca^{2+} concentration in the perfusate. Su *et al.*,[10] using skinned myocardial muscle preparations at 22° C, presented evidence that N_2O slightly decreases the affinity of myofibrils for activator Ca^{2+} and it also increases the uptake of Ca^{2+} by the sarcoplasmic reticulum (SR). In all, the data on N_2O leave doubt as to its ultimate direct effects on *in vitro* myocardial inotropy. Moreover, little can be said of the mechanisms by which N_2O may act.

To reexamine the hypothesis that N_2O significantly depresses myocardial contractility and to properly position it in the spectrum of anesthetic effects on *in vitro* myocardium, a series of experiments were conducted on guinea pig papillary muscle that were similar to those previously conducted using the volatile agents.[5,7] Experiments were also performed to determine if myocardial depression by halothane and isoflurane is modified by exposure to N_2O.

Methods

N_2O effects were studied using techniques previously described.[5,7] The heart was excised from methoxyflurane-anesthetized guinea pigs according to protocol approved by the University of Virginia Animal Research Committee. Right ventricular papillary muscles were horizontally mounted in a recirculating chamber maintained at 37 or 30° C (15 ml · min^{-1}) and superfused with modified Tyrode's solution (composition in mM: Na, 143; K, 5.0; Cl,

128; Ca, 2.5; Mg, 1.2; SO_4, 1.2; HCO_3, 25; glucose, 11; and EDTA, 0.1). All experiments were conducted with the recirculating muscle chamber enclosed within a 21.6-l Plexiglass® enclosure. Gas mixtures (6 l/min total flow) were bubbled through superfusate in a sealed 1-l reservoir maintained at 37 or 30° C and then directed into the enclosure before venting to an exhaust source. Gases used for the study were as follows: 95% O_2/5% CO_2, 50% N_2/45% O_2/5% CO_2, and 50% N_2O/45% O_2/5% CO_2. The latter two mixtures were composed by combining 90% O_2/10% CO_2 (±0.02%) with either 100% N_2 or 100% N_2O so that the final mixed gas flow contained 45% O_2 (±1%) as measured by a calibrated polarographic oxygen electrode. Oxygen levels (per cent) inside the enclosure were monitored continuously. Perfusate *p*H was also monitored continuously, and its maintenance at 7.45 ± 0.05 verified a continuous CO_2 in the gas phase of 5%. N_2O and N_2 were assumed to be 50% (within 2%) by subtraction of the other gases. Muscles were field stimulated with 0.5–1-ms pulses at current levels 10% above threshold. Isometric tension development was recorded at the minimum muscle length and rest tension that produced the maximal active tension. The stability of each muscle's contractile behavior was verified by equilibration for a 25–35-min period in 95% O_2, during which time intermittent stimulation at 0.5 Hz demonstrated identical developed force. The muscle was then kept inactive for 15–20 min.

Force–Frequency Protocol

The first contraction elicited after 20 min of rest was termed the rested-state contraction (RSC). Consistent muscle tension development was then elicited at 0.1, 0.25, 0.5, 1, 2, and 3 Hz stimulation rates with 20–60 s required at each frequency for stabilization. This stimulation profile produces the typical positive "staircase" or force–frequency relation: greater tension and more rapid tension development with each higher stimulation rate. The peak developed tension and the first derivative with respect to time (rate of tension development; dT/dt) were recorded continuously.

In all the protocols described, the muscle was equilibrated with gas in the following manner: 1) initial 95% O_2 control; 2) 50% nitrogen or 50% N_2O with 45% O_2; 3) intermediate 95% O_2 recovery; 4) 50% nitrogen or 50% N_2O with 45% O_2; and 5) final 95% O_2 recovery. N_2O or nitrogen was applied in random order, and every gas exposure period lasted 25–35 min (seven to nine exchanges of the enclosure volume). Muscle contractility, as determined by peak tension and by the maximum rate of tension development (dT/dt_{max}), decreased by 30–50% during a typical 2–3 h force–frequency experiment at 37° C; this decline was greater than past controls from this laboratory[5] when employing 95% O_2/5% CO_2. Such deterioration suggested that 45% O_2 resulted in some degree of hypoxic dysfunction.[11] Many laboratories routinely perform experiments at 30° C to increase the longevity of their superfused isolated muscle preparations; therefore, these experiments were completely duplicated in identical fashion at 30° C. In muscles studied at 30° C, little deterioration in contractile behavior was observed over the course of the experiment; at this temperature, contractility was enhanced at 0.5–3 Hz, as shown by the control force–frequency curves in figure 1. When standardized for cross-sectional area, peak tension in muscles at 30° C was approximately threefold greater than at 37° C; dT/dt_{max} at 1 and 3 Hz was approximately twofold greater in muscles studied at the lower temperature.

Rested-State Double-Stimulation Protocol

Tyrode's superfusate was altered for this protocol by the partial substitution of K^+ for Na^+ (in mM: Na, 97; K,

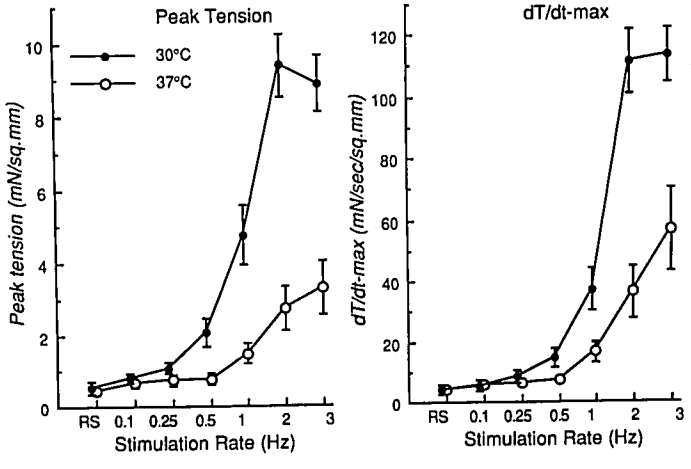

FIG. 1. Force–frequency relation of guinea pig papillary muscles in Tyrode solution at 37 and 30° C. Peak tension and maximum rate of tension development (dT/dt_{max}) standardized for the estimated muscle cross-sectional area. Error bars represent ± SEM (n = 5).

26) and the addition of 0.1 μM isoproterenol, with the bath at 37° C. Isoproterenol significantly enhances muscle inotropy and cell automaticity. Increased extracellular K^+ partially depolarizes the muscle, inactivates the Na^+ channels, and inhibits spontaneous papillary muscle activity, but permits propagation of slow (Ca^{2+}-channel-dependent) action potentials in response to electrical stimulation. After adjusting muscle tension and confirming stable contractile behavior as previously described, rested-state double-stimulation experiments were performed as follows: The first stimulus of the pair elicited an enhanced RSC that was characterized by modest initial tension development within 100 ms followed by rapid tension development resulting in late-peak tension. A second stimulus that was generated after an interval of 250, 300, 400, 500 or 600 ms elicited a second contraction (C2) that was notable for very rapid and strong early tension development without an initial delay. Each pair of stimuli was spaced by a 3- or 4-min rest so that reproducible RSCs were obtained. Contractile behavior with double stimulation was first determined under the following conditions: 1) initial 95% O_2 control; 2) 50% nitrogen or 50% N_2O with 45% O_2; 3) intermediate 95% O_2 recovery; 4) 50% nitrogen or 50% N_2O with 45% O_2; and 5) final 95% O_2 recovery. During the course of a 2- or 3-h experiment, a decline of 15–25% in peak tension was typically observed, with little change in the maximum rate of tension development.

To compare N_2O with the volatile anesthetics and to evaluate the additive effects of N_2O with those of the volatile anesthetics, a set of double-stimulation experiments was conducted using 1% isoflurane and another set using 0.5% halothane (approximately 0.66 MAC and 0.45 MAC, respectively, for the guinea pig[12]). The gas mixture exposure protocol was altered as follows: 1) 95% O_2; 2) 95% O_2 and volatile anesthetic (VA); 3) 50% nitrogen or 50% N_2O and VA with 45% O_2; 4) 95% O_2 and VA; 5) 50% nitrogen or 50% N_2O and VA with 45% O_2; 6) 95% O_2 and VA; and 7) 95% O_2 alone. As in the other experiments, the order in which N_2O or nitrogen was introduced was random, and for each gas mixture, rested-state double-stimulation contractions were observed (stimulus interval, 250–600 ms).

Low-Sodium Contractions

In a final set of experiments, the Na^+ concentration in Tyrode's solution was reduced (in mM: Na, 40; KCl, 5.0;) and superfusate osmolarity was maintained with 200 mM sucrose. This experimental preparation decreases the exchange of extracellular Na^+ for intracellular Ca^{2+} so that intracellular Ca^{2+} normally pumped out of the cell during rest accumulates within the SR, resulting in early tension development and large contractions.[5,13] After adjusting muscle tension and 20 min of stabilization with oxygen control, the muscles were stimulated at 0.1 Hz, achieving a steady-state response within 30–60 s. The first contraction after rest (RSC) and steady-state 0.1 Hz contractions were recorded. When stimulated at rates higher than 0.5–1 Hz, these muscles tended to develop some degree of contracture, a decrease in peak developed tension, and subsequent permanent loss in contractility. Therefore, higher stimulation rates were not employed. The gas mixtures were applied as previously described in the force–frequency protocol.

Data Analysis

At each stimulation rate or interval used in the force–frequency, double stimulation, and low-Na^+ experiments, peak tension and maximum rate of tension development (dT/dt_{max}) during N_2O or nitrogen exposure were expressed as a per cent of "control-recovery": the average of the values observed in the immediately preceding 95% O_2 (control) and in the subsequent 95% O_2 (recovery). This method corrects for nonreversible systematic changes in contractile characteristics of the muscle during the course of the experiment. In the presence of the volatile anesthetics, nitrogen or N_2O effects were also expressed as a per cent of average contractile behavior (control-recovery) in the preceding and subsequent 95% O_2 and VA.

To directly compare the relative potency of N_2O with those of isoflurane and halothane, contractions in the presence of either volatile anesthetic in 45% O_2 and N_2O in 45% O_2 were expressed as a per cent of the initial 95% O_2 control only (see fig. 7). Thus, all three anesthetics are comparable in the presence of 45% oxygen. To evaluate the combined pharmacologic action of the volatile agents with N_2O, the depressant action of N_2O with each volatile agent was estimated according to the formula:

$$\text{Predicted effect of combined nitrous oxide-volatile agent (as \% control)} = \frac{(N_2O \text{ effect as \% control}) \times (\text{VA in 45\% } O_2 \text{ effect as \% control})}{100\%} \quad (1)$$

This estimate assumes that each agent works independently, and the effects merely superimpose onto the measured variable (peak tension or dT/dt_{max}). The effects as predicted by equation 1 were compared with the actual observed results for 50% N_2O and either volatile agent.

A simple *t*-test was used to evaluate the significance of the effect of N_2O or nitrogen at each stimulation rate or interval (as per cent control-recovery) *versus* unity (100%). Comparisons between nitrogen and N_2O oxide employed a two-way analysis of variance for repeated measures with Duncan's multiple range test. Comparisons among the

anesthetics during rested-state double stimulation employed an analysis of variance with Duncan's multiple-range test.

Results

Force–Frequency Experiments

N_2O (50%) consistently and reversibly reduced contractile performance of the papillary muscles at 37° C to a greater extent than that caused by 50% N_2 after rest and at all stimulation rates (fig. 2). The presence of nitrogen depressed the peak tension developed by the papillary muscles to approximately 80% of the control-recovery value after rest and at all steady-state frequencies (0.1–3 Hz), which is a significant, partially irreversible reduction in muscle performance. N_2O depressed peak tension development at all frequencies to 59–64% of the control-recovery value and an average of 16 ± 6% more than with nitrogen; this additional depression was significant at all frequencies except 2 and 3 Hz. In contrast, the maximum rate of tension development (dT/dt_{max}) was more resistant to the effects of reduced oxygen concentrations and was reduced ($P < 0.05$) compared to control-recovery in the presence of nitrogen only at the 1 Hz stimulation rate. The maximum rate of tension development was inhibited by N_2O an additional 27 ± 5% compared to nitrogen, a difference that was significant at all frequencies. Although there appeared to be less difference between the actions of nitrogen and N_2O on dT/dt_{max} as the stimulation rate was increased to 3 Hz, the difference in depression (N_2 effect minus N_2O effect, as % of control) after rest (35 ± 8% of control) did not differ from that at 3 Hz (20 ± 5% of control).

The average change in peak tension and dT/dt_{max} for each stimulation rate between the initial 95% O_2 control and the final 95% O_2 recovery is shown in figure 3A. There was less deterioration of dT/dt_{max} than peak tension during the experiment. Past controls of guinea pig papillary muscle from this laboratory show a decline of 0–20% in peak tension development during a typical 3-h experiment in 95% O_2/5% CO_2 at 37° C.[5] However, over the course of these experiments, with two 25–35-min exposures to 45% O_2, the muscles showed a decline in contractile performance of up to 50% (fig. 3A). The contractile depression at 37° C associated with exposure to nitrogen and the significant overall deterioration in papillary muscle contractility led us to repeat the force–frequency protocol at 30° C with the expectation that oxygen consumption and muscle degradation would be reduced.[11] In this setting, despite stronger contractions (see fig. 1), the contractile behavior of the muscles showed little change over the course of the experiment between the initial 95% O_2 control and the final 95% O_2 recovery (fig. 3B).

The effects of nitrogen and N_2O on contractile performance at 30° C are shown in figure 2B. Nitrogen caused little depression of peak tension or dT/dt_{max} steady-state contractions except at 2 Hz where dT/dt_{max} was reduced 3% and 3 Hz where peak tension was reduced 5%. Enhanced peak tension development and dT/dt_{max} were seen in the RSC. N_2O significantly reduced peak tension across all frequencies an average of 19 ± 4% when compared to 95% O_2 control-recovery, and 20 ± 5% at 0.5, 1, and 3 Hz when compared to nitrogen. N_2O depressed dT/dt_{max} by an average 19 ± 3%, an effect that was significantly different from both nitrogen control and 95% O_2 control-recovery at all frequencies. Thus, at 30 and 37° C, 50% N_2O depressed papillary muscle contractions by 15–20% compared to that caused by nitrogen; however, at 30° C, there was no superimposed depression of contractility in association with repeated exposures to 45% O_2.

Rested-State Double-Stimulation Experiments

A typical experiment using the double-stimulation technique on muscles in 26 mM K^+ Tyrode's solution and 0.1 μM isoproterenol is shown in figure 4A. The first RSC showed modest tension development for approximately 100 ms before rapid strong tension development, a pattern typical for the RSC observed in guinea pig muscle when stimulated by drugs that increase intracellular cyclic adenosine monophosphate (cAMP).[13–15] The contraction (C2) elicited by the second stimulus (at an interval of 300 ms) showed rapid strong initial tension development with no initial delay. In this example, N_2O caused significant (18%) depression of both the RSC and of C2, with substantial recovery on return to 95% O_2. Subsequent application of 50% N_2 and return to 95% O_2 had little effect. Figure 4B plots the change observed in the dT/dt_{max} for the muscle shown in figure 4A across all the stimulation intervals; 50% N_2O caused the greatest depression in all instances.

The mean change observed for the rested-state and second contractions (C2) with either 50% N_2 or 50% N_2O are summarized in figure 5. Small significant changes (3 and 4%) were observed with nitrogen for peak tension and dT/dt_{max} for C2 at 250 ms. Unlike the behavior of the papillary muscles in the force–frequency protocol at 37° C, 50% N_2 had little deleterious effect on peak tension under the double-stimulation technique. The relatively short periods of muscle activity in this protocol (two contractions followed by 3 or 4 min of rest) appeared to prevent the deterioration of contractile performance seen in muscles in the force–frequency protocol at 37° C. N_2O significantly depressed all C2 peak tensions and all C2 dT/dt_{max} compared to the 95% O_2 control-recovery. N_2O also depressed C2 compared to nitrogen; peak tension was depressed by 10% compared to nitrogen at the

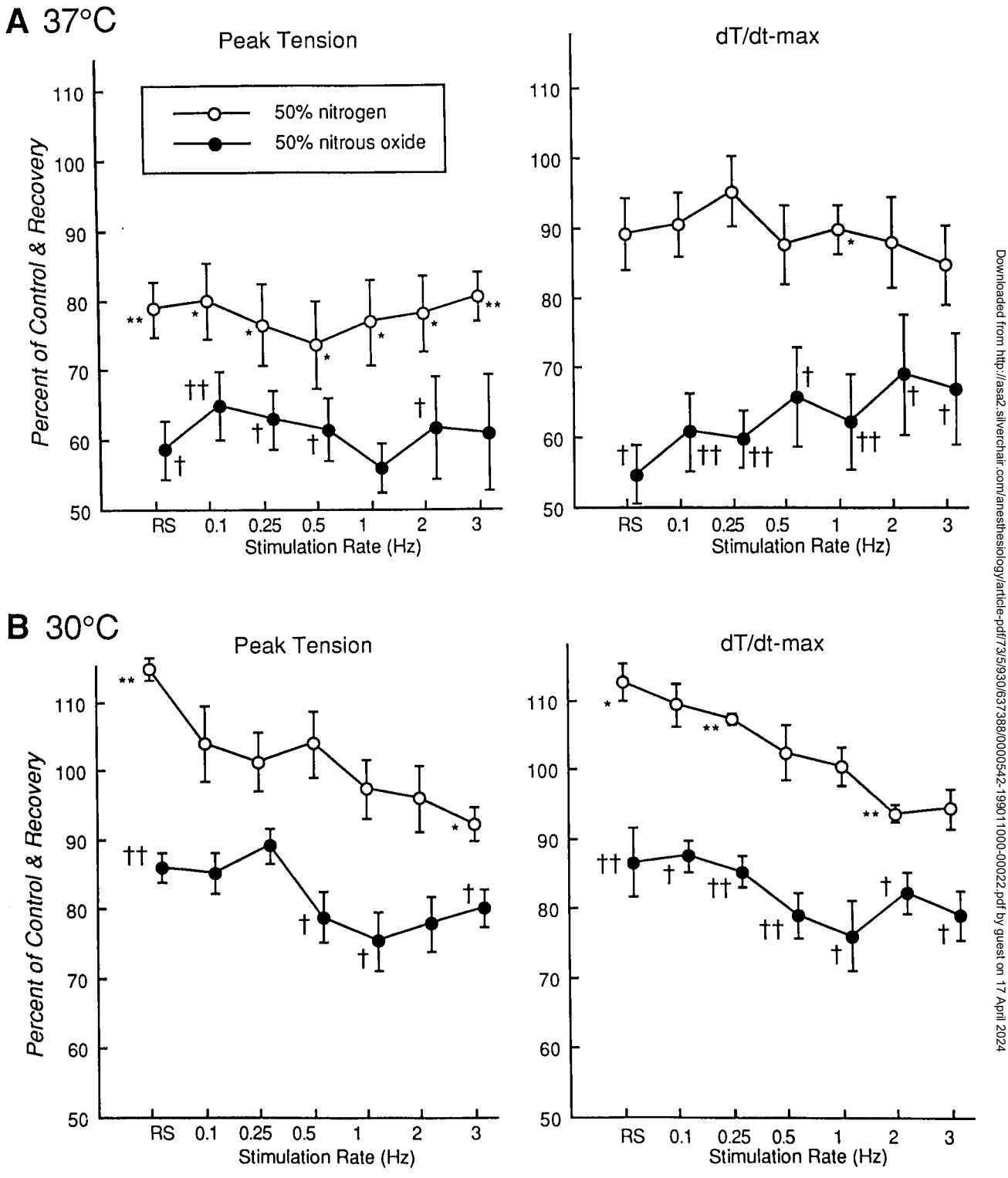

FIG. 2. Nitrous oxide effects on papillary muscles (mean CSA = 0.80 ± 0.11 mm^2 for 37° C experiments; mean CSA = 0.68 ± 0.06 mm^2 for 30° C experiments) during force–frequency contractions at 37 and 30° C. Peak tension and dT/dt_{max} are expressed as percent of the average of the values observed in 95% O_2 immediately preceding and following the N_2 or N_2O exposure (control and recovery). Error bars represent ±SEM; n = 5 at each temperature. (*A*) at 37° C N_2 (50%) significantly depressed peak tension, with less effect on dT/dt_{max}. N_2O (50%) was significantly more depressant than was N_2. (*B*) At 30° C, 50% N_2 caused no deleterious effects on the preparations. In this setting, N_2O still caused significant depression of contractility. *$P < 0.05$; **$P < 0.01$, different from control-recovery (100%) by simple *t* test; †$P < 0.05$; ††$P < 0.01$ by ANOVA between gases with Duncan's multiple range test.

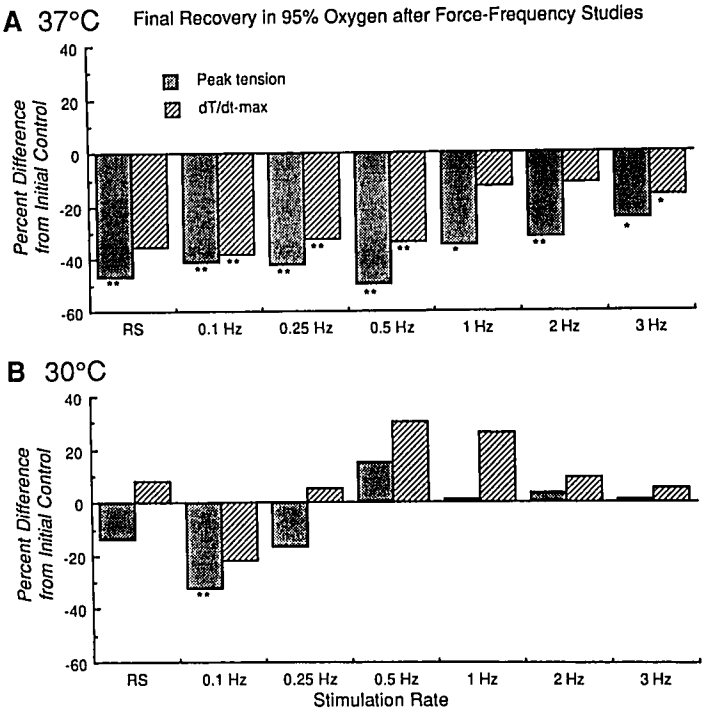

FIG. 3. Final papillary muscle contractile performance in 95% O_2 control after a 2–3-h experiment that included two 25–35-min periods of exposure to 50% N_2 and 50% N_2O with 45% O_2; n = 5 at each temperature. Contractility is expressed as the percent difference from the initial 95% O_2 control. (*A*) Two exposures to reduced (45%) oxygen at 37° C resulted in considerable loss of contractile performance. (*B*) Reduced O_2 at 30° C caused little significant alteration in overall contractility. $*P < 0.05$; $**P < 0.01$, difference from initial 95% oxygen control (100%) by simple *t* test.

250-, 400-, and 500-ms intervals; the C2 dT/dt_{max} at 250, 300, 400, and 600 ms was depressed by N_2O by 10–15% compared to nitrogen.

To examine N_2O depression in the presence of the other anesthetics, the effects of N_2O and nitrogen were determined in a stable anesthetic background of either isoflurane (1%) or halothane (0.5%). Nitrogen did not cause further inhibition of muscle performance during the RSC or C2 (fig. 6). However, the presence of N_2O caused significant additional depression of approximately 10–15% of peak tension and dT/dt_{max} during the RSC and all second contractions. This depressant effect was similar in magnitude to that observed in the absence of the volatile anesthetics (fig. 5), except for the RSC that appeared to be depressed only in the presence of anesthetics plus N_2O but not in 50% N_2O alone.

The minimal effect of 45% oxygen on the rested-state double-stimulation experiments allows a direct comparison among isoflurane, halothane, and N_2O in 45% O_2. Effects on muscle contractility of 50% N_2O, 1% isoflurane, and 0.5% halothane are plotted in figure 7 as per cent of initial muscle performance in 95% O_2 rather than as per cent of (95% O_2 control + 95% O_2 recovery)/2 as employed in figures 2, 5, and 6. Any intrinsic changes in muscle contractility with respect to time are not factored out by this analytic method and would thus be included as part of the anesthetic effect. This difference in contractile analysis may explain why N_2O appeared to significantly depress the RSC dT/dt_{max} (as per cent of initial 95% O_2 control in fig. 7A), yet contractility, as expressed as the mean of 95% O_2 control-recovery (fig. 5), revealed no significant N_2O effect. The depression of RSC peak tension amplitude did not significantly differ among N_2O, isoflurane, and halothane (fig. 7A); however, 1% isoflurane did increase the time to the RSC tension peak by 17% from 174 ± 6 to 203 ± 4 ms (n = 4; $P = 0.001$). By way of contrast, 50% N_2O and 0.5% halothane caused insignificant changes of 0.3 and 2.6% from control values of 160 ± 5 and 159 ± 16 ms, respectively. The 29-ms increase in time to late-peak RSC tension caused by isoflurane was sufficient to prevent the second stimulus at 250 ms from eliciting a second contraction in three of four muscles; therefore, values for C2 at 250-ms intervals are not shown for isoflurane. The second contraction at 300 ms in isoflurane was stronger than the preceding RSC and also stronger than the corresponding C2 contraction in halothane, but otherwise, the three anesthetics depressed C2 peak tension to a similar degree.

Differences among the anesthetics emerge on examination of the rates of tension development (dT/dt_{max}, fig. 7A). Although N_2O and halothane depress dT/dt_{max} during the RS contraction, isoflurane caused greater depression of the RSC dT/dt_{max} as is characteristic for this agent.[5,7] Isoflurane in 45% O_2 did not depress C2 dT/dt_{max} compared to the initial 95% O_2 control, whereas halothane and N_2O depression of C2 dT/dt_{max} was significantly greater than that of either isoflurane or oxygen control. In the case of halothane, the depression of C2 dT/dt_{max} at 500- and 600-ms stimulation intervals was greater than for the RSC.

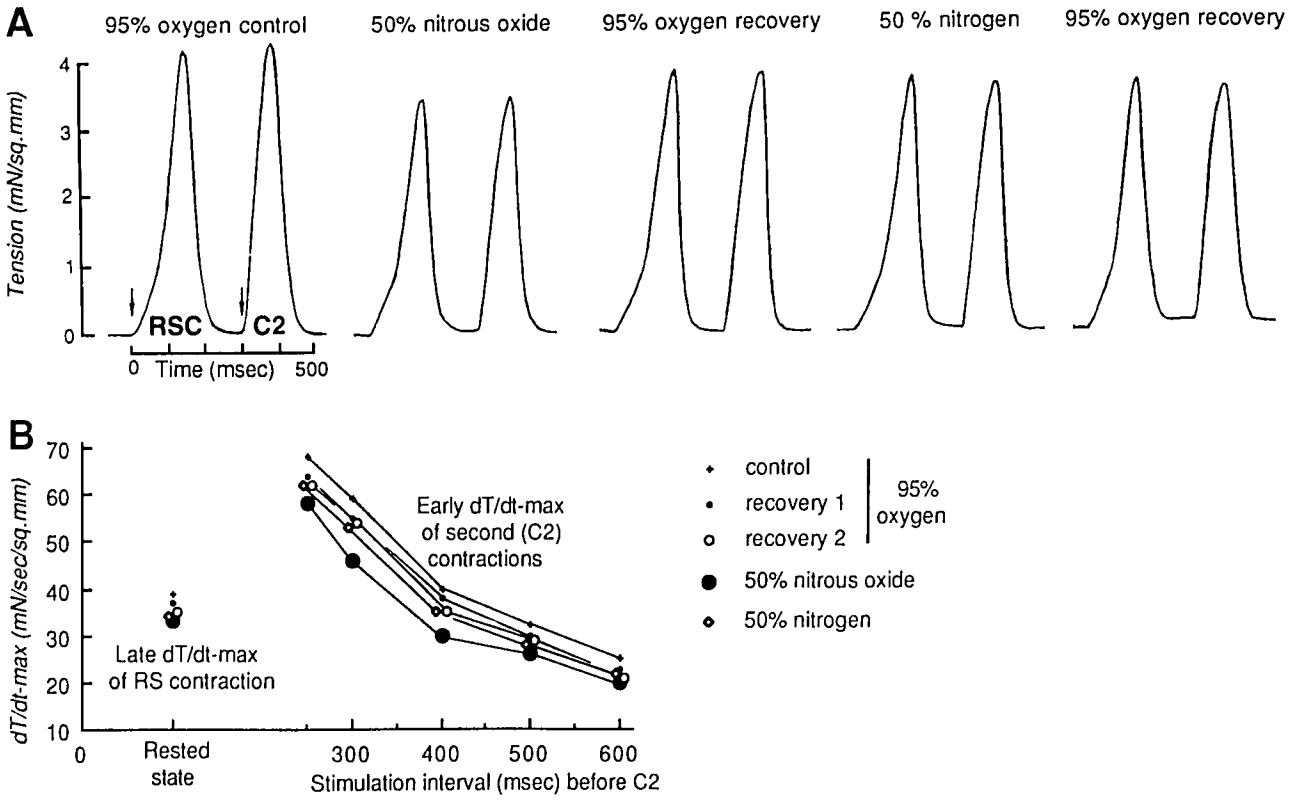

FIG. 4. Nitrous oxide effects on papillary muscles in 26 mM K^+ Tyrode solution with 0.1 μM isoproterenol at 37° C; depolarizations are mediated by slow (calcium channel-dependent) action potentials. Rested-state double-stimulation protocol employed two stimuli separated by 250, 300, 400, 500, or 600 ms with intervening periods of 3–4 min of rest. (*A*) Tension recordings of papillary muscle contractions with a stimulation interval of 300 ms (arrows in control tracing indicate time of stimulus). The rested state contraction (RSC) with late-peaking tension is followed by the second contraction (C2) with rapid initial tension development. Sequential traces for the same muscle are shown for 95% O_2 initial control, 50% N_2O, 95% O_2 recovery, 50% N_2, and 95% O_2 final recovery. (*B*) Maximum rate of tension development (dT/dt_{max}) of the muscle shown in fig. 4*A* at all stimulus intervals and standardized for cross-sectional area (0.40 mm^2).

The observed combined depressant action of N_2O and either halothane or isoflurane is plotted in figure 7B as a percentage of initial 95% O_2 control contractions. The estimated combined additive depression of N_2O–isoflurane and N_2O–halothane is also plotted, employing the per cent depression of each agent in 45% O_2 (fig. 7A) and calculated using equation 1 (see methods). Although slight differences from the prediction were noted in two of the 22 comparisons, the experimental combination of N_2O with either halothane or isoflurane did not signifi-

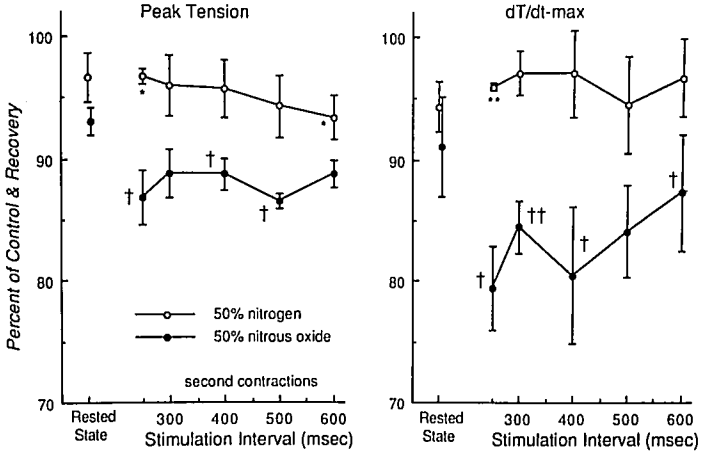

FIG. 5. N_2O effects on papillary muscles (mean CSA = 0.79 ± 0.17 mm^2) during rested-state double-stimulation experiments at 37° C (see fig. 4). Peak tension and dT/dt_{max} of rested state contraction (RSC) and second contraction (C2) are expressed as a percent of the average control-recovery values in 95% O_2. N_2 (50%) caused no consistent change in contractility, whereas N_2O depressed C2, with little effect on the RSC. Error bars represent ± SEM (n = 4). *$P < 0.05$; **$P < 0.01$ different from control-recovery (100%) by simple *t* test; †$P < 0.05$; ††$P < 0.01$ different than 50% N_2 by ANOVA with Duncan's multiple range test.

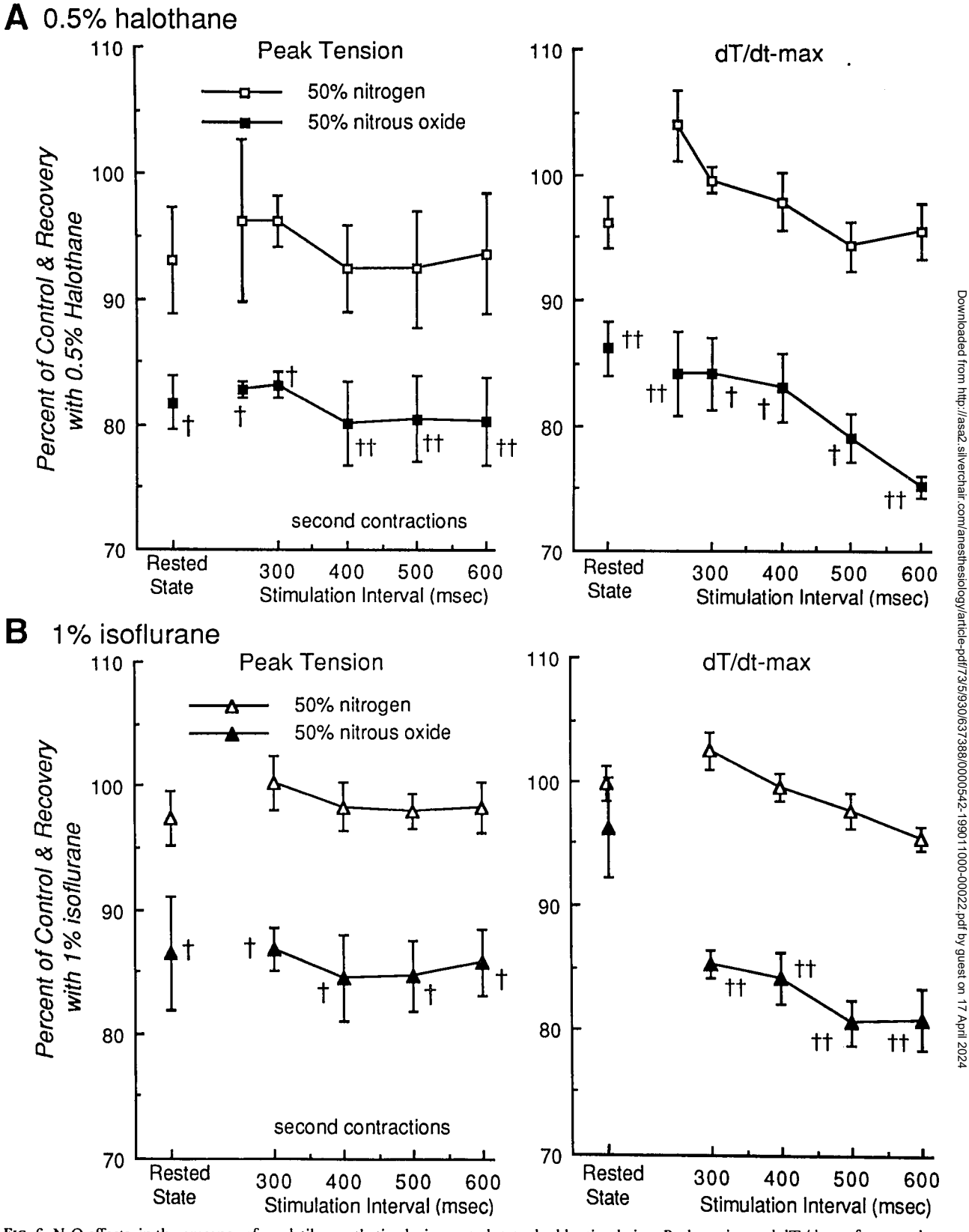

FIG. 6. N_2O effects, in the presence of a volatile anesthetic, during rested-state double stimulation. Peak tension and dT/dt_{max} of contractions are expressed as a percent of the average control-recovery values in 95% O_2 with either anesthetic. Error bars represent ±SEM; n = 4 for each anesthetic. N_2 had no additional effect on muscles previously depressed by anesthetic, whereas N_2O caused additional uniform depression in the presence of either anesthetic. (*A*) Effects in the presence of prior depression by 0.5% halothane. (*B*) Effects on the presence of 1.0% isoflurane. $P < 0.05$ compared to control-recovery by simple *t* test. †$P < 0.05$; ††$P < 0.01$ different from nitrogen by ANOVA using Duncan's multiple range test.

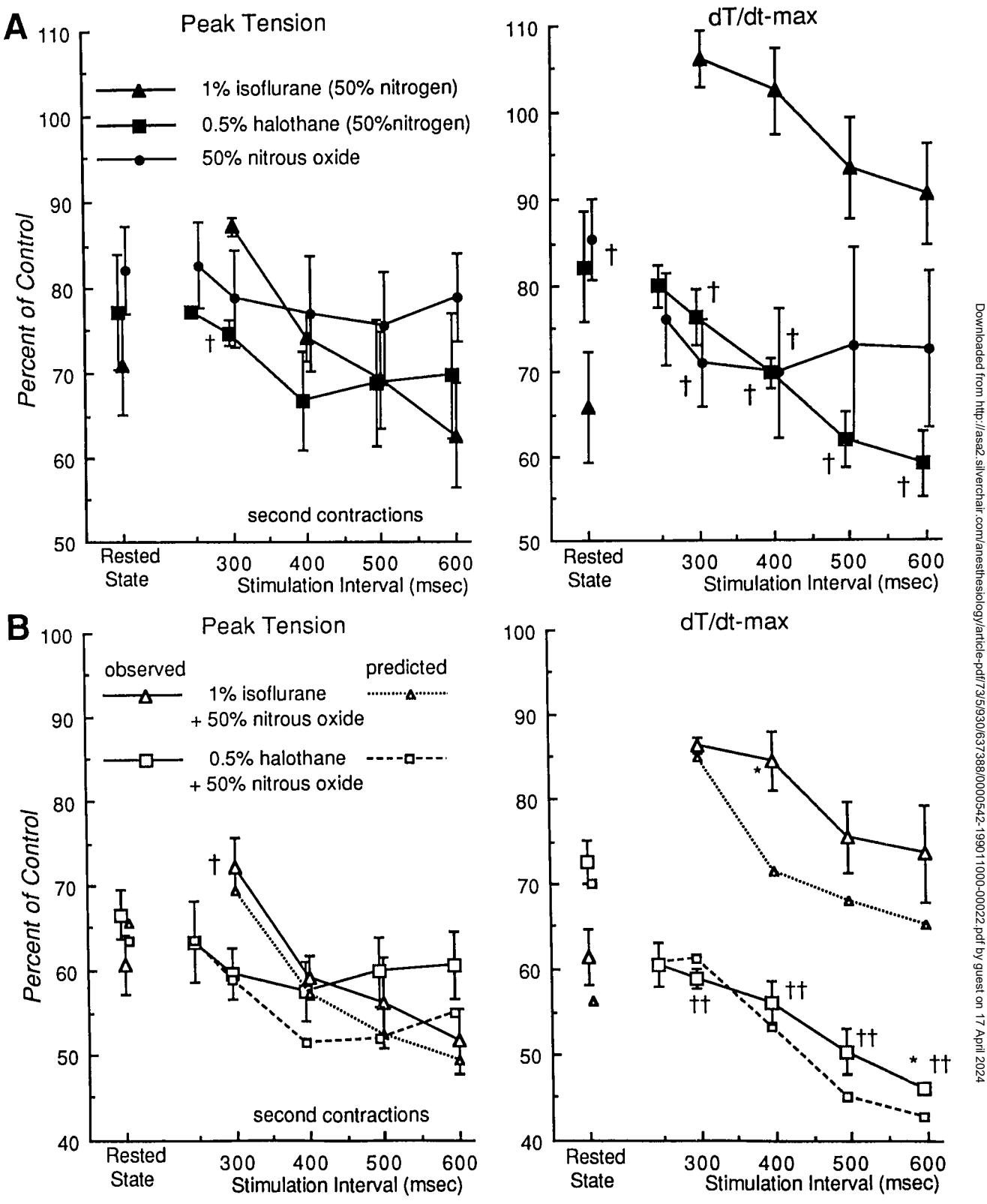

FIG. 7. N_2O, isoflurane, and halothane effects during rested-state double stimulation. The data for N_2O are derived from separately studied muscles shown in figure 5; data for the volatile anesthetics with and without N_2O are derived from separately studied muscles shown in figure 6. Peak tension and dT/dt_{max} of contractions are observed in 45% O_2 and are expressed as a percent of the initial 95% O_2 control. Error bars represent ±SEM; n = 4 for each anesthetic. (*A*) Anesthetics significantly depressed peak tension and dT/dt_{max} from 95% O_2 control in all cases ($P < 0.05$ by ANOVA between each anesthetic and initial control), except for isoflurane, which did not depressed the C2 dT/dt_{max}. (*B*) Predicted combined depression of 50% N_2O with 0.5% halothane and with 1% isoflurane, calculated according to equation 1 in methods, is plotted with the observed contractile behavior of muscles in the presence of N_2O and either agent. No synergy is apparent. †$P < 0.05$; ††$P < 0.01$ different from isoflurance by ANOVA with Duncan's multiple range test; *$P < 0.05$ difference between measured and predicted values by simple *t* test.

cantly vary from that predicted by simply adding the drugs' effects.

Low-sodium Contractions

In this experimental protocol, the presence of 50% N_2 did not significantly diminish the rapidly developed peak tension and early dT/dt_{max} of the RS or 0.1-Hz contractions (fig. 8). However, the exposure of the muscles to N_2O caused a depression of the RS and 0.1-Hz contractions, significantly diminishing dT/dt_{max} and peak tension.

Discussion

This study examined the effects of N_2O in a well-characterized model of isolated myocardial tissue (superfused guinea pig papillary muscles) in a manner intended to eliminate or reduce confounding variables such as nonphysiologic temperatures and possible muscle deterioration.[8,9,11] In addition, the magnitude and pattern of N_2O's contractile effects were contrasted with those of isoflurane and halothane, two anesthetics that cause distinctly different patterns of depression.[5,7]

Nitrous Oxide Depression, Reduced O_2, or Drug Effect?

The force–frequency stimulation protocol was conducted at 37° C because changes in the sources of activator Ca^{2+} may occur during contractions performed at temperatures lower than physiologic.[16] Unfortunately, periods of continuous muscle stimulation at 37° C during exposure to 25–30-min periods of 45% O_2 caused a 20–40% decline in baseline (95% O_2) contractility over a 2- or 3-h period. This decline was substantially greater than that observed in our previous experience,[5] despite the fact that the cross-sectional area (CSA) of the muscles was consistently less than 1.0 mm^2, which is, ostensibly, a small radial diffusion distance for oxygen. Paradise *et al.*[11] previously demonstrated in kitten papillary muscles that with decreasing solution P_{O_2}, nonlinear deterioration in contractile performance occurred with increases in muscle diameter or stimulation rate. These effects were more prominent at 37 than at 30° C. In the current study, at 37° C, peak tension and dT/dt_{max} were, to a large degree, irreversibly reduced across most frequencies after periods of exposure to 50% nitrogen, whereas a greater readily reversible depression was observed with N_2O (fig 2A). The depression recorded during nitrogen exposure, although relatively irreversible, did not lead to the contractures classically described in the presence of profound tissue hypoxia, metabolic dysfunction, and imminent cell death.[17]

Goldberg *et al.*[8] found no difference in muscle depression under 50% N_2 or 50% N_2O in rat trabeculae bathed at 37° C and stimulated at 15/minute (0.25 Hz). Although depression of dT/dt_{max} of 28.9% with 50% N_2 and 43.4% with 50% N_2O suggested an N_2O effect, the difference was not statistically significant. Unlike our protocol, Goldberg *et al.* made no attempt to wash out each gas administration with 95% O_2; thus, the full potential for reversibility was not assessed. They also exposed their preparations to 25% O_2 while testing for the effects of 75% N_2 and 75% N_2O; without high oxygen intervals separating these exposures, hypoxic depression and permanent damage to their preparation cannot be excluded.[11] Although the authors acknowledge that hypoxia adversely affected their preparation, they conclude that N_2O lacks pharmacologic potency. Our data at 37° C demonstrate additional depression on exposure to N_2O, despite some underlying tissue deterioration. The contractility data are reported as per cent of the mean 95% O_2 control-recovery for each nitrogen or N_2O administration; therefore, muscle deterioration is factored between each gas exposure, and drug washout and muscle recovery could be readily recorded.

The study by Price in cat papillary muscles presented evidence for an N_2O effect at 25° C.[9] Recognizing the effect of decreased oxygen tensions at 37° C in obscuring

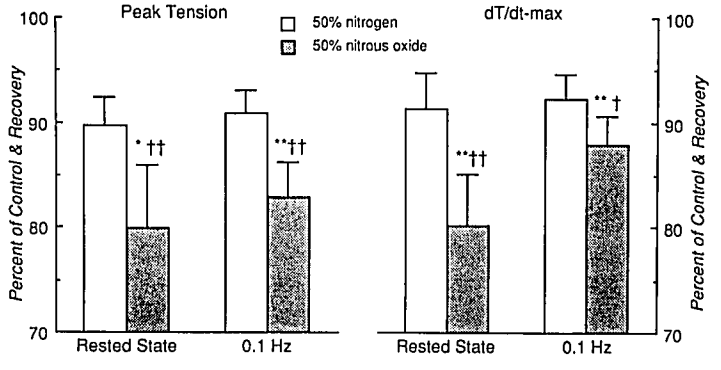

FIG. 8. N_2O effects on papillary muscle contractions elicited in isoosmotic 40 mM Na^+ Tyrode; contractions are mediated by the release of sequestered SR calcium. Peak tension and dT/dt_{max} are expressed as percent of the average control-recovery values in 95% O_2. Error bars represent ±SEM (n = 7). *$P < 0.05$ compared to control-recovery by simple t test; †$P < 0.05$; ††$P < 0.01$ different from nitrogen by ANOVA using Duncan's multiple range test.

the action of N_2O, we repeated the force–frequency protocol in a 30° C bath. If the average temperature coefficient (Q_{10}) for metabolic rate is at least two, then a 7° C decrease in bath temperature to 30° C should reduce oxygen demand by 35%. Although tension and dT/dt_{max} at 30° and 37° C are similar at low stimulation rates, both peak tension and dT/dt_{max} increased two- or threefold with successive stimulation rates above 0.5 Hz. Yet, the amplification of muscle contractility at 30° C and intermittent exposure to 45% O_2 did not cause a serious change in contractile performance over time. Muscle performance at the time of the first 95% O_2 control, compared with contractility during the last 95% O_2 control (as depicted in figure 3B), showed little evidence of run-down at 30° C, unlike muscle behavior at 37° C (fig. 3A). Steady-state contractions up to 1.0 Hz were unaffected by nitrogen at 30° C, while N_2O significantly decreased peak tension at selected stimulation rates and decreased dT/dt_{max} at all stimulation rates. At the physiologic frequencies of 2 and 3 Hz, nitrogen was associated with a slight reduction of dT/dt_{max} or peak tension, probably due to the moderate oxygen tension and high muscle workload. With greater contractility at 30° C, the absence of sustained detrimental effects of 45% O_2 following periods of 2 and 3 Hz contractions suggest that basal metabolic requirements (*e.g.*, maintenance of ion gradients, etc.) may be sufficiently decreased by the lower temperature so that metabolic reserves are maintained. It is also noteworthy that permanent deterioration in contractility at 37° C required a sustained myocardial workload, since the β-adrenergically enhanced muscles studied in the double-stimulation experiments at 37° C did not manifest signs of deterioration in 45% O_2. The increase in myocyte oxygen consumption during enhanced contractions in the double-stimulation experiments must have been offset by the infrequency of contractions. In this setting as well, N_2O was clearly depressant.

In isolated perfused whole guinea pig hearts at 37° C, Stowe *et al.*[18] demonstrated significant depression (25%) of left ventricular pressure generation by 48% N_2O (with 48% O_2, 4% CO_2), which significantly exceeded the depression (of 20%) caused by 48% N_2. These results in a vascularly perfused preparation of the same cardiac muscle underscore the impact of low oxygen concentrations at 37° C; it is unclear why the additional depression caused by N_2O was so modest. In contrast, Motomura *et al.*[19] used a blood-perfused isolated dog papillary muscle (maintained at 38° C and stimulated at a rate of 2 Hz) and found that substitution of 80% N_2O for 80% N_2 in the inspiratory gas of the donor dog caused a 25 ± 5% reduction in developed tension, which is consistent with the 15–20% additional depression caused by 50% N_2O in the current study.

In summary, the data collected at 30 and 37° C strongly suggest a reversible N_2O effect that is distinct and separable from any concurrent hypoxic effect. Although unlikely, one may argue that N_2O-induced depression may be dependent on the presence of a low oxygen tension, and such depression would not be observed at high oxygen tensions. Ultimately, experiments using hyperbaric chambers will be required to resolve such an objection.

Nitrous Oxide: Potency and Potential Mechanisms

We used the rested-state double-stimulation protocol at 37° C to further study N_2O because the force–frequency stimulation pattern used on the guinea pig muscles in 45% oxygen at 37° C caused substantial deterioration in contractility and because the contractile activation characteristics of myocardium are altered at 30° C.[16] Additionally, this method permitted a detailed comparison of N_2O and volatile inhalational anesthetics and determination of combined actions. This preparation relies on β-adrenergic stimulation using isoproterenol to increase cAMP levels, thereby increasing Ca^{2+} entry to the cell[20] and Ca^{2+} uptake by the SR.[21] Although the partially depolarized ($[K]_o$ = 26 mM), β-adrenergically enhanced muscles are clearly nonphysiologic, the observed anesthetic effects do correlate closely with those of the normal force–frequency response[5] while also permitting some mechanistic insights.[7]

The potency of 50% N_2O (approximately 0.33 MAC for guinea pig, as calculated from the halothane:N_2O and isoflurane:N_2O potency ratio for humans and the volatile anesthetic MAC values for guinea pig[12]) was relatively consistent in the various conditions under which it was employed; peak contractile tension and dT/dt_{max} were depressed approximately 15% as compared to nitrogen. If contractility in the rested-state double-stimulation experiments is expressed as the per cent of the initial 95% O_2 control and directly compared with the volatile agents, then depression of peak tension by 50% N_2O was similar to that of 0.45-MAC halothane and 0.66-MAC isoflurane in 45% O_2. This analytic representation (as per cent of initial 95% O_2 control only) may exaggerate drug-induced depressant effects by including intrinsic time-dependent decreases in contractility and reversible hypoxic changes; this analysis does lead to a modest increase in the estimate of N_2O depression from 10–15% to 15–25%. It may perhaps be surprising that 50% N_2O appears to be as potent as the higher doses of the volatile agents. N_2O closely resembles halothane in its relatively uniform depression of contractions after rest and at various stimulation intervals (fig. 7) and at various continuous stimulation rates.[5] In contrast, isoflurane caused little depression with in-

creases of the stimulation rate to 2 or 3 Hz[5] and a different pattern of rested-state contraction inhibition. Su *et al.*[10] found that 50% N_2O depressed maximal Ca-stimulated active tension of skinned rabbit myofibrils to 96% of control. While this effect may contribute to the depression observed in this study, it is insufficient to explain the greater effect observed.

In these studies of N_2O action on intact muscle, it is not possible to define with certainty the subcellular processes by which N_2O produces its effect. However, based on N_2O actions on initial or late elements of tension development, and its similarity to other anesthetics and drugs, certain mechanistic inferences are possible. Ryanodine is a plant alkaloid that binds to the Ca^{2+} release channel–"foot" protein complex of the junctional sarcoplasmic reticulum (SR) and causes a low conduction and prolonged open state that ultimately leads to the loss of Ca^{2+} from SR.[22,23] Ryanodine most profoundly depresses muscle contractility during steady-state 2 or 3 Hz contractions,[24] and it also depresses the rapid initial tension development of the second contraction of the rested-state double stimulation (C2).[7] These findings suggest that such contractions are heavily dependent on intact SR release of stored Ca^{2+}, and it is the resultant rapid initial tension development that is most relevant for tension generation at physiologic frequencies. In this study, the rate of tension development of C2 (dT/dt_{max}) is depressed by 0.5% halothane to ~75–65% of control, while 1.5% halothane was previously found to depress C2 contractions to ~40% of control.[7] N_2O (50%) depression of C2 dT/dt_{max} closely resembled 0.5% halothane, while isoflurane was not depressed. These results suggest that halothane and N_2O decrease the rapid Ca^{2+} release from the ryanodine-sensitive SR pool; this decrease may be due to Ca^{2+} depletion or blockade of release. A similar conclusion regarding halothane was recently reached by Komai and Rusy in their study of rabbit atria.[25]

The experiments conducted in a low extracellular Na^+ environment (40 mM) add support to the postulate that N_2O depression at physiologic frequencies is mediated in part at the SR. In tissue superfused with physiologic solutions (~140 mM Na^+), the large Na^+ gradient across the sarcolemma drives the entry of three extracellular Na^+ into the cell in exchange for one intracellular Ca^{2+} *via* a specific Na^+:Ca^{2+} exchange mechanism. At rest, a fraction of the intracellular Ca^{2+} sequestered by the SR to maintain relaxation is exchanged out of the cell; with prolonged rest, the ventricular myocyte of the guinea pig and certain other mammals actually becomes depleted of Ca^{2+}.[26] A low extracellular Na^+ concentration (40 mM) reduces the exchange of intracellular Ca^{2+} out of the cell so that all the intracellular Ca^{2+} is retained in the SR during prolonged periods of rest.[27] Drugs that can interfere with SR function will reduce the amplitude of an RSC in low Na^+ solutions, while drugs that reduce Ca^{2+} entry have no effect on the rate of rise of contractions in low Na^+.[13,28] N_2O (50%), but not 50% N_2, modestly reduced the dT/dt_{max} and peak tension of contraction in the low Na^+ experiments (fig. 8), implying that N_2O possesses a depressant effect on SR function. Su *et al.*[10] inferred from tension development of skinned rabbit myofibrils subjected to caffeine-induced Ca^{2+} release that Ca^{2+} uptake was enhanced by N_2O while release was unchanged. When N_2O was present during both the uptake and release phases, tension was enhanced. Since these experiments were performed at 22° C, direct application to the present results may be misleading.

As previously noted, guinea pig myocardium permitted to rest in physiologic solutions will become depleted of SR Ca^{2+}.[26,29] Therefore, the RSC is mediated by Ca^{2+} entering from the extracellular space through the surface membrane (sarcolemma) to activate the myofibrils. In the absence of inotropic stimulation, this contraction is small with delayed tension development. The force–frequency experiments conducted at both 37 and 30° C clearly demonstrate N_2O depression of the RSC, a possible result of decreased entry of extracellular Ca^{2+} into the muscle. Shattock and Bers[16] demonstrated that the positive inotropy observed in rat and rabbit ventricular muscle at lower temperatures, and similar to that seen in this study for guinea pig, is less sensitive to ryanodine (*i.e.*, SR Ca^{2+} depletion). This suggests that the SR does not contribute additional activator Ca^{2+} for the increased inotropy seen at lower temperatures, rather the Ca^{2+} is derived extracellularly. N_2O was as equally depressant at 30 and 37° C, thus one might infer that N_2O also altered the entry of external Ca^{2+}.

The application of β-adrenergic agents, or other drugs that increase intracellular cAMP, results in the development of a distinct late-peak tension RSC (fig. 4A).[5,7,13–15] Drugs that inhibit sarcolemmal Ca^{2+}-channel conductance, such as nifedipine, can reduce or eliminate this enhanced RSC.[7,13,28] However, substantial evidence suggests that entering extracellular Ca^{2+} is first sequestered by the SR and then released for activation of the contractile process.[7,13,29] The RSC late-peak tension is not inhibited by ryanodine; yet, it is depressed by the local anesthetics[7,30] that are known to inhibit certain forms of Ca^{2+}-induced Ca^{2+} release in isolated cardiac SR.[31] As previously demonstrated, isoflurane causes selective depression of the RSC dT/dt_{max},[5] and in our experiments, it introduced a sufficient delay so that a C2 contraction could not be elicited with a 250-ms interval.[7] The contractile delay caused by isoflurane in the face of modest effects on Ca^{2+} entry leads to the conclusion that isoflurane inhibits SR Ca^{2+} release in a manner distinct from

the rapid initial Ca^{2+} release that is sensitive to ryanodine. N_2O caused significantly less depression and no delay of the late peaking RSC dT/dt_{max} as compared to isoflurane (fig. 7A), and the effect of N_2O on the RSC was not significant when considered as a per cent of control-recovery values. The lack of significant depression of the enhanced RSC suggests that any effects of N_2O on the Ca^{2+} entry mechanisms responsible for the RSC must be small. Thus, the myocardial depression induced by N_2O is probably mediated by a decrease in the rapid SR release of Ca^{2+} and possibly by a modest decrease in the contribution of entering extracellular Ca^{2+} to total activator Ca^{2+}.

The observed depression caused by N_2O combined with either volatile agent agrees with the value predicted for each combination, assuming separate noninteracting effects (fig. 7B). This precludes any obvious synergy arising from the simultaneous presence of two depressants. The simple superimposition of effects (as defined by equation 1) occurred whether N_2O was combined with an agent with a similar pattern of effects (halothane) or whether the pattern of depression was distinctly different (isoflurane). These statements can only be made about the relatively modest concentrations studied (<1 MAC) and may not apply to higher concentrations.

In summary, 50% N_2O causes significant depression of myocardial tension development in isolated superfused guinea pig heart that is above and beyond any effect attributable to hypoxia. The pattern and magnitude of contractile depression are similar to that of 0.5% halothane and distinct from that seen with 1% isoflurane. One possible site of N_2O action may be the SR. Finally, the combination of N_2O with either anesthetic (<1 MAC) merely produces the additive superimposition of the individual depressant actions of each anesthetic.

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