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Nitrous oxide can enhance the hypnotic effect, but not the suppression of spinal motor neuron excitability by propofol in humans

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Abstract
Purpose. We investigated whether nitrous oxide can enhance the suppressive effect of propofol on spinal motor neuron excitability in humans.
Methods. Sixteen adult patients were prospectively randomly assigned to be given either propofol alone (group P; $n = 8$) or a supplement of 66% nitrous oxide with propofol (group PN; $n = 8$) for intraoperative sedation. Propofol was administered by a target-controlled infusion system to maintain sequentially increasing plasma propofol concentrations (Cpt) of 0.5, 0.8, 1.0, 1.3, 1.5 and 1.8 μg·ml^{-1} in all patients. Assessment of the patient's level of sedation in both groups was performed with the Wilson Sedation Scale (WSS). F-wave analysis on the left abductor pollicis brevis muscle was carried out for the assessment of spinal motor neuron excitability at each plasma propofol concentration.
Results. Significant differences in the WSS scores between group P and group PN were observed at 0.8, 1.0, 1.3, and 1.5 μg·ml^{-1} of Cpt (group P < group PN; $P < 0.01$). Cpt greater than 1.0 μg·ml^{-1} significantly reduced F-wave persistence in a concentration-dependent manner, and the ICpt 50 and ICpt 95 values for plasma propofol concentration (plasma propofol concentrations that produced 50% and 95% inhibition of the baseline, respectively) were 1.05 and 1.95 μg·ml^{-1} in group P, and 1.07 and 2.14 μg·ml^{-1} in group PN, respectively.
Conclusion. These results suggest that nitrous oxide can enhance the hypnotic effect, but not the suppression of spinal motoneuron excitability by propofol in humans at clinical levels of Cpt.

Key words Propofol · Nitrous oxide · F-wave · Spinal cord · Motor neuron excitability

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Introduction

Recent studies suggest that the spinal cord is as important as the brain as the site of anesthetic action [1–3]. Especially, the suppression of spinal motor neurons by volatile anesthetics appears to be associated with surgical immobility in humans [4].

The F-wave is evoked by a supramaximal electrical stimulus to peripheral nerves, generated by the antidromic activation of spinal motor neurons and recorded as late muscle potentials by electrodes placed on the muscle [5]. It is useful in evaluating spinal motor neuron excitability. Employment of the F-wave, therefore, is known to be a noninvasive electrophysiologic technique to measure the effect of anesthetics on the spinal cord. A human study [6] of F-wave analysis showed that propofol 2 mg·kg^{-1} administered intravenously (IV), but not ketamine 1 mg·kg^{-1} IV or fentanyl 5 μg·kg^{-1} IV, decreased F-wave persistence, suggesting that propofol can reduce spinal motor neuron excitability in humans. Our recent study [7] has shown that propofol predictably suppresses spinal motor neuron excitability in a concentration–dependent manner in humans.

It has also been demonstrated that isoflurane decreases spinal motor neuron excitability in humans [8,9]. It is controversial whether nitrous oxide can suppress spinal motor neuron excitability [9,10]. Friedman et al. [10] showed that nitrous oxide with or without isoflurane produced a dose-dependent suppression of the F-wave in rats. In humans, combining nitrous oxide and isoflurane depresses spinal motor neuron excitability, but the degree of the depression is not different from that produced by isoflurane alone [9]. According to the results of these studies [9,10], there appear to be no significant differences in either F-wave persistence or amplitude between with and without nitrous oxide (30% or 50%) under isoflurane (0.59–0.92) anesthesia, suggesting that the addition of nitrous oxide administra-

tion to isoflurane might not provide further suppression of spinal motor neuron excitability.

Propofol is known to have a less suppressive effect than the volatile anesthetics on transcranial motor evoked potentials, which might make it more suitable as a background agent when examining nitrous oxide's effects on the motor system. Although, of interest to us, it was reported that even nitrous oxide could reduce, to some degree, the amplitude of transcranial motor evoked potentials [11], it is still unclear whether this reduction by nitrous oxide is induced by its spinal or its supraspinal action. In the current study, we investigated whether nitrous oxide could enhance the suppressive effect of propofol on motor neuron excitability in humans, using F-wave analysis and a target-controlled infusion system for propofol.

Methods

The study was approved by the local ethics committee, and written informed consent was obtained from all the study participants. The subjects were 16 adult patients, aged 30–45 years (range, 38.1 ± 5.5 years; mean ± SD) who were classified as American Society of Anesthesiologists (ASA) physical status I or II and were to undergo elective surgery under epidural anesthesia, with the intravenous administration of propofol for intraoperative sedation. Patients with a history of neuromuscular disease were excluded. All patients were prospectively randomly assigned (by using sealed envelopes) to be given either propofol alone (group P) or propofol with a supplement of nitrous oxide (group PN) for intraoperative sedation. Premedication consisted of atropine 0.5 mg given intravenously (IV) 30 min before the epidural catheter insertion. The usual monitors, with continuous electrocardiography, heart rate determination, noninvasive blood pressure measurement, and pulse oximetry were used in the operating room. After baseline values were recorded, epidural catheters were inserted 3 cm into the epidural space at the L3–4 interspace. After 3 ml of a 1.5% lidocaine test dose containing 15 μg of epinephrine was injected, an additional 5–7 ml of 1.5% plain lidocaine with 1:200000 epinephrine was injected. The extent of dermatomal analgesia was assessed by pinprick and cold insensitivity every 5 min until sensory blockade was confirmed. Epidural infusion of 1.5% plain lidocaine at 4 ml·h^{-1} was begun and continued until the end of surgery.

Spinal motor neuron excitability was determined by measuring the left median nerve F-wave. A baseline F-wave was evoked with supramaximal electrical stimuli (10–16 mA) and recorded using Neuropack Σ (Nihon Koden, Tokyo, Japan). Two surface recording electrodes were placed 4–5 cm apart over the abductor pollicis brevis muscle. The stimulation intensity began at 0.2 mA and was gradually increased, in 0.2-mA increments, with 15 s between each stimulus, until the maximal amplitude on compound muscle action potentials (M-wave) was reached. The least stimulus intensity that produced a maximal M-wave was defined as the electrical supramaximal intensity. Supramaximal stimuli of 0.1-ms duration were applied percutaneously to the median nerve at the wrist joint, with a stimulation rate of 1.0 Hz. To distinguish F-waves from the background noise, we accepted only the appropriately timed (25–35 ms after electrical stimuli) deflections from baseline with an amplitude of at least 50 μV. The filter setting was 100–1500 Hz to remove background noise. To determine F-wave persistence (the number of measurable F-waves divided by the number of electrical stimuli), a series of 16 stimuli was delivered at an interstimulation interval of 1 s. M-wave amplitude and maximal peak-to-peak F-wave amplitude were recorded to determine the F/M ratio (maximal amplitude of F-wave divided by M-wave amplitude).

All patients received a computer-controlled infusion of propofol, administered by using an Apple Machintosh/Power Macintosh computer (Apple, Cupertino, CA, USA) that was loaded with three-compartment propofol pharmacokinetic data [12]. A target-controlled infusion (Graseby 3500 infusion pump; Graseby, Chicago, IL, USA) was used to rapidly attain and maintain a sequentially increasing plasma propofol concentration (Cpt) from 0.5 to 1.8 μg·ml^{-1}. Blood pressure was supported to maintain mean arterial pressure values of no less than 75% of preanesthetic values, using IV lactate Ringer's solution. End-tidal (ET) CO_2 through the face mask was continuously monitored and, if necessary, mask ventilation was given to maintain ET CO_2 at 38 ± 5 mmHg.

Nurses, who were unaware of the patient group assignment, assessed the patient's level of sedation in both groups, using the Wilson Sedation Scale (WSS) [13] (see Appendix).

After the preanesthetic F-wave was recorded as the control, the Cpt was then increased stepwise to 0.5, 0.8, 1.0, 1.3, 1.5, and 1.8 μg·ml^{-1} in all patients after an F-wave was evoked at each concentration. Each Cpt was maintained until the effect-site concentration reached the same level, and then the F-wave was recorded. In group PN, nitrous oxide 66% in 34% oxygen was given via a face mask after the preanesthetic F-wave was recorded. The F-wave was recorded after reaching a steady ET concentration of nitrous oxide. In group P, 0.7 l·min^{-1} of oxygen and 3.3 l·min^{-1} of air (fraction of inspired oxygen [$F_{I_{O_2}}$], 0.34) were given for at least 10 min before recording the F-wave.

For the demographic data, the unpaired Student's *t*-test was used to compare intergroup data for age and

body weight, and Fisher's exact test was used to ensure sex distribution, ASA physical status, and distribution of the peak block height. Spearman rank correlation was used to analyze the relationship between the sedation score and Cpt. The Mann-Whitney *U*-test was performed for statistical analysis of the sedation score for intergroup comparisons at each Cpt level. The average F-wave persistence at each level of Cpt was calculated. Dunnett's test was used for analysis of variance (ANOVA) before and after propofol administration. The Mann-Whitney *U*-test following two-way repeated-measures ANOVA was performed to analyze F-wave persistence for intergroup comparisons at each Cpt. Probability values of less than 0.05 were considered significant. Data from the plasma concentration-response curve were analyzed using a computer program, and ICpt 50 and ICpt 95 were calculated (representing the plasma propofol concentrations that produced 50% and 95% inhibition of the baseline, respectively).

Results

There were no significant differences in age, sex, body weight, ASA physical status, or the peak block height in the two groups (Table 1). The WSS scores were increased significantly corresponding to the increase in Cpt, in both groups (Fig. 1). Significant differences in the WSS scores between group P and group PN were observed at 0.8, 1.0, 1.3, and 1.5 μg·ml^{-1} of Cpt (group P < group PN; $P < 0.01$). One patient at a Cpt of 1.8 μg·ml^{-1} in group P and three at a Cpt of 1.5 μg·ml^{-1} in group PN, required a jaw-thrust maneuver to maintain an adequate airway. No blood pressure changes of more than 25% of baseline were observed throughout the study period. Mask ventilation was not required in either group.

F-waves were recorded in a reproducible manner in all the patients. The M-wave amplitude did not change in any patient during this study (Table 2). While propofol, at a Cpt of more than 1.3 μg·ml^{-1} in both groups, reduced the F/M ratio significantly compared with the baseline, there was no significant difference between the two groups in the F/M ratio (Table 2). In both groups, propofol produced a Cpt-dependent reduction of F-wave persistence. In group P, the F-wave persistence decreased significantly at a Cpt of more than 1.0 μg·ml^{-1} (Fig. 2). When the inhalation of nitrous oxide at 66% was added (group PN), the F-wave persistence also decreased, in a similar way to that in group P

Table 1. Demographic data

Group	P	PN
n	8	8
Age (years)	39.0 (4.6)	37.1 (6.4)
Sex (M/F)	3/5	4/4
Body weight (kg)	59.8 (15.7)	62.2 (18.0)
ASA physical status (I/II)	1/7	0/8
Peak block height		
Th10	5	6
Th11	1	2
Th12	1	0
L1	1	0

Figures in parentheses are SDs

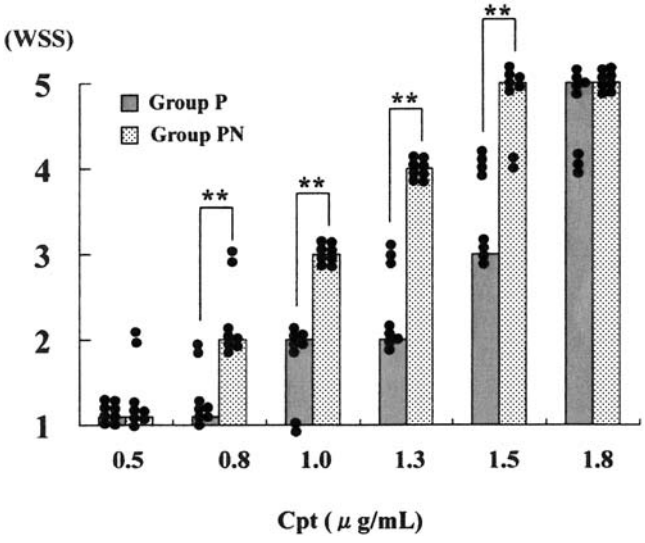

Fig. 1. Wilson Sedation Scale (*WSS*) scores during propofol infusion by target-controlled system. Data values are expressed as medians. WSS scores were increased significantly, corresponding to the increase in plasma propofol concentrations (Cpt), in both groups (Spearman rank correlation was used). **($P < 0.01$), Difference between group P (propofol alone) and group PN (propofol plus nitrous oxide) at a different Cpt

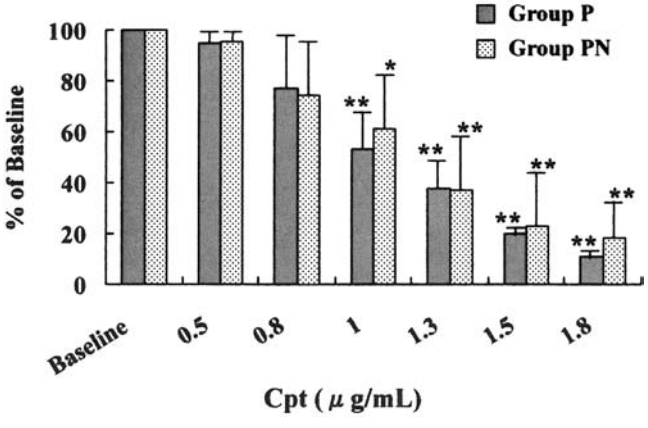

Fig. 2. F-wave persistence (percent of baseline) at each Cpt. There was a significant decrease in F-wave persistence at a Cpt of more than 1.0 μg·ml^{-1}. Data values are expressed as means ± SD. * $P < 0.05$ compared with baseline value; ** $P < 0.01$ compared with baseline value

Table 2. Data of M-wave amplitude, maximum F-wave amplitude, and F/M ratio

	Amp. of M-wave (mV)	Max. amp. of F-wave (mV)	F/M ratio (%)
Propofol without N_2O			
Baseline	3.35 (1.1)	0.25 (0.05)	8.0 (2.5)
0.5[a]	3.38 (1.1)	0.28 (0.07)	8.6 (1.7)
0.8	3.45 (1.1)	0.34 (0.09)	10.7 (3.0)
1.0	3.48 (1.2)	0.26 (0.10)	8.4 (4.5)
1.3	3.36 (1.0)	0.17 (0.08)	5.5 (3.1)*
1.5	3.38 (1.1)	0.12 (0.10)	3.9 (3.4)*
1.8	3.40 (1.1)	0.13 (0.06)	4.0 (2.5)*
Propofol with N_2O			
Baseline	3.19 (1.0)	0.27 (0.06)	8.9 (3.2)
0.5[a]	3.16 (1.0)	0.28 (0.07)	9.7 (5.1)
0.8	3.10 (1.0)	0.30 (0.11)	10.4 (6.1)
1.0	3.16 (1.0)	0.27 (0.09)	9.1 (4.4)
1.3	3.18 (0.9)	0.16 (0.14)	5.8 (6.2)*
1.5	3.18 (0.9)	0.12 (0.11)	3.4 (3.2)*
1.8	3.19 (0.9)	0.09 (0.08)	3.3 (3.4)*

* $P < 0.05$ compared with baseline value
Values are means (SD)
[a] Cpt values, in micrograms per milliliter

(Fig. 2). These changes were significant ($P < 0.05$ and $P < 0.01$) compared with baseline levels, but were not significant compared with those with propofol alone. In group P, the ICpt 50 and ICpt 95 values were 1.05 μg·ml^{-1} (95% confidence interval, 0.92–1.19) and 1.95 μg·ml^{-1} (95% confidence interval, 1.53–2.46), respectively (Fig. 3). In group PN, the ICpt 50 and ICpt 95 values were 1.07 μg·ml^{-1} (95% confidence interval, 0.97–1.19) and 2.14 μg·ml^{-1} (95% confidence interval, 1.74–2.46), respectively (Fig. 3).

Discussion

This study demonstrates that nitrous oxide added to propofol produces a deeper level of sedation than does propofol alone. It also demonstrated that propofol suppressed the F-wave persistence and F/M ratio, and that nitrous oxide did not affect the relationship between Cpt and F-wave persistence. In this study, propofol did not decrease the M-wave amplitude evoked by supramaximal electrical stimulation; therefore, the F-wave suppression was not the result of the effect of propofol on the neuromuscular junction or muscle.

Because propofol does not have a strong analgesic effect, it is usually administered in combination with opioids, and/or nitrous oxide. In addition, nitrous oxide 67% reduces the induction dose of propofol by 44% [13] and decreases the 50% effective concentration (EC_{50}) of propofol during maintenance by approxi-

Fig. 3. Cpt—response curves of the suppressive effect of propofol on F-wave persistence in both groups. Data values are expressed as means ± SD. The Cpt values that produced 50% ($ICpt_{50}$) and 95% ($ICpt_{95}$) inhibition of the baseline were 1.05 μg·ml^{-1} (95% confidence interval [*CI*], 0.92–1.19) and 1.95 μg·ml^{-1} (95% CI, 1.53–2.46), respectively, in group P, and these values were 1.07 μg·ml^{-1} (95% CI, 0.97–1.19) and 2.14 μg·ml^{-1} (95% CI, 1.74–2.46), respectively, in group PN

mately 30% [14]. In the present study of sedation levels obtained with propofol infusion, the results showing that nitrous oxide (group PN) produced a deeper level of sedation than did propofol alone (group P) are consistent with other reports [13,14].

The F-wave amplitude indicates the number of spinal motor neurons in which recurrent discharges occur in response to the antidromic potential [15]. F-wave persistence (i.e., the number of measurable F-wave responses divided by the number of stimuli) indicates the antidromic excitability of a particular motor neuron pool [5]. It has been reported in many articles that the F/M amplitude ratio was also one of the indicators for the excitability of motor neurons in spastic patients [16–18]. However, in normal subjects, it is known, that the F/M ratio indicates the number of spinal motor neurons activated by antidromic stimulation, but not the excitability of individual motor neurons. Therefore, it can be considered that the F/M ratio and F-wave persistence are indicators of the excitability of the whole motor neuronal pool in the spinal cord and the excitability of individual motor neurons, respectively.

The mechanisms by which propofol produces depression of spinal motor neuron excitability are hypothesized to be hyperpolarization [20], the activation of γ-aminobutyric acid (GABA)a receptors [21], and the suppression of L-type calcium channels in spinal motor neurons [22]. In the present study, supplemental nitrous oxide and propofol produced a deeper level of sedation than propofol alone. Nitrous oxide, however, cannot enhance the suppressive effect of propofol on spinal motor neuron excitability. Indeed, the effect of nitrous oxide on the spinal cord is still controversial. Friedman et al. [10] showed that nitrous oxide with or without isoflurane produced a dose-dependent suppression of the F-wave in rats. In a human study, although the combination of nitrous oxide and isoflurane depressed motor neuron excitability, the degree of the depression produced by this combination was not different from that produced by isoflurane alone [9]. In our present study, the suppression of motor neuron excitability by propofol was not enhanced when nitrous oxide was added to propofol anesthesia, although nitrous oxide enhanced the sedative effect of propofol. These results indicate that sensitivity to nitrous oxide may be different between the supraspinal and spinal levels. It has been thought that the F-wave is one of the indicators of the excitability of spinal motor neurons, which is independent of descending modulation from the supraspinal level. However, spinal motor neuronal excitability is dependent on the balance between excitatory and inhibitory pathways, which is mediated by a supraspinal system [23]. For example, the existence of noradrenergic neurons projecting to the spinal motor neurons has been shown in the locus ceruleus, and suppression of these neurons in the locus ceruleus depressed the excitability of spinal motor neurons in humans [24]. In addition, Sawamura et al. [25] demonstrated that nitrous oxide could activate a descending noradrenergic pathway, which stimulates adrenoceptors in the spinal cord through the released norepinephrine. Our results, together with these results, suggest that it is likely that nitrous oxide could induce a hypnotic action at the supraspinal level and, in contrast, could indirectly enhance, to some degree, spinal motor neuronal excitability through the suprapinal level. Detailed electrophysiological studies, including in vitro experiments using isolated spinal cord, should be performed to elucidate the mechanism of this difference.

In conclusion, we have demonstrated that propofol, at sedative doses, can decrease F-wave persistence and the F/M ratio in a Cpt-dependent manner, but the addition of nitrous oxide does not enhance this effect. Our data indicate that nitrous oxide can enhance the hypnotic effect, but not the suppression of the spinal motoneuron excitability by propofol in humans at clinical Cpt levels.

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Appendix

Wilson sedation scale

Score	Description
1	Fully awake and oriented
2	Drowsy
3	Eyes closed, but rousable to command
4	Eyes closed, but rousable to mild physical simulation (earlobe tug)
5	Eyes closed, but unrousable to mild physical stimulation