Dialysis Delivery of an Adenosine A1 Receptor Agonist to the Pontine Reticular Formation Decreases Acetylcholine Release and Increases Anesthesia Recovery Time

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Background: Adenosine modulates cell excitability, acetylcholine release, nociception, and sleep. Pontine cholinergic neurotransmission contributes to the generation and maintenance of electroencephalographic and behavioral arousal. Adenosine A1 receptors inhibit arousal-promoting, pontine cholinergic neurons, and adenosine enhances sleep. No previous studies have determined whether pontine adenosine also modulates recovery from anesthesia. Therefore, the current study tested the hypotheses that dialysis delivery of the adenosine A1 receptor agonist N6-p-sulphophenyladenosine (SPA) into the pontine reticular formation would decrease acetylcholine release and increase the time needed for recovery from halothane anesthesia.

Methods: A microdialysis probe was positioned in the pontine reticular formation of halothane-anesthetized cats. Probes were perfused with Ringer’s solution (control) followed by the adenosine A1 receptor agonist SPA (0.088 or 8.8 mM). Dependent measures included acetylcholine release and a numeric assessment of recovery from anesthesia. An intensive, within-subjects design and analysis of variance evaluated SPA’s main effect on acetylcholine release and anesthetic recovery. The adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 μM) was coadministered with SPA to test for antagonist blocking of SPA’s effects.

Results: SPA significantly (P < 0.0001) decreased acetylcholine release in the pontine reticular formation and significantly (P < 0.0001) delayed recovery from anesthesia. Coadministration of SPA and DPCPX caused no decrease in acetylcholine release or delay in postanesthetic recovery. Dialysis delivery of SPA into the cerebellar cortex confirmed that the SPA effects were site-specific to the pontine reticular formation.

Conclusion: The results provide a novel extension of the sleep-promoting effects of adenosine by showing that pontine delivery of an adenosine A1 receptor agonist delays resumption of wakefulness following halothane anesthesia. This extension is consistent with a potentially larger relevance of the current findings for efforts to specify neurons and molecules causing physiologic and behavioral traits comprising anesthetic states. These data support the conclusion that adenosine A1 receptors in medial regions of the pontine reticular formation, known to modulate sleep, also contribute to the generation and/or maintenance of halothane anesthesia.

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Materials and Methods

Animal Model

All procedures involving animals were reviewed and approved by the University of Michigan Committee on Use and Care of Animals (Ann Arbor, Michigan) and strictly adhered to the recommendations in the Guide for the Care and Use of Laboratory Animals. Four adult male cats were anesthetized with isoflurane and implanted with electrodes to enable objective recording of arousal states. Multiple lines of previous evidence made the cat the logical choice for these preclinical studies. First, most of the cellular-level data regarding brain stem regulation of arousal states have been derived from the cat. Second, use of the cat made it pos-
Tissue adjacent to the injection. Diffusion rates and tissue area affected by drug delivery in adenosinergic drugs is unknown. One of the advantages of microdialysis delivery of a known concentration of acetylcholine is unknown, and the acetylcholine values are therefore expressed as pmol/10 min of dialysis. Likewise, the precise amount of pontine tissue affected by dialysis delivery of adenosinergic drugs is unknown. One of the advantages of microdialysis over microinjection is that dialysis drug delivery influences a smaller area of tissue than microinjection. Diffusion rates and tissue area affected by drug vary as a function of drug properties and tissue. In the medial pontine reticular formation, microinjection effects have been shown to vary within 1 mm. One may speculate that microdialysis affects a more limited region of tissue surrounding the probe.

Each 30-μl sample was injected into an LC4C amperometric detector (Bioanalytical Systems, West Lafayette, IN). The electroactive product (H₂O₂) produced by an immobilized enzyme reactor column passed over the electrochemical detector and generated chromatograms proportional to the amount of acetylcholine in the dialysis sample. The area under each chromatographic peak was integrated with a computer using the ChromGraph Report and Control programs (Bioanalytical Systems). The percentages of acetylcholine recovery from the probe were compared by t test to values obtained after removing the probe from the brain. This comparison of preexperimental and postexperimental probe recoveries ensured that variation in acetylcholine reflected neurochemical changes rather than changes in performance of the dialysis probe.

**Drug Preparation**

The adenosine receptor agonist N⁶-p-sulphophenyladenosine (SPA; Sigma-Aldrich Corp., St. Louis, MO) was used because of its high selectivity for A₁ versus A₂ receptors (affinity ratio of A₁/A₂ = 120).26 SPA was prepared in Ringer's solution in concentrations of 0.088 or 8.8 mM. The adenosine receptor antagonist 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX; Sigma-Aldrich) shows a 700-fold selectivity for the A₁ receptor versus the A₂ receptor.29 DPCPX was dissolved in dimethyl sulfoxide (Sigma-Aldrich) and then diluted to 100 μM in Ringer's solution, with a final dimethyl sulfoxide concentration of 0.008%. Antagonist + agonist coadministration consisted of 8.8 mM SPA and 100 μM DPCPX prepared in Ringer's solution. Drug solutions were made fresh prior to every experiment. Drug solutions were filtered, pH adjusted to 6.0 ± 0.4, maintained at room temperature, and protected from light. These drug concentrations were chosen as equimolar to the concentration of cholinergic agonist shown by previous studies to alter sleep or acetylcholine release when administered into the pontine reticular formation. A limitation of microdialysis drug delivery is the inability to specify the quantity of administered drug. Since the current dialysis probes exhibit an 8% acetylcholine recovery, one can assume dialysis drug delivery of approximately 8% of the dialyzed concentration. If this assumption is correct, the delivered drug concentrations would have been approximately 7 or 700 μM SPA and 8 μM DPCPX.

**Experimental Procedures and Design**

Each experiment began by anesthetizing a cat with 3% halothane. The trachea then was intubated with a cuffed pediatric endotracheal tube (size 3.5–4.5 French), and

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Text continues with detailed procedures and protocols for microdialysis experiments.
the cat was placed in a Kopf stereotaxic frame and connected to a mechanical ventilator. End-tidal halothane and carbon dioxide fractions were monitored continuously with an Ohmeda Rascal II spectrometer (Datex-Ohmeda, Madison, WI). Ventilation and inspired halothane concentration were adjusted to maintain end-tidal halothane at 1.5% and end-tidal carbon dioxide at 30 mmHg. Oxygen saturation and heart rate were monitored with an Ohmeda Biox 3700 Pulse Oximeter (Datex-Ohmeda). Blood pressure was monitored using a Critikon Dinamap (Critikon, Tampa, FL), and body temperature was measured with a rectal thermometer throughout the experiment. A T500 T/Pump Heat Therapy System (Gaymar, Orchard Park, NY) was used to maintain body temperature at 37°C. A microdialysis probe was aimed for the mPRF cerebellar cortex using a stereotaxic atlas. Stereotaxic coordinates for the mPRF dialysis sites were posterior 1–3, lateral 1–3, and horizontal −5. Microdialysis sites in the cerebellar cortex were posterior 10, lateral 2–3, and horizontal 5.

This study used an intensive within-subjects design. Each experiment was divided into two phases (fig. 1). Care was taken to ensure that evaluation of the effects of adenosinergic compounds was not confounded by significant variations in duration of halothane anesthesia (fig. 1, lines A–D). The first phase involved microdialysis for simultaneous collection of acetylcholine and drug delivery (fig. 1, lines B–C). The second phase involved quantification of postanesthetic recovery time (fig. 1, lines D–E). Prior to the microdialysis portion of the study, the probe was positioned stereotaxically in the mPRF (fig. 1, lines A–B). The probe was perfused with one of four different solutions: Ringer’s solution alone, 0.088 mM SPA, 8.8 mM SPA, or SPA (8.8 mM) plus DPCPX (100 μM). During drug delivery, sequential 30-μl mPRF dialysis samples were collected on ice for quantifying acetylcholine release (fig. 1, lines B–C). The duration of sample collection (fig. 1, lines B–C) always was 180 min. After the microdialysis phase of the experiment was complete (fig. 1, line C), the dialysis probe was removed from the brain (fig. 1, lines C–D). Time required for removal of dialysis probe to cessation of halothane (fig. 1 lines C–D) averaged approximately 15 min. After the halothane anesthesia was discontinued (fig. 1, line D), recovery time from anesthesia was quantified (fig. 1, lines D–E). The results quantify variations in time for recovery (interval between lines D and E in fig. 1) as a function of dialysis drug delivery (conditions shown between lines B and C in fig. 1).

Quantification of Postanesthetic Recovery
To evaluate postanesthetic emergence, a recovery score developed for humans was modified for use in the current study. The recovery score was determined at each of the following time points after turning off the halothane: every min for the first 10 min, every 5 min up to 1 h, every 15 min up to 1.5 h, every hour up to 4 h, and at 24 h. This resulted in a total of 26 time points per experiment at which anesthetic recovery was evaluated. The five criteria for recovery score were provided by measures of (1) motor activity, (2) respiration, (3) blood pressure (BP) and heart rate (HR), (4) responsiveness, and (5) oxygen saturation and skin color. These variables were evaluated and scored as follows:

1. Motor activity: Spontaneous motor activity was scored as 0 if no movement was observed; 1 when the head or one or two limbs moved; and 2 when all four limbs moved and the cat started walking.

2. Respiration: No spontaneous respiratory activity was scored as 0; apparent dyspnea, superficial breathing, or effort to breathe were scored as 1; and deep, regular breathing was scored as 2.

3. Blood pressure and heart rate. Systolic arterial BP and/or HR variation greater than 50% of the initial values was scored as 0; a 20–50% variation from initial values was scored as 1; and less than 20% variation from initial values was scored as 2.

4. Responsiveness: Failure to respond to auditory stimulation (head or eye movement toward the sound) was scored as 0; any behavioral sign of responsiveness to auditory stimulation was scored as 1; and full alertness and responsiveness to visual and auditory stimuli was scored as 2.

5. Oxygen saturation and skin color: Cyanosis was scored as 0; alterations from the normal color, such as pale, dusky, or blotchy discoloration, were scored as 1; and normal pink skin coloration and/or oxygen saturation values above 90% were scored as 2.

For each experiment, at each time point, the total recovery score was determined by summing the scores for each variable.
for dependent measures 1–5. Thus, the maximal score possible was 10 and indicated complete recovery from anesthesia.

**Histologic and Statistical Analysis**

On completion of in vivo experiments, brains from all animals were examined histologically to confirm dialysis probe placement in the mPRF or cerebellar cortex. Cats were deeply anesthetized with sodium pentobarbital (35–40 mg/kg intraperitoneal) and perfused transcardially with isotonic saline followed by 10% formalin. Brains were removed, fixed, serially sectioned (40 μm thick), and stained with cresyl violet for histologic localization of microdialysis probe sites. All tissue sections containing dialysis probe-induced lesions were digitized and compared with a brain stem atlas.32

Postanesthetic recovery scores are reported as mean ± SD. Recovery scores from experiments in which the same drug was administered were averaged at each of the 26 time points. Linear regression analysis was used to quantify recovery score as a function of time for Ringer’s solution (control), SPA, and SPA + DPCPX dialysis conditions. Analyses were performed using multiple linear comparisons to evaluate significant differences in slopes and intercepts (GraphPad Prism 3.0 Software Inc., San Diego, CA). One-way analysis of variance (ANOVA) and Tukey-Kramer procedures compared recovery scores following Ringer’s solution, 0.088 mM SPA, and 8.8 mM SPA at 15 min, 45 min, and 24 h after drug dialysis completion. Acetylcholine release as a function of dialysis drug delivery was evaluated using ANOVA and the Tukey-Kramer procedure. Probability values of $P < 0.05$ were regarded as statistically significant.

**Results**

**Pontine Dialysis with SPA Delayed Emergence from Halothane Anesthesia**

The pontine region where drugs were delivered by microdialysis is illustrated in figure 2. Histologic analyses confirmed that all pontine dialysis sites were localized to the mPRF, which lies within the gigantocellular tegmental field as described by Berman.32

Figure 3 shows that dialysis delivery of 8.8 mM SPA into the mPRF significantly increased time required for recovery from halothane anesthesia compared to recovery time following dialysis with Ringer’s solution alone. The three functions shown in figure 3 were determined from recovery scores obtained after dialysis with Ringer’s solution, 0.088 mM SPA, or 8.8 mM SPA (fig. 1, lines B–C). The function labeled Ringer’s had a slope of 0.18 and an intercept of 5.4, representing the control profile of emergence from halothane anesthesia (fig. 3). Following control dialysis with Ringer’s solution, all cats completely recovered in 30 min after delivery of halothane ended.

Dialysis delivery of 0.088 mM SPA produced a recovery score profile similar to the control, with a slope of 0.14 and an intercept of 4.95 (fig. 3). All cats that received the low concentration of SPA (0.088 mM) recovered within 30–45 min after cessation of halothane. Dialysis delivery of 8.8 mM SPA increased postanesthetic recovery time. The recovery score determined following treatment with 8.8 mM SPA had a slope of 0.05 ± 0.007 and a y-axis

![Fig. 2. Histologic localization of a dialysis site. Sagittal section of cat brain stem shows a typical microdialysis site (arrow) localized within the medial pontine reticular formation (mPRF). Inset indicates location of the mPRF on a midsagittal schematic of the cat brain and schematizes a microdialysis probe used to deliver drugs and collect endogenously released acetylcholine. Comparison of histologic sections from all cats to a cat brain stem atlas confirmed mPRF dialysis probe placement for all animals used in this study. TB = trapezoid body.](image)

![Fig. 3. Time course of recovery from anesthesia following pontine microdialysis. For recovery score (ordinate), 10 = full recovery and 0 = no recovery. Each data point represents the mean ± SD of at least three individual recovery scores from different experiments in different animals. Regression analyses for each of these treatment conditions generated linear functions.](image)
intercept of 3.24 ± 0.19, reflecting the delayed emergence from anesthesia (Fig. 3). Multiple regression comparisons indicated a significant difference ($F = 22.7; df = 2, 83; P < 0.0001$) between the recovery score slopes for SPA (8.8 mM) and Ringer's solution (control). The histograms of figure 4 show that dialysis delivery of 8.8 mM SPA significantly delayed recovery for up to 4 h following discontinuation of halothane. The figure 4 recovery scores were obtained during the interval indicated in figure 1 between lines D and E.

**Coadministration of DPCPX Partially Blocked the SPA-induced Increase in Halothane Recovery Time**

The SPA-induced delay in recovery from halothane anesthesia was significantly reduced by coadministration of the adenosine $A_1$ receptor antagonist DPCPX. Figure 5 summarizes the functions generated by linear regression analysis of recovery scores obtained after dialysis with Ringer's solution, DPCPX + SPA, and SPA alone. By min 60, the mean recovery score following administration of DPCPX + SPA was 9.67, indicating complete postanesthetic recovery. The linear function representing postanesthetic recovery scores following DPCPX + SPA had a slope of 0.09 ± 0.01 and an intercept of 4.89 ± 0.27. These values were intermediate between slope and intercept values for Ringer's solution and 8.8 mM SPA. Multiple linear regression analysis demonstrated that DPCPX and SPA coadministration caused a significantly shorter ($F = 21; df = 2, 78; P < 0.0001$) wake-up time than 8.8 mM SPA dialysis alone.

**Dialysis Administration of 8.8 mM SPA into the Cerebellar Cortex Did Not Delay Recovery from Anesthesia**

Figure 6 compares recovery scores obtained following cerebellar SPA administration with recovery scores obtained following mPRF Ringer's solution (control) and
mPRF SPA administration. Following dialysis delivery of SPA into cerebellum, two cats achieved complete recovery by min 30, and the third cat recovered completely by min 45. Multiple linear regression analyses demonstrate that cerebellar administration of SPA failed to delay recovery from anesthesia, and that the recovery score was significantly different from the delayed recovery caused by 8.8 mM SPA delivered to mPRF ($F = 20.53; df = 2, 75; P < 0.0001$). Thus, the delay in recovery was significantly dependent on SPA administration into the medial pontine reticular formation. There was no significant difference in the duration of halothane administration between experiments that dialyzed the mPRF with Ringer’s solution (control), experiments that dialyzed the mPRF with SPA or SPA + DPCPX, and experiments that dialyzed the cerebellar cortex with SPA.

**Dialysis Delivery of SPA Decreased Acetylcholine Release, and This Effect Was Blocked by DPCPX**

Figure 7A plots acetylcholine release measured during a typical experiment in which dialysis delivery of 8.8 mM SPA decreased mPRF acetylcholine. Figure 7B summarizes mPRF acetylcholine release during dialysis with Ringer’s solution (control) and during dialysis delivery of 0.088 or 8.8 mM SPA. ANOVA revealed a significant SPA-induced decrease in acetylcholine release ($F = 22.4; df = 2, 117; P < 0.0001$). Dialysis with 8.8 mM SPA (fig. 7B, solid bar) decreased acetylcholine release to 78.6% of control (fig. 7B, hatched bar). Multiple comparison statistic showed that the lower concentration of SPA (0.088 mM) did not significantly alter acetylcholine release (fig. 7B, shaded bar). Figure 8 shows that coadministration of DPCPX with SPA blocked the SPA-induced decrease in mPRF acetylcholine release ($F = 39.9; df = 2, 102; P < 0.0001$).

**Discussion**

Adenosine is relevant to anesthesiology in part because of compelling evidence that adenosine alters hyperalgesia and neuropathic pain.34-38 Adenosine infusion reduces the requirement for isoflurane,39 and the current results show that time required for recovery from halothane anesthesia was significantly increased by pontine delivery of an adenosine A<sub>1</sub> receptor agonist. Normal brain acetylcholine concentrations are required for electroencephalographic and behavioral arousal.40 Thus, the finding that pontine acetylcholine was decreased by the adenosine A<sub>1</sub> receptor agonist is considered below as one potential mechanism modulating the delayed recovery from halothane anesthesia.

**Adenosine A<sub>1</sub> Receptor Agonist Increased Time Required for Recovery from Halothane Anesthesia**

$N$-methylated xanthine molecules such as caffeine, theobromine, and theophylline all promote arousal. Caffeine ingestion by humans increases latency to sleep onset and reduces the electroencephalographic $\delta$ power of non-REM sleep.41 The ability of caffeine to promote wakefulness is mediated in part by A<sub>1</sub> adenosine receptors.5 The data reported here show a significant delay in posthalothane recovery caused by dialysis delivery of the adenosine A<sub>1</sub> receptor agonist SPA into the medial pontine reticular formation (figs. 3 and 4). The results provide additional support for the view that medial regions of the pontine reticular formation, known to modulate sleep,12 also contribute to the loss of wakefulness caused by a chemically diverse set of anesthetic agents. As noted...
Adenosine A1 Receptors Modulate Arousal via Cholinergic Neurotransmission

Studies of sensory afferent neurons, central nervous system neurons, and efferent motor systems show that adenosine A1 receptors alter cholinergic neurotransmission. In sensory neurons, purinergic neuromodulators such as ATP and acetylcholine can activate similar non-selective cation channels via common intracellular pathways. Adenosine A1 receptors inhibit synaptic transmission by decreasing N-type Ca2+ current. Adenosine A1 receptors exert an inhibitory action on Ca2+ entry coupled to acetylcholine release in retina cells. In motor systems, ATP suppresses acetylcholine release via protein kinase C-coupled purinergic receptors, and adenosine alters quantal acetylcholine release.

Previously, acetylcholine release in the pontine reticular formation is significantly decreased by morphine, halothane, fentanyl, and ketamine.

The results are consistent with the interpretation that the SPA-induced increase in postanesthetic recovery time can be modulated by adenosine A1 receptors. The delay in postanesthetic recovery caused by dialysis delivery of the adenosine A1 receptor agonist SPA varied significantly as a function of agonist concentration (figs. 3 and 4) and was blocked by coadministration of the adenosine A1 receptor antagonist DPCPX (fig. 5). Dialysis delivery of the agonist into the cerebellar cortex failed to prolong emergence (fig. 6). Thus, the delayed recovery from halothane anesthesia was site-specific to the mPRF, varied significantly with SPA concentration, and was blocked by an adenosine agonist.

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By what cellular mechanisms might adenosine alter acetylcholine release and arousal? Adenosine inhibits electroencephalographic and behavioral arousal, which are modulated by forebrain and brain stem cholinergic neurons. Basal forebrain cholinergic neurons provide acetylcholine to the cortex, and cortically activated states of wakefulness and REM sleep are associated with greater concentrations of cortical acetylcholine than are states of NREM sleep. Measures of basal forebrain acetylcholine also show that acetylcholine release is greater during wakefulness and REM sleep than during NREM sleep. These microdialysis data are consistent with studies showing that administering adenosine to the basal forebrain decreases wakefulness. In contrast, wakefulness is increased by administering an adenosine A1 receptor antagonist to the basal forebrain. The foregoing neurochemical studies are supported by electrophysiological evidence. Putatively cholinergic neurons of the magnocellular basal forebrain exhibit the greatest discharge frequency during wakefulness and REM sleep. The discharge of these wake-active basal forebrain neurons is inhibited by an adenosine A1 receptor agonist.

Cholinergic neurons in the pontine brain stem also modulate electroencephalographic and behavioral arousal. These cholinergic neurons are located in the laterodorsal tegmental and pedunculopontine tegmental nuclei (LDT/PPT) and provide acetylcholine to the mPRF. Neurons in the mPRF do not produce acetylcholine and, in the current study, the acetylcholine measured in the mPRF is known to originate from LDT/PPT neurons. In vitro electrophysiological recordings found that adenosine A1 receptors cause postsynaptic inhibition in approximately two thirds of LDT/PPT neurons. LDT/PPT neuronal excitability also is presynaptically disfacilitated by adenosine A1 receptors on presynaptic glutamatergic terminals that innervate the LDT/PPT. Microdialysis delivery of adenosine to the LDT/PPT decreases wakefulness. The foregoing evidence fits well with the current findings that dialysis delivery to the mPRF of the adenosine A1 agonist SPA decreased acetylcholine release in the mPRF (figs. 7 and 8). These results also are consistent with the sleep-enhancing effects of microinjecting an adenosine agonist into the pontine reticular formation. The current results provide a novel extension of the sleep-promoting effects of adenosine by showing that an adenosine A1 agonist delays resumption of wakefulness following halothane anesthesia. This extension is consistent with a potentially larger relevance of the current findings for efforts to elucidate the specific neurons and molecules causing traits that characterize the state of anesthesia. The current data support the working hypothesis that adenosine A1 receptors in medial regions of the pontine reticular formation, known to modulate

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sleep states, also contribute to the generation of anesthetic states.22,23

Limitations and Conclusions

Four adenosine receptors (A_1, A_2a, A_2b, A_3) have been cloned,48 and use in this study of the agonist SPA restricts conclusions to inferences regarding the A_1 subtype. Adenosine A_1 receptors inhibit the release of “virtually every classic neurotransmitter (including glutamate, γ-aminobutyric acid, acetylcholine, norepinephrine, 5-hydroxytryptamine, dopamine, and other transmitters as well).”44 Dialysis delivery of SPA was limited to concentrations that were ineffective (0.088 μM) or effective (8.8 μM) in decreasing acetylcholine release. A complete concentration-response curve is required to determine potency and maximal efficacy of SPA. Limitations associated with applying Aldrete and Kroulik’s53 clinical assessment tool to the evaluation of feline posthalothane emergence also are acknowledged. The current data, however, demonstrate the face validity of modifying the human recovery score53 for operationally quantifying posthalothane recovery in felines (fig. 4). The results encourage future studies aiming to determine if quantitative electroencephalographic analyses can provide a more objective assessment of SPA-induced alterations in levels of arousal.62

The current data cannot specify the cellular mechanisms by which the adenosine agonist SPA prolonged the recovery from halothane anesthesia for 4 h (figs. 3–6). Available data, however, permit speculation regarding the long duration of SPA effects on anesthetic recovery time and acetylcholine release. The adenosine A_1 receptor is coupled to an inhibitory guanine nucleotide-binding (G) protein which inhibits adenyl cyclase and activates calcium and potassium channels.63 One function of G proteins is signal amplification in the time domain, which may account for the long-duration SPA actions. SPA causes a concentration-dependent activation of G proteins in the medial pontine reticular formation.64 Although acetylcholine is a neurotransmitter that alters cell excitability with a time course of milliseconds, the cholinergic regulation of sleep and wakefulness has a time course of minutes and hours.25,30 The modulation of arousal by muscarinic cholinergic receptors in the pRF involves a signal transduction cascade comprised of adenylate cyclase, a pertussis toxin-sensitive G protein, cyclic AMP, and protein kinase A.55–67 This cholinergic transduction pathway would be anticipated to cause slow and long-lasting changes in neuronal excitability.12 The clinical relevance of these cholinergic pathways is illustrated by the ability of acetylcholinesterase inhibitors to reverse propofol-induced unconsciousness.68 Thus, the current results suggest the working hypothesis that SPA caused a long-lasting depression in acetylcholine and anesthetic recovery time by interacting with cholinergically activated G proteins. This hypothesis is testable and consistent with data showing that

in the pons, SPA and the μ-opioid agonist DAMGO cause a partially additive activation of G proteins.64

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References


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