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NITROUS OXIDE AND ISOFLURANE ARE SYNERGISTIC WITH RESPECT TO AMPLITUDE AND LATENCY EFFECTS ON SENSORY EVOKED POTENTIALS

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Sloan T, Sloan H, Rogers J. Nitrous oxide and isoflurane are synergistic with respect to amplitude and latency effects on sensory evoked potentials.

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ABSTRACT. Objective. Combinations of anesthetic agents are frequently employed to produce the desired clinical effect. No systematic study has been conducted on the effect of the combination of nitrous oxide with a potent inhalational agent such as isoflurane on sensory evoked responses. **Methods.** Median nerve somatosensory evoked responses from the cervical and cortical regions (SSEP), auditory brainstem responses (ABR) and flash visual evoked responses (VEP) were tested in baboons. The latency and amplitude of the major response peaks were recorded at five proportionate mixtures of isoflurane (I) and nitrous oxide (N_2O) (0.8% I only, 0.6% I/20% N_2O, 0.4% I/40% N_2O, 0.2% I/60% N_2O, and 79% N_2O only). A similar set of experiments were also conducted with 0.8% isoflurane and 0.6% halothane. All data were normalized to 0.8% isoflurane only and Dunnett's method of analysis used to determine which mixtures deviated from the reference values with 0.8% isoflurane. **Results.** Several combinations of isoflurane with nitrous oxide produced increases in latency (ABR: wave V, VEP, SSEP cervical and cortical) and decreases in amplitude (ABR: amplitude ratio V/I, VEP, cortical SSEP) from that expected if the effects were additive. No deviations were observed with combinations of isoflurane and halothane. **Conclusions.** These studies are consistent with drug synergy when isoflurane is mixed with nitrous oxide. This suggests that if these agents are considered for anesthesia when sensory evoked responses are to be monitored that the combination of these agents may produce more amplitude and latency changes than expected from a proportionate mixture of the individual agents.

KEY WORDS. halothane, isoflurane, nitrous oxide, evoked potential, baboon.

INTRODUCTION

In many respects, anesthesiology is the art of mixing different medications to take advantage of their combination to accomplish the needed pharmacologic and physiologic state for surgery [1]. The choice of anesthesia medications is extremely important when intraoperative monitoring of neurophysiological function is used [2–4]. The use of inhalational agents in this circumstance is of substantial importance due to the significant depressant effects they have on sensory and motor evoked potentials (MEP). Accordingly the choice and dosage of these agents is paramount to the success of monitoring as well as the clinical state of anesthesia.

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The current potent inhalational agents (isoflurane, desflurane, sevoflurane) and nitrous oxide have been used for anesthesia, with a combination of a potent agent commonly mixed with nitrous oxide. Observations of the effects of the latter combination on clinical endpoints has shown variable results compared to the pure agents depending on the specific neurological endpoint and the species studied. Since systematic studies with sensory evoked responses have not been conducted, we evaluated the effects of isoflurane with nitrous oxide on visual, auditory and somatosensory evoked responses in a primate model to determine the implications for intraoperative neurophysiological monitoring.

METHODS AND MATERIALS

In a study approved by the institutional animal care and use committee, we examined the effect of combinations of isoflurane with nitrous oxide and combinations of isoflurane with halothane in five male and female baboons (17–24 kg) (Papio hamadryas anubis). After an overnight fast the animals were given ketamine (15 mg/kg) with atropine (0.02 mg/kg) intramuscularly. An intravenous line was then placed in a leg vein and balanced salt solution was infused continuously. Lidocaine (approximately 1 mg/kg of a 4% solution) was sprayed on the vocal cords during direct laryngoscopy, and the trachea was intubated approximately 2 min later using a 5.0 mm (inside diameter) cuffed endotracheal tube. The lungs were ventilated mechanically (Harvard Respirator 665, South Natick, MA) with 40% oxygen and a tidal volume of 12 ml/kg at a rate sufficient to produce an end-tidal carbon dioxide tension of 38–42 mm Hg.

The animal was placed in the right lateral decubitus position on a padded table with the head elevated on a soft pad and the left arm resting in a padded support. Blankets and hot water warming pads (Aquamatic K module, Ruleville, OH) were used to maintain an esophageal temperature of 36–37° C (Monotherm 6500, Precision Biomedical, Piano, TX). The electrocardiogram (Grass Instrument Co., Model 7D Polygraph, Quincy, MA), blood pressure (Omega 1400 NIBP, Tulsa, OK), hemoglobin oxygen saturation (Ohmeda Biox 3700, Boulder, CO), and end-tidal carbon dioxide (Instrumentation Laboratories IL 200, Lexington, MA) were monitored continuously during the study. End-tidal concentrations of anesthetic agents (halothane, isoflurane, nitrous oxide) were measured using an Ohmeda 5250 RGM gas analyzer (Soma Technology, Inc. Bloomfield, CT).

In the first set of experiments to explore the effect of combining nitrous oxide with isoflurane, isoflurane was mixed with nitrous oxide in varying proportions. In these studies the anesthesia started with 0.8% isoflurane (0.6 MAC [5, 6]) in an air/oxygen mixture from an anesthesia machine using a non-rebreathing circuit and an inspired oxygen concentration of 21%. Approximately 1 h later triplicate sensory evoked responses (as below) were recorded in random order. Then the flow from a second anesthesia machine delivering 80% nitrous oxide (MAC is approximately 200% [7]) was mixed with the output of the anesthesia machine delivering the isoflurane. The proportions of flow from these two machines allowed proportionate anesthetic mixtures of 0.8–0% isoflurane with 0–80% nitrous oxide. When pure nitrous oxide was delivered the concentration was adjusted to 79%. Five anesthetic mixtures were tested: 0.8% ISO (no N_2O), 0.6% ISO/20% N_2O, 0.4% ISO/40% N_2O, 0.2% ISO/60% N_2O, and 79% N_2O (no ISO). All five animals were tested at these mixtures after equilibration at each mixture for 1 h. Each animal was also tested using the reverse paradigm (i.e., starting with 79% nitrous oxide) after 1 month's rest. After each testing the animals were returned to their housing for recovery.

In a second set of studies similar to the first study, more than 1 month later, the mixture of isoflurane and halothane was studied. Initially, isoflurane at 0.8% was delivered by an anesthesia machine to produce general anesthesia and testing conducted 1 h later. The anesthetic concentration was then changed by adding flow from a second anesthesia machine delivering 0.6% halothane (0.6 MAC [5, 7]) to produce proportionate mixtures of the two anesthetics. Five anesthetic mixtures were tested: 0.8% ISO (no HAL), 0.6% ISO/0.15% HAL, 0.4% ISO/0.3% HAL, 0.2% ISO/0.45% HAL, and 0.6% HAL (no ISO). After equilibration at each new anesthetic mixture for 1 h, triplicate recordings of the sensory responses were made. Each of the five animals were tested in this paradigm were also tested more than 1 month later using a paradigm that started at 0.6% halothane (the reverse order of above).

Sensory evoked potentials were recorded in triplicate using a Biologic Navigator (Mundelein, IL). Median nerve somatosensory evoked potentials (SSEP) were produced using subdermal needle electrodes placed at the wrist using 300 μs square wave pulses at 5.7 Hertz (Hz) and a constant current twice that necessary to produce a motor response. Cortical responses were recorded from a subdermal needle placed at C3′ (0.5 cm behind C3 in the international 10–20 system) and cervical responses recorded on the skin posterior to the second cervical vertebrae. These responses were referenced to a subdermal needle over Fz and a ground placed at the shoulder. 500 averages were recorded using a recording window of 60 ms, filtration of 10–500 Hz (no notch filter), and

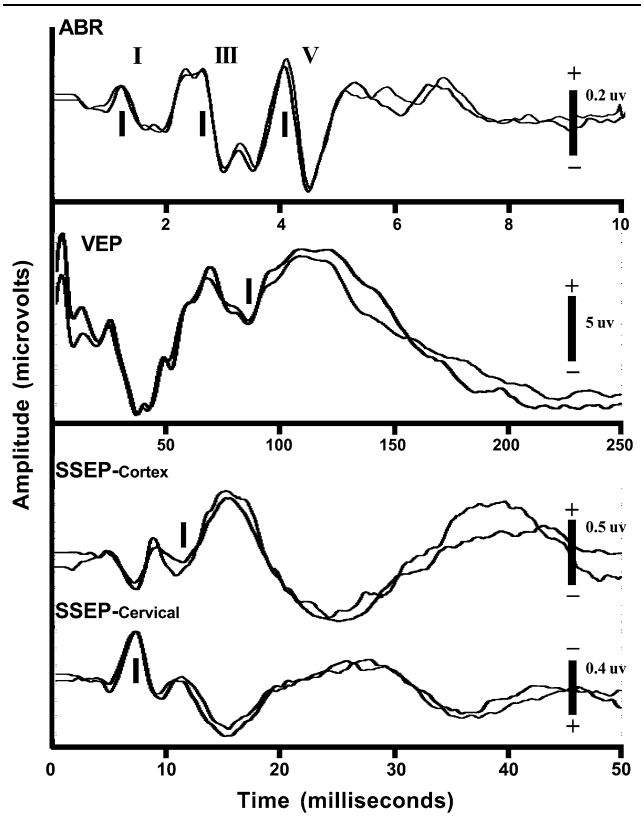

Fig. 1. Examples of the sensory evoked responses recorded in this study from the baboon when recorded at 0.8% isoflurane. The time base is shown along the bottom for each waveform and the vertical calibration in microvolts (μv). The top panel shows the auditory brainstem response plotted and the three major peaks (I, III, V) used in the study. Shown in the middle panel is the visual evoked response recorded from LED flash stimulation and the primary cortical negative peak (indicated) used in the study. The bottom panel shows the median nerve somatosensory response. Indicated is primary negative peak at cortex and the major negative response from recordings over the cervical spine which were used in the study. All responses are plotted positive up at the recording electrode with the exception of the SSEP response recorded over the cervical spine which is plotted negative up.

amplification of 10,000. The latency and amplitude of the primary negative cortical response and the latency and amplitude of the major negative peak of the cervical response were recorded (shown in Figure 1).

Auditory brainstem responses (ABR) were recorded in triplicate after application of 100 μs alternating polarity clicks at 95 dB SPL through a unilateral ear insert (ER-3A, Biologic Inc.) at a rate of 17.1 Hz. The response was recorded from subdermal needle electrodes at the ipsilateral ear, referenced to Cz and a shoulder ground. 2,000 averages were recorded using a 10 ms window, filtration of 100–3,000 (no notch filter), and amplification of 75,000. The latency of the three major peaks (I, III, V) and the amplitude ratio of peak V divided by peak I was recorded (see Figure 1).

Visual evoked potentials (VEP) were recorded in triplicate after application of light flashes from goggles using light emitting diodes (Biologic Inc.) at full output at 1.9 Hz over closed eyes. The response was recorded from subdermal needles placed at Oz, referenced to Fz and shoulder ground. 100 averages were recorded using a recording window of 256 ms, filtration of 3–100 Hz (no notch filter), and an amplification of 10,000. The latency and amplitude of the major negative peak was recorded (see Figure 1).

Data were analyzed by later evaluation of the recorded responses by an observer blinded to the details of the signal acquisition. All data were normalized by comparing each response to the average value recorded at 0.8% isoflurane for that dataset. All data were then pooled and the average value was assessed at each anesthetic mixture. Dunnett's method of analysis was used with each dataset to determine if any anesthetic mixture deviated sufficiently ($P < 0.05$) from the reference dataset with isoflurane alone. This was used to infer that the value of latency or amplitude at any mixture deviated from the value expected in a linear extrapolation between the values recorded at the pure anesthetics.

RESULTS

All animals tolerated the recording sessions without complications. Examples of the recorded sensory evoked potentials at 0.8% isoflurane and the peaks used for analysis are shown in Figure 1. These responses were recordable in all animals at all concentrations and combinations of anesthetic agents.

Shown in Tables 1, 2, and 3 are the average values (±sem) for the latencies and amplitudes recorded in this study for the ABR, VEP, and SSEP (respectively). As indicated by asterisks, some values in the amplitude and latency of the ABR wave III, VEP, cortical SSEP, and latency of the cervical SSEP statistically deviated from the reference values with isoflurane alone. No amplitude or latency values of any responses reached statistical significance when halothane was mixed with isoflurane.

Since the most clinically relevant results are those of the cortical SSEP, Figure 2 shows plots of the relative latency and amplitude data for the SSEP response measured over the cerebral cortex. As can be seen, the mixtures of isoflurane and nitrous oxide increased latency and decreased amplitude such that the effect of mixtures was greater than the pure agents alone.

Shown in Figure 3 are recordings of the cortical somatosensory evoked response from one animal at the several concentrations of isoflurane and nitrous oxide.

Table 1. Auditory brainstem response data

Isoflurane	Halothane	Latency I	Latency III	Latency V	Amplitude V/I
0.80	0.00	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)
0.60	0.15	0.960 (0.015)	0.960 (0.121)	0.950 (0.031)	2.255 (0.854)
0.40	0.30	1.000 (0.036)	0.960 (0.075)	0.950 (0.029)	1.333 (0.715)
0.20	0.45	1.150 (0.193)	1.030 (0.200)	1.000 (0.070)	2.834 (1.358)
0.00	0.60	0.900 (0.033)	0.970 (0.133)	0.950 (0.049)	1.842 (1.447)
Isoflurane	Nitrous oxide	Latency I	Latency III	Latency V	Amplitude V/I
0.80	0	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)
0.60	20	1.107 (0.059)	1.037 (0.013)	1.024 (0.005)	0.731 (0.148)⋆
0.40	40	1.074 (0.022)	1.059 (0.013)	1.040 (0.006)⋆	0.825 (0.282)⋆
0.20	60	1.052 (0.019)	1.051 (0.008)	1.039 (0.008)⋆	0.532 (0.068)⋆
0.00	79	1.141 (0.040)	1.064 (0.026)	1.024 (0.005)	4.126 (1.554)

Data shown as mean (sem), ⋆ $P < 0.05$ deviation from 0.8% isoflurane.

Table 2. Visual evoked potential data

Isoflurane	Halothane	Latency	Amplitude
0.80	0.00	1.000 (0.000)	1.000 (0.000)
0.60	0.15	1.000 (0.020)	1.030 (0.238)
0.40	0.30	1.070 (0.039)	1.000 (0.212)
0.20	0.45	1.040 (0.038)	1.480 (0.277)
0.00	0.60	1.020 (0.055	1.990 (0.522)
Isoflurane	Nitrous oxide	Latency	Amplitude
0.80	0	1.000 (0.000)	1.000 (0.000)
0.60	20	1.078 (0.020)⋆	0.757 (0.093)
0.40	40	1.195 (0.041)⋆	0.400 (0.063)⋆
0.20	60	1.177 (0.031)⋆	0.372 (0.297)⋆
0.00	79	1.047 (0.025)	0.757 (0.170)

Data shown as mean (sem), ⋆ $P < 0.05$ deviation from 0.8% isoflurane.

DISCUSSION

These studies suggest that although combinations of potent anesthetic agents (e.g., isoflurane with halothane) are additive with respect to the effects on the amplitude and latency of sensory evoked responses, combinations of isoflurane with nitrous oxide is synergistic or supra-additive (i.e., the effect is more than expected from the sum of the individual effects). This is contrary to the popularly held belief that inhalational anesthetics are additive in their anesthetic effects. In other words, many anesthesiologists would suggest that one-half MAC (the minimum alveolar concentration where 50% of subjects move in response to a painful skin incision) of a potent agent (e.g., isoflurane) with one-half MAC of nitrous oxide would equal one MAC effect. This belief is also contrary to numerous studies described below which suggest that the combination of isoflurane and nitrous oxide may actually be antagonistic (i.e., that the combination has less effect than predicted by the sum of the individual effects).

Assessing the effect of the combinations of two drugs is usually done with an isobologram where a defined clinical endpoint (such as 50% immobility with a painful stimulus) is measured with amounts of the pure drugs titrated to the same endpoint and then with intermediate mixtures of equally effective dose pairs [8, 9]. If the mixtures are such that the effect equals the sum of the components it is said to be "additive." If the effect is less than predicted by the sum of the components it is said to be "infra-additive" (e.g., antagonistic) and if the effect exceeds the sum of the individual components the effect is said to be "synergistic." Generally a 10% difference from additivity is used as a threshold for the definition of synergism or infra-additivity. It is important to note that in studies of immobility, the MAC of the agents needs to be known; some of the differences in animal studies have been explained by inaccurate knowledge of the MAC of nitrous oxide since its measurement is made difficult because it exceeds 100% [10, 11]. Since the MAC of these drugs is not known accurately for these particular animals, a more practical approach was taken in this study to mimic the addition of nitrous oxide and isoflurane by adding concentrations such as might be done clinically.

Table 3. Somatosensory evoked potential data

Isoflurane	Halothane	Latency-cervical	Ampltude-cervical	Latency-cortex	Amplitude-cortex
0.80	0.00	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)
0.60	0.15	1.000 (0.018)	1.330 (0.332)	1.010 (0.022)	1.140 (0.165)
0.40	0.30	1.030 (0.024)	1.420 (0.467)	0.990 (0.049)	1.610 (0.620)
0.20	0.45	1.000 (0.071)	1.190 (0.204)	0.990 (0.043)	1.950 (0.440)
0.00	0.60	1.050 (0.000)	2.850 (0.920)	0.970 (0.000)	2.390 (0.621)

Isoflurane	Nitrous oxide	Latency-cervical	Amplitude-cervical	Latency-cortex	Amplitude-cortex
0.80	0	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)
0.60	20	1.012 (0.005)	0.831 (0.051)	1.124 (0.015)★	0.735 (0.077)
0.40	40	1.018 (0.009)★	1.217 (0.220)	1.141 (0.028)★	0.710 (0.165)★
0.20	60	1.013 (0.007)★	1.176 (0.265)	1.136 (0.031)★	1.596 (0.444)
0.00	79	0.994 (0.007)	1.183 (0.245)	1.075 (0.015)	2.815 (0.823)

Data shown as mean (sem), ★ $P < 0.05$ deviation from 0.8% isoflurane.

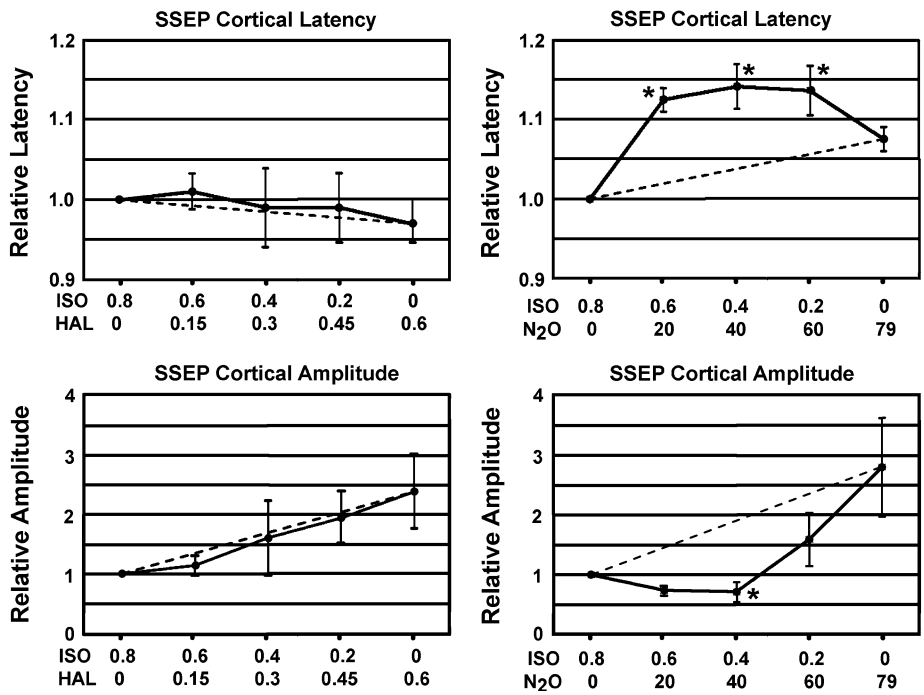

Fig. 2. Plot of the relative latency (top) and amplitude (bottom) for the median nerve SSEP responses recorded over the sensory cortex. The mean (±sem) of the data at the combinations of isoflurane with halothane (left) and with isoflurane and nitrous oxide (right) is shown. The dotted line shows the predicted mean value if the effects were additive. Mean data which deviates significantly from the reference dataset (0.8% isoflurane) using Dunnett's analysis is marked with an asterisk (★).

The interaction of drug combinations which are not additive could be the result of pharmacokinetic or pharmacodynamic effects. We believe that the interaction in our study is likely pharmacodynamic since it is unlikely that the drugs alter each other's absorption, distribution, metabolism or elimination at steady state. Alternatively the effect could be the result of hysteresis or because the effect of these drugs on the sensory evoked responses are

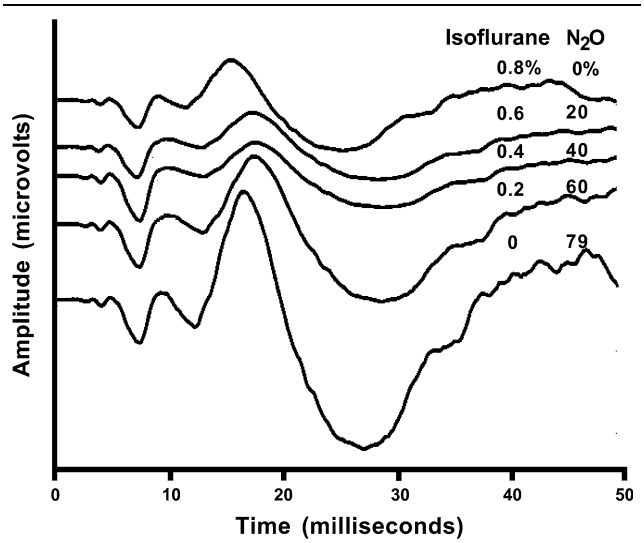

Fig. 3. Plot of the cortical SSEP response in one animal at the five concentrations of isoflurane and nitrous used in the study. As shown, the amplitude and latency of the response is affected more at the intermediate mixtures than in the pure agents.

non-linear such that linear extrapolation of the intermediate doses is not appropriate [9]. We believe that the testing paradigm of increasing and then later decreasing isoflurane concentrations has minimized hysteresis effects. We also believe that the results of testing the combination of isoflurane with halothane suggest that hysteresis and nonlinear effects are not dominant contributors to the effect seen. Hence we believe that the effects seen when combining isoflurane with nitrous oxide represent pharmacodynamic drugs effects within the sensory neural pathways.

When observing the pharmacodynamic effect of drug combinations additivity is often taken as an indication that the drugs are acting at the same drug receptor by the same mechanism. When agents have differing potencies at receptors involved in the pathway or act at different receptors (i.e., by different mechanisms), synergism or infra-additivity would be predicted [12, 13]. This has been observed in combinations of a large number of anesthetic agents. Examples of synergy are abundant with the intravenous agents such as propofol combined with midazolam and propofol combined with sevoflurane [1, 14]. For example, a mixture of barbiturates and benzodiazepines have an effect 1.8 times the predicted effect of the combination [14]. This effect frequently underlies the effective delivery of anesthesia since excellent drug effects can be achieved while minimizing side effects that would occur at higher individual drug dosages [1, 15].

Whereas synergism is often seen with intravenous agents (or combinations of intravenous agents with inhalational agents [14]), additivity or infra-additivity appear to be the rule for combinations of potent inhalational agents [12]. In a systematic review of drug combinations on immobility (the MAC effect), Hendrickx noted halothane and isoflurane were additive and isoflurane and nitrous oxide were either additive or infra-additive (depending on the study) [11]. He noted that most human studies suggested additivity for nitrous oxide with potent agents but infra-additivity is consistently seen in animals. Studies in the rat have demonstrated that isoflurane and halothane are additive with respect to immobility [12, 15]. In similar studies, Eger found that the combination of nitrous oxide and isoflurane which were infra-additive [12]. Other studies in rats on immobility showed that nitrous oxide was infra-additive when mixed with enflurane or cyclopropane [16–18].

The lack of additivity of nitrous oxide and potent inhalational agents suggests that these agents have different molecular mechanisms of action with respect to the pathways mediating immobility in the spinal cord. However, with the measurement of other endpoints, such as the effects on the brainstem or cerebral cortex, it is possible a different kind of relationship might exist since different neural transmitters, receptors, and receptor subunits may be involved in the response. A variety of studies have shown that anesthetic drug effects differ at the cortical level from the spinal cord level [19, 20]. To the extent that different drug receptors are involved in each pathway and clinical endpoint, there may be differences in drug effects and the combinations of agents. Hence, the effect of anesthetic mixtures on cortical endpoints (such as cortical sensory evoked responses) may be different from the effects on the spinal cord (immobility and MAC). This difference has been seen when comparing the effect of agents on immobility with the effects on the raw electroencephalogram (EEG) and processed EEG (BIS and entropy) [21, 22]. When the processed EEG was examined, studies show that the effects of isoflurane with nitrous oxide are also not additive [21]. With respect to hypnosis (response to verbal commands), human studies of nitrous oxide and isoflurane were infra-additive [11]. Similarly, infra-additivity has also been seen for MAC-awake and for learning and memory [23].

Thus, the drug interaction noted with nitrous oxide and isoflurane in this study on sensory evoked responses are likely due to differences in the actions of the drugs at drug receptors or subtypes of drug receptors [24]. Isoflurane is thought to have its major potentiation effects on the GABAa, 2 pore potassium channels, glycine, serotonin and kainate channels and with major inhibition effects at the inwardly rectifying potassium channels and AMPA channels. Nitrous oxide is thought to have its major potentiation at the 2 pore potassium channels and major

inhibition effects at the NMDA, nicotinic acetylcholine and kainate channels. The differences between nitrous oxide and isoflurane would be consistent with a non-additive effect of the combination whereas additivity would be more likely with isoflurane and halothane where the drug actions are thought to be very similar [24].

In order that the study be relevant to the effects of anesthesia used in operative monitoring, the technical aspects of recording were similar to those used during monitoring. It is unknown if the results would be different if a different methodology were employed. The amplitude and latencies chosen are also similar to those used in operative monitoring with the exception of the amplitude ratio in the ABR which may have less monitoring implications than relative amplitude changes from an operative baseline. However, this ratio does give insight into the relative anesthetic effects along the auditory pathway.

Studies in humans on sensory evoked responses have consistently demonstrated decreases in cortical amplitude with inhalational agents or nitrous oxide when they were used alone [2–4]. In this study the design was to compare the effects of the anesthetic agents at the various mixtures studied. As such we used the amplitude and latency values at 0.8% isoflurane as the basis for comparison because it was the only anesthetic state that was common between the two parallel studies (isoflurane when mixed with nitrous oxide and isoflurane mixed with halothane). Because these agents have been demonstrated to produce depression of sensory responses, an alternate approach could have been to evaluate the amplitude and latencies at the various mixtures with the animal in the unanesthetized state except that this was not possible with these animals. The depression produced by these agents compounded by the additional depression produced by the mixture can produce a response amplitude small enough that the variability could make the response unreliable for detecting the changes used to alert possible neurological compromise.

The effects of these agents has been described in numerous reviews and chapters and is thought to be mediated by interaction with synaptic receptors which reduce the effectiveness of synaptic transmission and interrupt neural processing responsible for consciousness, amnesia and response to painful stimuli [2, 25–38]. Early studies focused on the effects on latency which are less than the impact on amplitude which later became the focus of study. Studying a broad range of agents, Angel [39] studied the cortical SSEP from forepaw stimulation in the rat. All of the agents studied produced a dose-dependent decrease in amplitude and increase in latency which paralleled the anesthetic depth. Further studies noted that the effects of anesthetics varied with the specific agent involved depending on the specific synapses and the loci of neural structures that may be excited or depressed. This effect was nicely was demonstrated by Rosner [40] who also demonstrated that differences in neural depression and excitation correlated with differences in EEG patterns with increasing doses of the agents studied. It is of interest that Rosner noted that nitrous oxide depressed cortical evoked responses more than halothane when compared on an anesthetic potency basis (e.g., by minimal alveolar concentration MAC). More recent studies have included isoflurane and suggest a relative potency based on MAC equivalents in the order nitrous oxide (most potent) > isoflurane, sevoflurane, desflurane > enflurane > halothane [41–45].

The most prominent anesthetic effects on evoked responses during clinical anesthesia are those of the potent inhalational agents including isoflurane and halothane which have a broad spectrum of synaptic interactions. The effects on sensory responses are consistent with the location of synapses in the anatomic pathway of the responses. For example, studies of recordings at Erb's point (brachial plexus from upper extremity stimulation) and over the cervical spine (from lower extremity stimulation) show minimal changes (0–9%), that are not dose related [46, 47]. Major changes are seen above the thalamus and from the cerebral cortex. Consistent with ''thalamic gating'' of the anesthetic model, the responses above the thalamus are disproportionately affected, as seen in several studies [48–51]. Studies in children demonstrate that the predominant effect is above the level of the thalamus as predicted (N_{19}-P_{22} and above) [52] and specific studies of the spontaneous and evoked output of the thalamic relay nuclei (VPM, VPL) suggest that these nuclei may be an important location for the anesthetic modulation of afferent stimuli [53]. Since this level of anesthetic is 0.3–0.5 MAC it may explain why many cortical sensory evoked responses (such as the SSEP) are often disproportionately affected above concentrations of 0.5–1 MAC. Interestingly the nonlinear effect is also supported by neuronal network modeling of the SSEP effect based on the known effect of anesthetic agents on neurons [54]. The effect on the visual evoked response is among the most dramatic, perhaps also due to the multiple synapses involved [55]. This is also consistent with the synergistic effects of these agents occurring in the responses recorded in the cortical SSEP and cortical VEP.

Some studies of the subcortical responses show that anesthetic effects appear to plateau at low concentrations consistent with a minimal effect on pathways without synapses. For example, the major latency increase often occurs at 0.5–1% inspired isoflurane with minimal effects at higher concentrations. This is consistent with the major effect of anesthetic agents on amplitude not latency.

However, that latency effects have been seen are consistent with the synergistic effects seen in this study, however, the magnitude of these changes is less than the changes seen in the amplitude. This anesthetic effect has also been observed with the auditory response [48, 55–66]. The changes seen in the brainstem response show a progressive increase in effect as the number of synapses increases along the auditory pathway, with the major changes in wave V. This is also consistent with the synergistic changes seen in wave V but not waves I and III.

Studies have also been conducted with nitrous oxide which is believed to have primary actions of antagonizing the NMDA receptor, inhibiting the neuronal nicotinic acetylcholine receptor, and exhibiting opioid-like effects on the opioid receptors. Because its' MAC value exceeds 100%, it is generally considered a weak anesthetic, however, it is a more potent depressant of the P_{15}-N_{20} SSEP response than isoflurane [44]. When various studies are compared, the relative effects of the agents on latency and amplitude of the cortical SSEP have been observed [38]. For example, at 1.5 MAC halothane increases latency 10–15% and decreases amplitude about 70%. For isoflurane at 1.6 MAC the increase is latency is 15–20% and amplitude decrease 60–70%. For 60–65% nitrous oxide the amplitude decrease is about 50–55% with negligible latency change. When 0.5 MAC isoflurane is combined with 60% nitrous oxide, the latency increase is less than 10% with an amplitude decrease of 50–70%.

Hence, when anesthetic mixtures in the midrange of this study are used, the sensory response amplitude may be reduced by over one half. In this case the normal signal variability may reduce the ability of the monitoring to effectively detect subtle response changes. This may be further complicated by pathology in the nervous system that reduces the amplitude further resulting in an apparent response where the amplitude is extremely reduced, morphology broadened and ill defined. This creates a suboptimal situation for uncompromised interpretation of intraoperative change, particularly when viewed in light of the amplitude variability that accompanies responses collected under these anesthetic conditions.

Very few studies have evaluated the effect of the combination of isoflurane with nitrous oxide on sensory evoked responses compared to the pure agents. Perhaps of more importance in the current monitoring environment is studies of the combination motor evoked responses (MEP) are also very few since the profound depression of these agents on MEP make them extremely undesirable as single agents or in combination. However, one clinical study observed transcranial MEP during isoflurane and during the combination of isoflurane and nitrous oxide [67]. The authors noted that when the combination of isoflurane (0.78% end-tidal) with nitrous oxide (59% end-tidal) was used a substantial number of patients were not monitorable compared to when nitrous oxide (68% end-tidal) was used as a sole agent with propofol (7 mg kg^{-1} h^{-1}). This is consistent with a synergy of drug depressant effect on MEP similar to the effect on sensory evoked responses and also emphasizes the importance of intravenous anesthetics when MEP is to be monitored.

To the extent that the results in these primates are similar to human, the results in this study have substantial implications for anesthetic management in humans when evoked responses are monitored. With respect to sensory evoked responses, the reduction in amplitude of the combination of nitrous oxide with isoflurane on the cortical SSEP is 63% from that predicted by additive effects. It is important to note that the effect seen in the human may be more prominent than in the baboon since the MAC of nitrous oxide is lower in the human than in the baboon (104% [68] vs. 200% [7]) suggesting almost twice the potency on the spinal pathways mediating immobility.

This depressant effect of nitrous oxide is further important if the concentration of nitrous oxide is changed during the monitoring (e.g., such as turning it off during a period where the oxygen concentration needs to be increased) and a marked change in amplitude occurs making it necessary to reset the baseline values. If the nitrous oxide is turned off in a clinical emergency then a marked increase in amplitude might occur at a time when the monitoring would be watched for a drop in amplitude to signal adverse neural environment. In essence this increase in amplitude might mask an otherwise significant drop in amplitude indicative or potential neural compromise.

It is important to note that this study did not examine the effect of a combination of nitrous oxide with desflurane or sevoflurane, the other two commonly available potent inhalational agents. Further studies will be necessary to specifically examine the effect of the combination of these agents with nitrous oxide. However, the similarity of the mechanisms of action of these drugs with isoflurane and studies in the rat suggesting they are similar to isoflurane in other respects suggest a similar synergistic effect on sensory evoked responses might be seen in humans.

Since it is known that the intravenous agents potentiate the potent inhalational agents [14], it is possible that the effect seen in this study might actually be more profound when the three drug types are mixed together (e.g., isoflurane, nitrous oxide and an intravenous agent such as propofol). This synergistic effect may be more profound when monitoring motor evoked responses where the responses are more sensitive to anesthetic agents; it is not surprising that a total intravenous anesthetic is usually recommended when motor evoked responses are to be monitored [2–4].

It is important to recognize that the anesthetic effects mentioned above of nitrous oxide and isoflurane on other clinical anesthesia endpoints (immobility, hypnosis, MAC-awake, memory, and learning) are often infra-additivity (or antagonism). This means that the anesthetic effect is less clinically effective at the same time that it is more potent in reducing the amplitude of sensory evoked responses thus compounding the implications for intraoperative monitoring.

CONCLUSION

This study demonstrated in the baboon that the combination of nitrous oxide and isoflurane has synergistic effects on sensory evoked responses. In particular, the amplitude decrease of the cortical somatosensory evoked response is greater than predicted if the effects of these agents was additive.

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