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Physostigmine Reverses Propofol-induced Unconsciousness and Attenuation of the Auditory Steady State Response and Bispectral Index in Human Volunteers

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Background: **It is postulated that alteration of central cholinergic transmission plays an important role in the mechanism by which anesthetics produce unconsciousness. The authors investigated the effect of altering central cholinergic transmission, by physostigmine and scopolamine, on unconsciousness produced by propofol.**

Methods: **Propofol was administered to American Society of Anesthesiologists physical status 1 (n = 17) volunteers with use of a computer-controlled infusion pump at increasing concentrations until unconsciousness resulted (inability to respond to verbal commands, abolition of spontaneous movement). Central nervous system function was assessed by use of the Auditory Steady State Response (ASSR) and Bispectral Index (BIS) analysis of electrooculogram. During continuous administration of propofol, reversal of unconsciousness produced by physostigmine (28 μg/kg) and block of this reversal by scopolamine (8.6 μg/kg) were evaluated.**

Results: **Propofol produced unconsciousness at a plasma concentration of 3.2 ± 0.8 (± SD) μg/ml (n = 17). Unconsciousness was associated with reductions in ASSR (0.10 ± 0.08 μV [awake baseline 0.32 ± 0.18 μV], $P < 0.001$) and BIS (55.7 ± 8.8 [awake baseline 92.4 ± 3.9], $P < 0.001$). Physostigmine restored consciousness in 9 of 11 subjects, with concomitant increases in ASSR (0.38 ± 0.17 μV, $P < 0.01$) and BIS (75.3 ± 8.3, $P < 0.001$). In all subjects (n = 6) scopolamine blocked the physostigmine-induced reversal of unconsciousness and the increase of the ASSR and BIS (ASSR and BIS during propofol-induced unconsciousness: 0.09 ± 0.09 μV and 58.2 ± 7.5, respectively; ASSR and BIS after physostigmine administration: 0.08 ± 0.06 μV and 56.8 ± 6.7, respectively, NS).**

Conclusions: **These findings suggest that the unconsciousness produced by propofol is mediated at least in part *via* interruption of central cholinergic muscarinic transmission. (Key words: Awareness; EEG; anticholinesterase.)**

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IT is postulated that alteration of central cholinergic transmission may play an important role in the mechanism by which general anesthetic drugs produce unconsciousness.[1,2] Data from sleep–wake studies in humans[3,4] and animals[5,6] strongly implicate altered central cholinergic transmission in mediating changes in the level of consciousness associated with the sleep–wake cycle. By analogy, it is argued that altered central cholinergic transmission also mediates the loss of consciousness induced by anesthetic drugs.[2] This argument is supported by results from animal studies, which show that anesthetic agents reduce acetylcholine turnover and release[7–10] and inhibit cholinergic nicotinic and muscarinic transmission.[11–17]

Drugs specifically affecting central cholinergic transmission are exploited clinically to alter the level of consciousness of patients. For example, scopolamine, a competitive nonselective muscarinic antagonist that crosses the blood–brain barrier, is administered preoperatively to induce sedation, and high doses can produce

unconsciousness as part of the central anticholinergic syndrome.[18] Physostigmine, an anticholinesterase inhibiting drug that crosses the blood–brain barrier, has been used to reverse the central anticholinergic syndrome[19] and the central nervous system (CNS) depressant effects of a variety of anesthetic drugs. For example, physostigmine decreases the time necessary for return to consciousness after anesthesia with use of halothane[20] and ketamine,[21] and reverses prolonged postoperative somnolence after induction of anesthesia with use of midazolam.[22] A case report describes a patient who showed delayed arousal after administration of halothane and in whom physostigmine produced abrupt awakening after 2 min.[23] Physostigmine also diminishes the time to recover cognitive function after sedation induced by meperidine, propiomazine, and scopolamine administered to parturients.[24] Physostigmine has recently been shown to increase the dose of propofol necessary to induce loss of consciousness.[25]

These clinical observations are difficult to interpret because of multiple confounding variables, including administration of more than one drug, different end points used to assess the level of consciousness, and an unknown, inconsistent and varying level of anesthetic drug at CNS target sites. In the current study, we investigated the effect of altering central cholinergic transmission on unconsciousness produced by propofol in healthy human volunteers.

Methods

Subjects

After approval by the Royal Victoria Hospital Ethics Board, 17 American Society of Anesthesiologists physical status I paid volunteers (18–35 yr [24.9 ± 4.6 yr]; 7 men and 10 women) were recruited. Causes for exclusion, in otherwise healthy volunteers, included anticipated difficulty with tracheal intubation or mask ventilation, obesity, history of gastroesophageal reflux, and drug or alcohol abuse. Subjects gave written consent and underwent a complete medical evaluation (history, physical examination, and blood tests including complete blood count, biochemistry and β-human chorionic gonadotropin (βHCG) level [women]) before participation. Subjects had no history of neurologic or hearing disorders. Otoscopy and pure tone audiometry results were normal (MA40; Maico Hearing Instruments, Minneapolis, MN). Participants were required to fast for a minimum of 8 h before testing, which always occurred in the morning.

Anesthesia Monitoring

An 18G Jelco catheter (Ethicon Inc., Arlington, TX) was inserted into a large vein in the right forearm for drug administration, and a 20-gauge catheter was inserted into the right radial artery for blood sampling and monitoring of systemic arterial pressure. Additional monitoring included electrocardiography (3-lead), pulse oximetry, and concentration of expired carbon dioxide sampled through use of nasal prongs.

Design

Initial baseline (awake) auditory steady state response (ASSR), Bispectral Index (BIS), and behavioral and electrooculogram (EOG) responses to verbal command were determined and a blood sample was obtained for plasma propofol concentration analysis, as a control check for zero-concentration. Plasma propofol level was determined using high-performance liquid chromatography[26] (HPLC; duplicate assay for each determination). Propofol was administered with a computer-controlled Harvard 22 syringe pump (Harvard Apparatus, Inc., South Natick, MA) with use of the infusion program STANPUMP (Dr. S. Shafer, Stanford University, Palo Alto, CA) and pharmacokinetic data of Tackley *et al.*[27] The STANPUMP program incorporates[28] an effect-site equilibration constant (k_{eo}) of 0.25 min^{-1} ($t_{1/2}$ k_{eo} = 2.8 min).[29] Published $t_{1/2}$ k_{eo} values for propofol range[28] from 1.5 to 3.3 min. An initial plasma concentration of 1.0 μg/ml was targeted, followed by a 10-min period for blood–brain equilibration. If subjects were still awake or if they displayed spontaneous movement, the infusion rate was increased to achieve increases in plasma concentration of 1.0-μg/ml increments (each followed by a 10-min equilibration period) until loss of consciousness was produced and subjects were motionless. The requirement that subjects remain motionless during the study helped to reduce the likelihood of difficulty in evaluating responsiveness to verbal command and to eliminate movement artifact, which could contaminate ASSR, EOG, and BIS recordings. Assessment of responsiveness to verbal commands, electrophysiolgic recordings, and blood samples for propofol assay were obtained after loss of consciousness.

With loss of consciousness maintained by the continuous administration of propofol at a rate producing a stable target plasma concentration, subjects received an injection of saline (control) or physostigmine (28 μg/kg) + glycopyrrolate (4.2 μg/kg) in a random, double-blind order (n = 11). The dose of physostigmine was that which has been used clinically to reverse the sedative effects of anes-

thesia and the central anticholinergic syndrome.[18,20–25] Glycopyrrolate was administered with physostigmine to block the peripheral muscarinic side effects of the anticholinesterase. After injection of saline or physostigmine–glycopyrrolate, ASSR and BIS were measured and the level of consciousness assessed by the behavioral and EOG responses to verbal commands. Subsequently, the propofol infusion was discontinued, and ASSR and BIS values were determined after return to consciousness.

In six additional subjects, the effect of scopolamine pretreatment on the physostigmine-induced return to consciousness was assessed. The experimental protocol was similar to that described previously herein, except that after loss of consciousness was produced, scopolamine (8.6 μg/kg) was administered, followed by a 1-h delay before the administration of physostigmine and glycopyrrolate (28 μg/kg and 4.2 μg/kg, respectively). The dose of scopolamine (8.6 μg/kg) is in the range of that used clinically to produce sedation and has been shown to depress cognitive function in humans.[30–32] The administration of scopolamine 1 h before physostigmine is based on the latency to its peak amnesic effect[30] and on our unpublished observation that scopolamine reduces the amplitude of the ASSR and BIS maximally at 60–70 min.

Assessment of Level of Consciousness

Consciousness was assessed by the responsiveness to the spoken verbal commands "open your eyes" and "squeeze my fingers." The commands were spoken in a loud voice and repeated up to 3 times if the subject did not respond. To objectively evaluate responsiveness, the electrooculogram produced in response to prerecorded, digitized (digitizing frequency: 16 kHz, 16-bit resolution) commands "open your eyes" and "close your eyes" was measured. The commands were presented to the right ear *via* an inserted earphone at an intensity of 70 dB (SPL; Bruel-Kjaer sonometer, model 2203; Bruel-Kjaer, Naerum, Denmark, and Norcross, GA) 5 times at 6-s intervals. The signal recorded from gold-cup electrodes placed above and below the right orbit (impedance < 3 kΩ) was amplified (model 12A5; Grass Instruments, Inc., Quincy, MA; bandpass, 0.1–30 Hz), digitized (90 Hz for 5,277 ms) and stored on disk for off-line analysis. Only the response to the "open your eyes" command was measured. The "close your eyes" command was used to ensure that the subject closed the eyes before the "open your eyes" command. The five tracings from each assessment were evaluated individually and averaged. The onset latency was measured from the average tracing at the start of the deflection indicating eye opening.

Auditory Steady State Response and Bispectral Index

Gold-cup electrodes filled with conductive gel were affixed to the scalp with use of collodion-impregnated gauze for recording the ASSR and BIS (impedance < 3 kΩ). An insert earphone ("E-A-R TONE" 3A; Cabot Corp., Indianapolis, IN) was placed in the right ear for delivery of tone bursts or verbal commands. A nonactive earphone was placed in the left ear to attenuate ambient sound. A computer (Intel Pentium microprocessor; Intel, Santa Clara, CA) equipped with two analog-to-digital– digital-to-analog cards (DT2821 series; Transduction, Mississauga, Ontario, Canada) was used for evoking and recording the ASSR. Stimuli were 500 Hz tone bursts (10 ms, 82 dB peak equivalent sound pressure level) delivered to the right ear *via* the inserted earphone at the rate of 34–44 s. The stimulus rate producing the largest response during pretesting screening for a particular subject was chosen. A recording, with the right ear earphone disconnected (no stimulus), was also obtained to estimate baseline EEG noise. The EEG was recorded from T7, C5, C3, Cz, C4, C6, and T8 with reference to the right mastoid. The signal was amplified (bandpass, 0.1–300 Hz, model 12A5 amplifier; Grass Instrument Corp.), and the analog-to-digital conversion rate was adjusted to obtain 32 points/epoch (1 epoch = 1 ASSR cycle) that lasted 29.4–22.7 s, depending on the stimulus rate. Epochs contaminated by artifacts (±100 μV) were automatically rejected. For each assessment, we obtained 10 replicate averages. Each average, consisting of responses to 2,000 stimuli (responses automatically rejected as artifact were replaced), required 45–75 s and were stored on disk for off-line analysis. In one subject, the recordings were reformatted to have T7 as a reference instead of M2 to attenuate myogenic artifacts. Replicate averages that were clearly deviant from the others were eliminated during off-line review (number of discarded averages: 63 of 730 [8.3%]; maximum number of discarded average per subject = 3). The replicate averages were combined for each period and each subject to produce a single tracing (12–20,0000 stimuli). The amplitude and phase (relative to stimulus onset) of the ASSR were measured by fast Fourier transform of the single averaged tracings.

The BIS was recorded from electrodes placed at C3 and C4, with reference to the right mastoid using an A-1000 EEG Monitor (bandpass 1.0–30.0 Hz; Aspect Medical Systems, Natick, MA, software version 3.12). The electrodes were attached to the A-1000 monitor *via*

"jumper" cables connected from the Grass model headstage. BIS was recorded before and after the block of 10 ASSR recordings. The mean of the two measures was used for analysis.

Statistics

Statistical analysis was performed with use of GraphPad InStat (version 2.0, GraphPad Software, San Diego, CA) unless otherwise indicated. Differences between recording periods were evaluated using analysis of variance for repeated measures. The Tukey honest significance difference test was used for *post hoc* comparisons. A two-tailed, paired *t* test was used to compare ASSR and BIS values obtained during baseline and unconsciousness. The comparison of duration of loss of consciousness before physostigmine administration was performed using a two-tailed, unpaired *t* test. Comparison of the ability of physostigmine *versus* saline and of physostigmine *versus* scopolamine pretreatment + physostigmine, to reverse the propofol-induced unconsciousness, were evaluated using 2-×-2 contingency tables and the Fisher exact test.

We adopted the prediction probability (P_k) method of Smith *et al.*[33] (Excel, version 5, Microsoft Corp., Redmond, CA) to quantify the efficiency of the amplitude of the ASSR or the BIS to predict responsiveness to verbal commands. P_k is a nonparametric, rank-order measure of association, ranging from 0.5 (chance level) to 1.0 (perfect concordance). The P_k value was based on 73 observations for ASSR and BIS; all subjects and periods of recording were included. We calculated the standard error of the estimate with use of the jackknife approach, as if the observations were independent, as was done by Leslie *et al.*[34]

Results are expressed as mean ± SD, unless otherwise indicated. The criterion for statistical significance was $P < 0.05$.

Results

Reversal of Propofol-induced Loss of Consciousness by Physostigmine

The loss of consciousness and abolition of spontaneous movement was produced by propofol at a target plasma concentration of 3.1 ± 0.6 μg/ml (n = 17), which was similar to the measured plasma concentration of 3.2 ± 0.8 μg/ml (sample drawn after the 10-min equilibration period after loss of consciousness). The target and measured plasma concentrations of propofol differed by 16.4 ± 16.0%. Because of the persistence of spontaneous movement, target propofol concentrations had to be increased by 1 μg/ml from the concentration that produced loss of responsiveness to verbal commands in 4 of 17 subjects. Loss of consciousness was associated with the inability of the subjects to respond to the command "open your eyes" and "squeeze my fingers" and with the abolition of the EOG response (baseline awake: latency, 576.3 ± 297.8 ms; amplitude, 250.2 ± 138.1 μV; fig. 1). In addition, loss of consciousness was associated with reductions in the ASSR and BIS amplitudes compared with the awake baseline values (figs. 2 and 3).

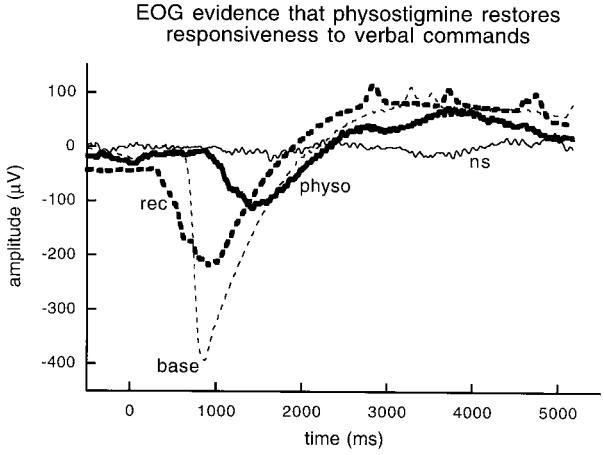

Fig. 1. Electrooculogram from a subject during baseline awake (base), during propofol-induced loss of consciousness after administration of saline (ns), when unconsciousness was reversed 10 min after administration of physostigmine (physo), and during recovery when fully awake (rec). Note that the electrooculoencephalography (EOG) response during physostigmine-evoked reversal of unconsciousness shows a longer latency and a smaller amplitude. Each trace is an average of five EOG responses recorded from the same subject.

Depending on the randomized order of physostigmine–saline (control) administration, physostigmine was administered 27.8 ± 12.4 (n = 5) or 70.7 ± 18.7 min (n = 6) after loss of consciousness and abolition of movement produced by propofol (51.2 ± 27.2 min for all 11 subjects). The propofol plasma concentration after the 10-min equilibration period after the loss of consciousness and mobility (3.3 ± 0.9 μg/ml) was similar to those before (2.9 ± 0.8 μg/ml) and after (3.0 ± 1.0 μg/ml) the time of administration of physostigmine or saline, indicating that the plasma propofol concentrations were stable during this period. Although saline was without effect (n = 5), physostigmine restored consciousness in 9 of 11 subjects ($P = 0.004$). Return to consciousness was determined by the reappearance of the EOG response to the verbal command "open your

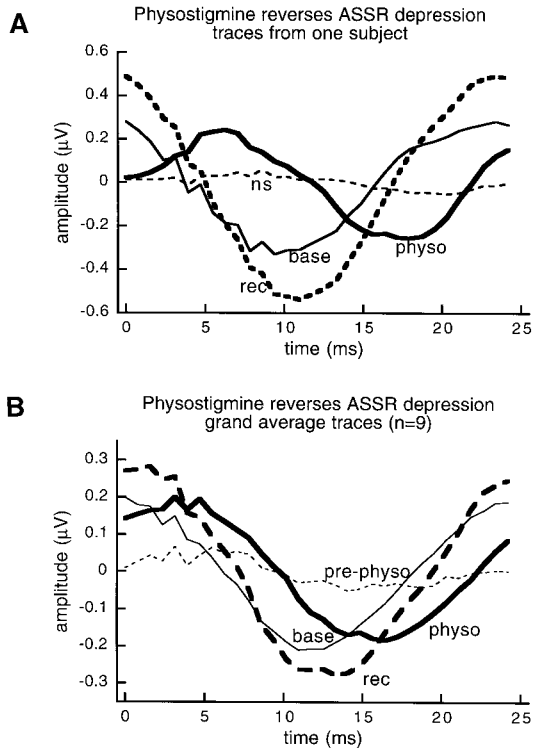

Fig. 2. Amplitude of the auditory steady state response (ASSR) as a function of time. (*A*) ASSR waveforms are shown for a single subject. Each waveform is the average response to 20,000 stimuli. Responses are shown during awake baseline (base), propofol-induced unconsciousness after administration of normal saline (ns), 10 min after administration of physostigmine (physo), and when fully awake during recovery (rec). (*B*) The averaged ASSR waveforms of all subjects are shown (n = 9). Responses are shown during awake baseline (base), propofol-induced unconsciousness before administration of physostigmine (pre-physo), 10 min after administration of physostigmine (physo), and when fully awake during recovery (rec).

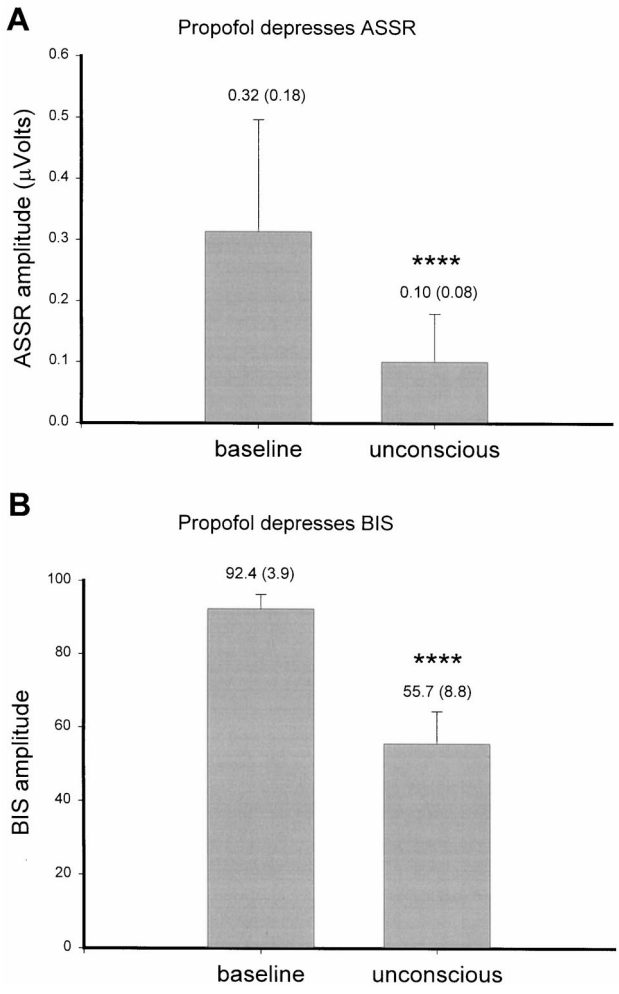

Fig. 3. (*A*) Auditory steady state response (ASSR) and (*B*) Bispectral index (BIS) amplitudes (mean ± SD) for all subjects (n = 17) during baseline (awake) and propofol-induced unconsciousness. Amplitudes were significantly reduced when subjects were unconscious compared with when they were awake (**$P < 0.0001$). Numbers above each histogram plot indicate (SD).**

eyes" (latency: 687.5 ± 526.7 ms; amplitude: 60.11 ± 114.4 μV; n = 9; fig. 1). The physostigmine-induced return to consciousness was accompanied by an increase in the ASSR, which was greater than that recorded during the unconscious state but was similar to the baseline value and to that observed 30 min after discontinuation of propofol administration, when the subjects were fully awake, oriented, and conversant (figs. 2 and 4). The physostigmine-induced return to consciousness was also accompanied by an increase in the BIS, which was greater than that recorded during the unconscious state (fig. 5). The physostigmine-induced increase in the BIS was less than the baseline value and that observed approximately 30 min after discontinuation of propofol administration (fig. 5B).

Of the remaining 2 of 11 subjects in whom physostigmine did not reverse the propofol-induced loss of consciousness, 1 remained fully unresponsive, and the propofol-induced reductions in the ASSR and BIS were not reversed by administration of physostigmine (figs. 4A and 5A). In this subject, physostigmine was administered 20 min after onset of unconsciousness. The other subject followed commands in an equivocal manner, demonstrating uncoordinated movements to the verbal command. In this case, the propofol-induced reductions in the ASSR and BIS were reversed by physostigmine (figs. 4A and 5A). Physostigmine was administered 49 min after onset of unconsciousness.

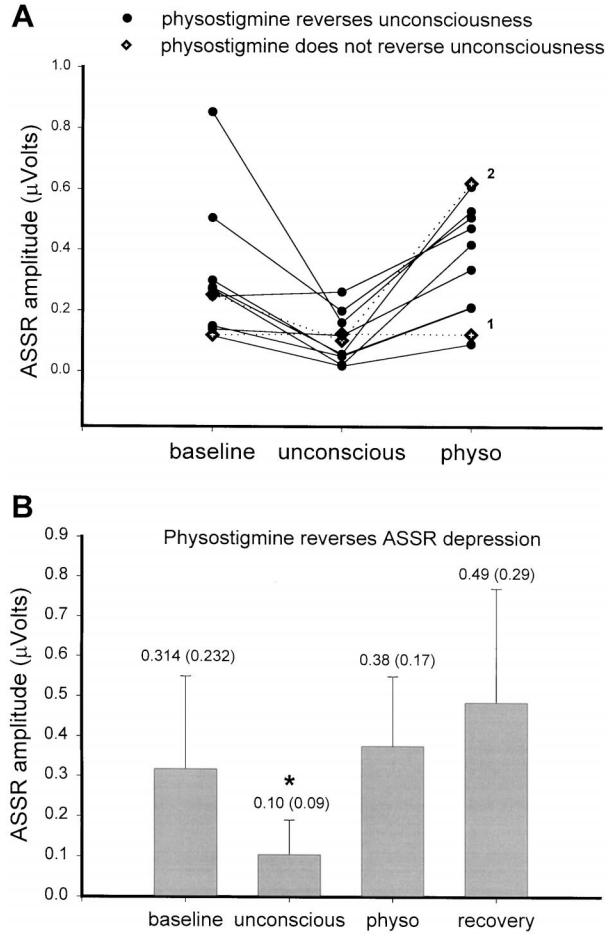

Fig. 4. Effect of physostigmine on the propofol-induced reduction of the auditory steady state response (ASSR) during loss of consciousness. (*A*) ASSR amplitudes of individual subjects are shown. Filled circles denote patients in whom loss of consciousness was reversed by the physostigmine (n = 9). Diamonds indicate patients in whom unconsciousness was not reversed (n = 2). One of these two patients remained clearly unconscious after physostigmine administration (subject 1), whereas the other showed an equivocal level of consciousness (subject 2). (*B*) The ASSR amplitude (mean ± SD, n = 9) during baseline, propofol-induced unconsciousness, physostigmine-induced reversal of the unconsciousness, and recovery. ASSR amplitude was significantly reduced when subjects were unconscious compared with the baseline, after physostigmine administration, and during recovery (P* < 0.05, 0.01, and 0.001, respectively). After administration of physostigmine, amplitude returned toward baseline values. Numbers above each histogram plot indicate ± (SD).**

Scopolamine Block of the Physostigmine-induced Return to Consciousness

In six additional subjects, scopolamine was administered after loss of consciousness, and abolition of movement produced by propofol and the ability of physostigmine to reverse the loss of consciousness was then

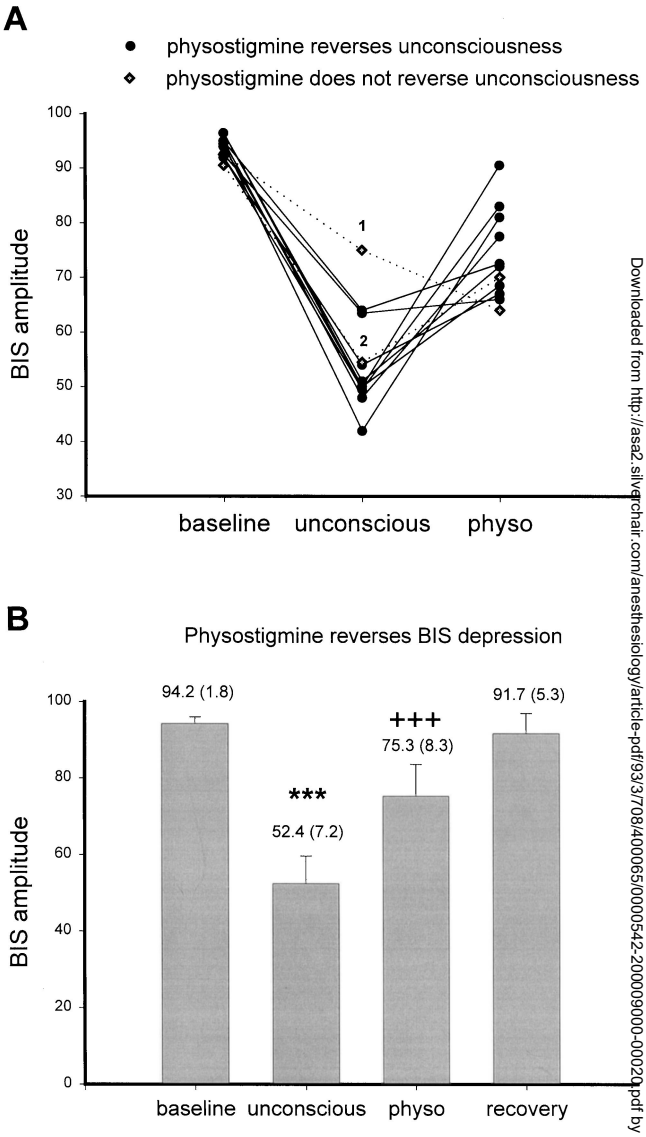

Fig. 5. Effect of physostigmine on the propofol-induced reduction of the Bispectral Index (BIS) amplitude during loss of consciousness. (*A*) BIS amplitudes of individual subjects are shown. Filled circles denote patients in whom loss of consciousness was reversed by the physostigmine (n = 9). Diamonds indicate patients in whom unconsciousness was not reversed (n = 2). One of these two patients remained unconscious after physostigmine administration (subject 1), whereas the other showed an equivocal level of consciousness (subject 2). (*B*) The BIS amplitude (mean ± SD, n = 9) during baseline, propofol-induced unconsciousness, physostigmine-induced reversal of the unconsciousness, and recovery. BIS amplitude was significantly reduced when subjects were unconscious compared with baseline, after physostigmine administration, and during recovery (**P* < 0.001). After administration of physostigmine, although the BIS amplitude increased, it was smaller compared with the baseline and recovery values (+++*P* < 0.001). Numbers above each histogram plot indicate (SD).**

studied 60 min after scopolamine administration. Physostigmine was administered 98.7 ± 13.5 min after the propofol-induced loss of consciousness, which was significantly greater than that for the subjects who did not receive scopolamine ($P = 0.001$). The plasma propofol concentrations after the 10-min equilibration period after the loss of consciousness and immobility (3.2 ± 0.6 μg/ml) and just before (3.0 ± 0.4) and after (3.0 ± 0.58) administration of physostigmine were similar, indicating that the plasma concentrations of propofol were stable during this period. Moreover, these concentrations were similar to those observed in patients who did not receive pretreatment with scopolamine before reversal of the loss of consciousness by physostigmine. Scopolamine blocked the physostigmine-induced reversal of unconsciousness in all six subjects, in marked contrast to the reversal of unconsciousness produced by physostigmine in 9 of 11 subjects reported previously herein ($P < 0.002$). Similarly, scopolamine pretreatment also prevented the recovery of the ASSR and BIS (fig. 6). Interestingly, scopolamine appeared to have no additional inhibitory effect on the ASSR and BIS, which were reduced during loss of consciousness and immobility produced by propofol administration (fig. 6). When subjects were fully awake, oriented, and conversant 30 min after discontinuation of propofol administration, the ASSR and BIS were similar to baseline levels (fig. 6).

Relation between Auditory Steady State Response, Bispectral Index, and Consciousness

Parallel changes in the sate of consciousness and amplitude of the ASSR and BIS produced by propofol, physostigmine, and scopolamine administration were observed. This is indicated by the high correlation of the amplitude of the ASSR ($P_K = 0.925$; SEM = 0.033) and BIS ($P_K = 0.984$; SEM = 0.011; P = NS) with the presence and absence of consciousness.

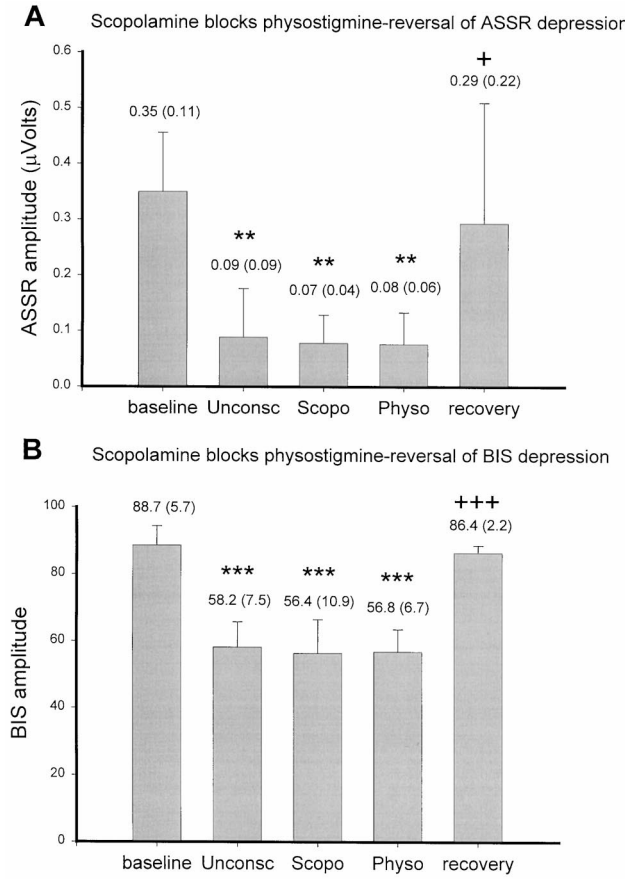

Fig. 6. (*A*) Auditory steady state response (ASSR) and (*B*) Bispectral Index (BIS) amplitudes (mean ± SD, n = 6) during baseline and after the propofol-induced unconsciousness, the administration of scopolamine, the physostigmine-induced reversal of the unconsciousness, and during recovery. ASSR and BIS amplitudes were significantly higher during baseline than during unconsciousness, after scopolamine administration, and after physostigmine administration ($P < 0.01$; ***$P < 0.001$). ASSR and BIS amplitudes were significantly higher during recovery than during unconsciousness, after scopolamine administration, and after physostigmine administration (+$P < 0.05$; +++$P < 0.001$). Numbers above each histogram plot indicate (SD).**

Discussion

This study shows that physostigmine, a carbamyl tertiary amine anticholinesterase that crosses the blood–brain barrier, reverses the propofol-induced unconsciousness and associated depression of the ASSR and BIS in human volunteers. Reversal of the loss of consciousness and depression of the ASSR and BIS produced by physostigmine was blocked by pretreatment with scopolamine, a nonselective muscarinic antagonist that also crosses the blood–brain barrier. These findings are consistent with the hypothesis that the loss of consciousness produced by propofol is mediated, at least in part, *via* interruption of central cholinergic muscarinic transmission.

To assess level of consciousness, we used responsiveness to verbal commands, which has been used previously.[35] To objectively measure responsiveness to the verbal command "open your eyes," we recorded the EOG. The electrooculogram confirmed our subjective impressions but showed no increase in sensitivity. The requirement of a motionless subject necessitated that, in

4 of 17 subjects, the target propofol concentration be increased by 1.0 μg/ml from the previous dose, which produced loss of responsiveness to verbal commands. It can be argued, therefore, that with these four subjects (and, perhaps, with others) a greater dose of propofol was administered than that which just produces loss of consciousness.

The experimental design of a study in which human volunteers are used is constrained by safety issues related to the dose of drugs that can be administered. We considered it impractical to achieve steady state concentrations of physostigmine and scopolamine. As explained in the Methods section, the single bolus doses of physostigmine and scopolamine were chosen on the basis of their demonstrated clinical effectiveness to reverse the central cholinergic syndrome (physostigmine) or to depress cognitive function and produce sedation (scopolamine). Conversely, the rate of propofol infusion was titrated to produce loss of consciousness and abolition of spontaneous movement. Larger doses of propofol were not administered to help avoid depression of the sensorium beyond that which produces loss of consciousness. This had practical relevance, in that subjects were able to maintain spontaneous respiration despite unconsciousness. This also may have theoretical relevance because it is anticipated that with any drug there is a greater degree of nonselective action as the dose increases. Given these caveats, it is fortuitous that the single bolus doses of physostigmine and scopolamine were, in almost all cases, of sufficient magnitude such that they produced the intended effect, indicating that the dose–effect relations of the relevant endogenous ligands and receptors were amenable to these pharmacologic manipulations.

The reversal of the propofol-induced loss of consciousness by physostigmine is compatible with the hypothesis that propofol produces the loss of consciousness by interfering, directly or indirectly, with central cholinergic transmission. We did not, however, directly assess whether propofol alters such transmission. Therefore, we cannot discount the possibility that physostigmine may produce a return to consciousness, *via* enhancement of central cholinergic drive, by altering neuronal activity in a pathway or neuronal system not affected by propofol. For example, it is known that noxious stimulation may improve the level of consciousness in a sedated patient,[36] yet this cannot be interpreted as proof that the somnolence was produced by block of sensory input. Moreover, consideration must be given to the possibility that physostigmine may produce a return to consciousness by mechanisms other than inhibition of central cholinesterase activity. For example, it has been suggested that physostigmine and other carbamyl containing anticholinesterase drugs directly activate muscarinic receptors.[37]

That scopolamine blocks the reversal of the loss of consciousness by physostigmine implicates muscarinic receptor subtypes. Of course, other possibilities must be considered. Again, we cannot discount the possibility that scopolamine alters neuronal activity in a pathway or neural system not affected by propofol, a possibility considered herein previously with physostigmine. Conceivably, propofol could mediate its effect on consciousness by interfering with nicotinic transmission, and the administration of scopolamine, *via* inhibition of central muscarinic transmission, simply augments the depth of anesthesia, such that physostigmine at the dose administered no longer reverses the loss of consciousness. However, scopolamine did not depress the ASSR or BIS beyond that produced by propofol, arguing against the possibility of a greater depth of anesthesia. If propofol mediates the loss of consciousness by block of central cholinergic muscarinic transmission, it may be anticipated that the addition of a muscarinic antagonist, such as scopolamine, should not further reduce transmission. This assumes that the block of central cholinergic transmission produced by propofol is at or near 100%.

Another consideration is that, in subjects pretreated with scopolamine, physostigmine was administered after a longer duration of unconsciousness than in those who did not receive the muscarinic antagonist. However, there was considerable overlap in the duration of unconsciousness in subjects pretreated with scopolamine (89, 89, 91, 92, 110, and 121 min) and in the non-pretreated subjects in whom unconsciousness was reversed by physostigmine administered after saline control (53, 54, 56, 82, 82, and 97 min), as dictated by the randomized administration protocol. The administration of scopolamine after achieving propofol-induced loss of consciousness ensured that the plasma concentration of propofol in this group was similar to that in the group of subjects that did not receive the muscarinic antagonist. Had the scopolamine been administered before propofol, then as a result of the additive CNS depressant effects, it is possible that a lower dose of propofol would have been necessary to produce unconsciousness. As reversal of the loss of consciousness by physostigmine would then have been evaluated in patients with different plasma concentrations of propofol, it would have been difficult to draw any inferences.

The site of action of propofol in the CNS to produce unconsciousness is not known. It has been proposed that cholinergic projections to the cortex and forebrain arising from the caudal mesencephalic–pontine reticular formation and the basal forebrain may play an important role in regulating the level of consciousness,[38,39] and one may speculate that propofol interferes with these projection systems. The mechanism of interference, too, is a matter of speculation. Evidence, as cited in the Introduction, suggests that propofol may directly interfere with cholinergic transmission. Alternatively, the effect may be indirect, involving other neurotransmitter and modulator substances. For example, it has been proposed that propofol acts by binding directly to the γ-aminobutyric acid receptor A ($GABA_A$) chloride channel or to the channel regulatory proteins.[40]

The close correlation of the amplitude of the ASSR and BIS with the presence or absence of consciousness, while propofol concentrations were held constant, suggest that these neurophysiologic measurements reflect CNS processes mediating consciousness rather than simply propofol concentration at CNS effector sites. In particular, that close correlations were observed when central cholinergic transmission was altered by block of cholinesterase activity or muscarinic receptors suggest that a neurophysiologic substrate mediating the ASSR and BIS involves cholinergic muscarinic processes. Muscarinic processes in the cortex[41] and thalamus[42] have been linked to the potentiation of endogenous γ oscillations. Enhancement of endogenous γ oscillations have been linked to states of high vigilance,[43,44] and generation of endogenous γ oscillations and the ASSR may involve similar cellular mechanisms.[45] The somewhat higher P_K of the BIS compared with that of the ASSR indicates that the BIS may be more closely correlated to the presence or absence of consciousness. The possibility that BIS recordings may have been contaminated by electromyogram artifact cannot be discounted because subjects were not paralyzed. Such contamination is less of a concern with an evoked averaged response such as the ASSR.

In summary, we have shown that physostigmine reverses the propofol-induced unconsciousness and associated depression of the ASSR and BIS in human volunteers. The reversal of the unconsciousness and depression of the ASSR and BIS was blocked by pretreatment with scopolamine. These findings support the hypothesis that the loss of consciousness produced by propofol and, by analogy, other anesthetic drugs is mediated, at least in part, *via* interruption of central cholinergic muscarinic transmission.

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