Caffeine accelerates recovery from general anesthesia

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There are no drugs available to the clinician or scientist that reverses the coma-like state induced by general anesthetics (Solt et al. 2011). Such drugs might be useful in a clinical or laboratory setting. In several recent articles Solt and colleagues demonstrated that intravenous administration of methylphenidate could shorten the time it took adult rats to emerge from anesthesia (Chemali et al. 2012; Solt et al. 2011; Taylor et al. 2013). Methylphenidate is known to inhibit dopamine transport; Solt and colleagues suggested that inhibition of dopamine transport results in elevated extracellular dopamine levels that accelerate recovery from anesthesia (Taylor et al. 2013) by dopamine receptor activation.

Anesthetics are thought to produce anesthesia, in part, by potentiating GABA_A receptor activity (Concas et al. 1990; Reynolds et al. 2003; Solt and Forman 2007). Additionally, there is increasing evidence for a presynaptic locus for the action of anesthetics (Hemmings 2009; Hemmings et al. 2005; Takahashi et al. 1999; Trudeau et al. 1996, 1998, 1999). From these observations we hypothesize that drugs that reverse anesthesia. Forskolin, theophylline, and caffeine are all drugs that elevate cAMP by different mechanisms. Theophylline and caffeine also inhibit adenosine receptors (Lazarus et al. 2011; Ribeiro et al. 2002). In this study, all three drugs were tested for their ability to accelerate emergence from anesthesia.

Materials and Methods

PC12 Cell Culture

PC12 cells were grown on collagen-coated 10 cm Petri dishes in culture medium that consisted of RPMI-1640, 10% heat-inactivated horse serum, 5% fetal bovine serum, 2 mM glutamine, and 10 μg/ml gentamicin in a humidified 7% CO2 incubator at 37°C. Culture medium was replaced every other day, and cells were passaged once per week. Cells were replated on poly-lysine-coated glass coverslips 24 hours before recording.

Amperometric Measurement of Catecholamine Release

Carbon fiber electrodes were fabricated and used as previously described by Grabner et al. (2005). The detection threshold for amperometric events was set at five times the baseline root mean squared noise, and the spikes were automatically detected. Ampero-
metric spike features, quantal size, and kinetic parameters were analyzed with a series of macros written in Igor Pro (Wavemetrics) and kindly supplied to us by Dr. Eugene Mosharov.

There can be some variation between experiments. Although there is significant variation week-to-week and even day-to-day, we observed modest cell-to-cell variation in experiments done on the same day using the same cultures. Thus, for each recording for an experimental group, a control cell was added on the same day at about the same time. Without a matching control the experiment was not used. A Student’s t-test was used to assess differences between populations of cells.

**PC12 Cell Permeabilization and Stimulation**

An amperometric electrode was placed gently against a cell. Following 2 min in a Ca\(^{2+}\)-free solution (step 1), the cell was permeabilized with 20 μM digitonin (Ca\(^{2+}\)-free) for 25 s (step 2) and then stimulated for 2–3 min with a solution containing 100 μM Ca\(^{2+}\) (step 3). The cell was allowed to recover for 2 min in Ca\(^{2+}\)-free media (step 4), and the cycle began again at step 2. Cells were stimulated four to five times in this way. The recording solutions had standard compositions previously described in (Grabner et al. 2005).

**Measurement of the Isoflurane Concentration**

Isoflurane solutions were prepared and measured as previously described (Jones et al. 1992; Jones and Harrison 1993). Isoflurane was prepared in sealed plastic intravenous bags. We have previously found that isoflurane concentrations in the bags and in the bath are remarkably constant for up to 1.5 h when measured in representative experiments using gas chromatography (Xie et al. 2006). All isoflurane concentrations in this article are provided in millimolars. The iso- flurane concentrations in the bags and in the bath are remarkably constant in 50% of trials (Eger et al. 1965) equivalents of isoflurane have been reported to be in the range of ~0.3 mM (Franks and Lieb 1996) to ~0.5 mM (Franks and Lieb 1996; Jones and Harrison 1993) at 25°C.

**Anesthetizing Adult Rats**

All studies on rats were approved by the University of Chicago Animal Use Committee.

*Isoflurane.* Adult Sprague-Dawley rats, weighing 420–510 g, were placed in a gas-tight anesthesia box where they were exposed to 3% isoflurane (in 3 l/min O\(_2\)) for 8 min. During this time the rats became unconscious and were insensitive to tail pinch. Isoflurane was dialed into the rat tail, while the nose cone and 2% isoflurane (2 l/min O\(_2\)) were maintained. Drugs were applied as a bolus in saline, and the intravenous line was then removed. Five minutes after the drug was injected vital signs were again acquired. Five minutes later they were measured again. This second measurement also lasted ~10 min. After the measurements were complete, anesthesia was discontinued and the rats were returned to their cages. The data from both measurements before drug administration were averaged as was the data from the two measurements after drug administration. Pulse oximetry was also measured during the experiments but not in all animals. Pulse oximetry was consistently at 100% with an occasional reading of 99%.

An unpaired t-test was used to test for statistical significance.

**RESULTS**

Isoflurane Blocks Neurotransmitter Release in PC12 Cells: Forskolin, Theophylline, and Caffeine Reverse This Block

Anesthetics modulate the activity of various channels and receptors, thereby altering neurotransmitter release. For the studies outlined in this article, PC12 cells were permeabilized with digitonin, and then cells were stimulated by exposing them to Ca\(^{2+}\). Cytoplasmic constituents stay intact in digitonin permeabilized cells (Holz et al. 1994; Li et al. 2003). Permeabilization disrupts the cell resting potential and equilibrates the intracellular and extracellular Ca\(^{2+}\) levels. Under these conditions, modulation of channels or receptors should have no effect on neurotransmitter release. In earlier studies, PC12 cells were also dialyzed with known Ca\(^{2+}\) concentrations, via a patch pipette from a constant holding potential of ~65 mV; these studies validated the digitonin methodology (Herring et al. 2009, 2011). Exocytosis was elicited in the presence and absence of isoflurane (0.5 mM). Basal (Ca\(^{2+}\)-independent) neurotransmitter release is virtually nonexistent in digitonin permeabilized PC12 cells in Ca\(^{2+}\)-free conditions, but robust release is observed upon exposing cells to Ca\(^{2+}\)-containing solutions (Graham et al. 2002; Jankowski et al. 1992). Physiologically, release is evoked by the activation of voltage-gated Ca\(^{2+}\) channels. The proximity of Ca\(^{2+}\) channels to synaptic release sites suggests that intracellular Ca\(^{2+}\) concentration may rise to levels >100 μM at the vesicle (Llinas et al. 1992). To mimic these levels in our experiments, evoked neurotransmitter release was elicited by exposing digitonin-permeabilized cells to 100 μM Ca\(^{2+}\), for 2 min, in the absence or presence of isoflurane.

In previous studies we have shown that 0.5 mM (~1.5 minimum alveolar concentration) isoflurane inhibits neu-
release by ~39% (Herring et al. 2009, 2011). Figure 1 shows that isoflurane (0.5 mM) significantly inhibited neurotransmitter release by ~36% ($P < 0.05$) in this study. Three drugs that elevate intracellular cAMP were then tested for their ability to reverse the inhibition produced by isoflurane. Figure 1 shows that all three drugs, forskolin (5 µM), theophylline (50 µM), or caffeine (50 µM), reversed the isoflurane-mediated inhibition of neurotransmitter release.

Interestingly, methylphenidate also appears to reverse the inhibition of neurotransmitter release produced by isoflurane (Fig. 2), perhaps by elevating [cAMP], (Pascoli et al. 2005).

### Recovery from Isoflurane Anesthesia in Behaving Rats

These results suggest that elevating intracellular cAMP reverses the inhibition of neurotransmitter release produced by isoflurane in vitro. Does elevating cAMP alter recovery from anesthesia in animals? To address this question, we examined emergence from anesthesia in rats. First, we assessed waking from anesthesia in the absence of any other drug to assess population variability. Rats were placed in an anesthetizing apparatus where they were exposed to 3% isoflurane (in 3 l/min O2) for 8 min and then to 2% isoflurane for an additional 45 min. Recovery time from anesthesia for rats is defined as the time from when the animals are removed from the anesthetizing chamber to when they stand upright with four paws on the table. Figure 3A shows data from 10 animals subjected to this protocol (filled square). A week later the experiment was repeated in an identical manner in the same group of rats (open circle). Each filled square or open circle in the graph represents data from a single trial. Note that although there is some variability in recovery time from week 1 exposure to the week 2 exposure, average recovery times for week 1 (437 s) and week 2 (465 s) are similar. Figure 3B plots averaged data from 3 groups of 10 control animals. Each group was exposed to isoflurane anesthesia twice. The average recovery times between groups were significantly different, but within each group there was no significant difference. These data suggest that the optimal method for studying alteration in waking times is to use the same animals as controls and test subjects, which is how data were gathered for this study. The same cohort of animals was used for each of the drugs studied. Figure 3C plots the distribution of recovery times for all 30 animals. Although additional studies are required for a definitive statement, the data in Fig. 3C may not be normally distributed.

There are risks associated with multiple applications of anesthesia, especially in the very young, the very old, and the critically ill. In the elderly there is postoperative cognitive decline following anesthesia and there is a possible link between anesthetic exposure and Alzheimer’s disease; in vitro studies suggest anesthetics promote apoptosis and decreased neurogenesis (Bittner et al. 2011; Hudson and Hemmings 2011; Run et al. 2010). Children younger than 2 yr who undergo multiple surgeries requiring general anesthesia may be up to three times more likely than other children to develop speech and language problems (Flick et al. 2011; Sun 2010). The literature on multiple rounds of anesthesia in healthy adults is sparse. Our studies in rats found no obvious changes in rats exposed to multiple rounds of anesthesia nor were there any changes in the response to anesthesia.

### Forskolin Accelerates Recovery from Isoflurane Anesthesia

Forskolin (5 µM), a drug that elevates cAMP by stimulating adenylate cyclase activity, was tested to determine whether elevating cAMP could alter recovery from anesthesia. Adult rats were anesthetized as above. An intravenous line was inserted into a tail vein. For the forskolin studies the animals received an intravenous injection of either saline (control, closed square) or saline with drug (open circle) 5 min before discontinuing the anesthetic. The anesthetic was then terminated, and the animals were allowed to wake and right themselves, breathing room air. On average, recovery time was reduced significantly by ~39% in animals injected with forskolin (open circles), 0.1 mg/kg, compared with control (Fig. 4A). Figure 4B
shows the average recovery time at different concentrations of forskolin normalized to the control value. Figure 4B, inset, shows the dose-response curve fit to the averaged recovery data showing that the midpoint of the response occurs at $\sim 0.021$ mg/kg forskolin.

**Theophylline Accelerates Recovery from Isoflurane Anesthesia**

Theophylline was tested to determine whether it too could alter recovery from anesthesia. Theophylline elevates intracellular cAMP by inhibiting phosphodiesterase, the enzyme that normally degrades cAMP. Thus theophylline elevates cAMP levels by a mechanism distinct from that of forskolin. Figure 5A shows a plot of recovery from anesthesia times from control rats (closed squares) or from animals injected with theophylline (open circle; 10 mg/kg). Theophylline was injected 30 min before discontinuing the isoflurane. On average recovery time was reduced by a significant $\sim 43\%$. Figure 5B shows a plot of the average normalized recovery time at different concentrations of theophylline. Figure 5B, inset, shows the dose-response curve fit to the data showing that the midpoint occurs at $\sim 0.28$ mg/kg theophylline.

**Caffeine Accelerates Recovery from Isoflurane Anesthesia**

Caffeine elevates intracellular cAMP by inhibiting phosphodiesterase. Caffeine is of particular practical interest as it is such a widely used and largely innocuous drug. Figure 6A shows a graph of recovery times from isoflurane anesthesia for control rats (closed squares) or for animals injected with caffeine (open circles; 25 mg/kg), 5 min before discontinuing the isoflurane. Caffeine dramatically sped recovery from anesthesia by $\sim 60\%$. Figure 6B shows the average recovery time at different concentrations of caffeine. Figure 6B, inset, shows a plot of the dose-response curve fit to the data showing that the midpoint occurs at $\sim 0.9$ mg/kg. Of note, an average person consumes $>0.9$ mg/kg of caffeine in a cup of coffee.

**Caffeine, Forskolin, and Theophylline Do not Alter Heart Rate, Blood Pressure, or Breathing Rate**

Figure 7 shows a plot of blood pressure, heart rate, and breathing rate immediately before or 10 min after injection of forskolin, theophylline, or caffeine, in isoflurane-anesthetized rats. No significant changes were observed as a result of forskolin (0.1 mg/kg) or theophylline (10 mg/kg) or caffeine administration (25 mg/kg). In all cases $O_2$ blood saturation was maintained between 99 and 100% (see MATERIALS AND METHODS).
primarily through enhancing activation of GABA<sub>A</sub> receptors in response to GABA (Concas et al. 1990; Solt and Forman 2007). We tested the ability of caffeine to accelerate recovery from propofol anesthesia to determine whether caffeine might accelerate recovery from anesthesia for different classes of anesthetics. Figure 8 shows a plot of recovery times from propofol anesthesia from control rats (closed square) or from rats injected with 25 mg/kg caffeine (open circle). Adult rats were placed in the anesthetizing chamber where they were exposed to 3% isoflurane for 8 min. Intravenous lines were inserted into a tail vein and the rats were allowed to wake. The rats then received a single bolus injection of propofol (4 mg/kg) and either saline (control) or caffeine in saline (25 mg/kg). All rats became unconscious within 5 s of propofol injection. The rats were placed on their backs on a table and

![Graph](http://jn.physiology.org/)

**Fig. 4.** Forskolin accelerated recovery from isoflurane anesthesia. A: adult rats were anesthetized with 3% isoflurane (3 l/min O<sub>2</sub>) for 8 min and were then exposed to 2% isoflurane (2 l/min O<sub>2</sub>) for 45 min. During the anesthesia an intravenous line was inserted into a tail vein, while anesthesia was maintained with a nose cone. Five minutes before discontinuing the anesthetic the animals received an intravenous injection of either saline (control, ■) or saline with 0.1 mg/kg forskolin (○). The anesthetic was then terminated and the animals were allowed to wake up. Every symbol represents a single trial. The same animals were used as controls and to test forskolin. B: average waking time normalized to the control value, set to 100%, at different forskolin concentrations.

* Waking times that were significantly different than control. (n = 14; control, n = 16; 0.02 mg/kg, n = 16; 0.1 mg/kg, n = 20; 0.5 mg/kg, n = 9; 1 mg/kg).

**Inset:** percent reduction in waking time as a function of the forskolin concentration. The data are fit with a standard dose-response function, with a midpoint at 0.021 mg/kg forskolin and a maximal reduction of waking time of ~41.2%.

![Graph](http://jn.physiology.org/)

**Fig. 5.** Theophylline accelerated recovery from isoflurane anesthesia. A: adult rats were anesthetized as described in Fig. 3. Thirty minutes before discontinuing the anesthetic the animals received an intravenous injection of either saline (control, ■) or saline with 10 mg/kg theophylline (○). The anesthetic was then terminated and the animals were allowed to recover. Each symbol represents a single trial of 1 rat. B: average waking time normalized to control value, set to 100%, at different theophylline concentrations. (n = 12; control, n = 21; 0.1 mg/kg, n = 11; 1 mg/kg, n = 15; 10 mg/kg, n = 12; 100 mg/kg).

**Inset:** percent reduction in waking time as a function of the theophylline concentration. The midpoint was ~0.28 mg/kg forskolin, max reduction in recovery time ~41%. *Waking times significantly different from control.
DISCUSSION

Anesthetics have been used successfully for over a century and a half, but until recently there have been no methods to accelerate emergence from anesthesia. In several recent studies, Solt and colleagues showed that the methylphenidate, used to treat attention deficit hyperactivity disorder and other disorders, could accelerate the emergence from isoflurane and propofol anesthesia (Chemali et al. 2012; Solt et al. 2011). Methylphenidate is known to inhibit the reuptake of dopamine and norepinephrine (Heal et al. 2009). Solt and colleagues proposed that inhibition of dopamine transport and the subsequent elevation of extracellular dopamine and activation of dopamine receptors were key to the ability of methylphenidate to accelerate the emergence from anesthesia (Chemali et al. 2012; Solt et al. 2011; Taylor et al. 2013). Methylphenidate also appears to reverse the inhibition of neurotransmitter release produced by isoflurane (Fig. 2), perhaps by elevating [cAMP], (Pascoli et al. 2005). This ability of methylphenidate to reverse the inhibition of neurotransmitter release may play a role in accelerating recovery from anesthesia.

In the past, other groups have attempted to accelerate recovery from anesthesia or to reverse anesthesia. Alkire et al. (2009) infused via a cannula, a K+ channel blocking antibody into rats; the antibody restored consciousness while animals were still receiving anesthesia. Unfortunately, about a third of animals treated in this manner exhibited seizures. Activation of histamine receptors also altered anesthetic action; injection of histaminergic agonists into nucleus basalis magnocellularis dramatically accelerated emergence from anesthesia (Luo and Leung 2009). Cholinergic activation has also emerged in the literature as a potential means of reversing anesthesia (Leung et al. 2011; Tai et al. 2013). Hudetz et al. (2003) infused either a cholinesterase inhibitor or a muscarinic agonist intracerebroventricularly to decrease anesthesia. In a different study, Alkier et al. (2007) microinjected nicotine, via a cannula, into the thalamus of rats and showed that these animals were aroused from anesthesia. Although compelling, these studies are of limited clinical utility as they involve injecting drugs directly into the brain. Several human trials have explored cholinergic activation as a means to reverse anesthesia. These studies have shown that physostigmine, a cholinesterase inhibitor, reversed postoperative somnolence (Hill et al. 1977) and that physostigmine could reverse propofol anesthesia (Meure et al. 2000). Unfortunately, physostigmine was less reliable when used with the popular volatile anesthetic sevoflurane (Plourde et al. 2003). Physostigmine usefulness would also be limited by significant peripheral autonomic side-effects.

Comparing the effectiveness of reversing anesthesia in these earlier studies to the data shown in this article is complicated since the methodologies are so diverse. For instance, Alkier et al. (2009) injected an antibody to the K1.2 channel into the thalamus of anesthetized rats. Only 17% of the rats woke during the injection. In that study the authors did not terminate anesthesia and determine whether animals emerged more rapidly from anesthesia. In a second study by Alkier et al. (2007) nicotine was injected into thalamus in an effort to reverse the effects of anesthesia. The therapeutic window was small. Too little nicotine had no effect and too much induced seizures. Even the optimal dose produced either no effect or seizures at higher rates than it did arousal. In that study the authors did not
terminate anesthesia and determine whether animals emerged more rapidly from anesthesia. In the study by Hudetz et al. (2003) arousal from anesthesia was never measured. In that study brain wave activity was monitored. Similarly, Hill et al. (1977) employed a cholinesterase inhibitor to alter postoperative somnolence, not emergence time. Our work is most comparable to the studies of methylphenidate applied intravenously (Chemali et al. 2012; Solt et al. 2011) and of histamine agonists directly infused into nucleus basalis (Luo and Leung 2009); both paradigms dramatically accelerated emergence from anesthesia. In the methylphenidate studies, the rats were more lightly anesthetized than in our study. That makes the comparison between our work and these prior studies somewhat difficult. On the other hand, we ourselves studied methylphenidate, using conditions identical to those illustrated in this manuscript for caffeine (data not shown). In our hands, methylphenidate dramatically accelerated emergence from anesthesia following termination of anesthesia, as previously reported (Chemali et al. 2012; Solt et al. 2011). Interestingly, methylphenidate produced a slightly smaller response than did caffeine, but this effect was not statistically significant nor were the studies carried out in the same cohort of animals. Additional studies carried in the same cohort of animals will be required to determine whether caffeine or methylphenidate is the more effective drug or whether they have equal efficacy. Interestingly, the study by Luo and Leung (2009) used a 60-min exposure to 2.1% isoflurane, conditions very similar to our own; in this study injection of histamine agonists accelerated emergence from isoflurane anesthesia by over 50%, a result quite similar to our own.

Fig. 7. There was no significant change in blood pressure, heart rate or respiratory rate in rats exposed to forskolin, theophylline, or caffeine. Rats were anesthetized as described in Fig. 3. A: forskolin (0.1 mg/kg) was injected into each animal. Just before forskolin injection blood pressure, heart rate and respiratory rate was measured. Ten minutes after forskolin, the parameters were remeasured. B: theophylline (10 mg/kg) was injected into each animal. Just before theophylline injection blood pressure, heart rate and respiratory rate were measured. Ten minutes after theophylline, the parameters were remeasured. C: caffeine (25 mg/kg) was injected into each animal. Just before caffeine injection blood pressure, heart rate and respiratory rate was measured, 10 min after caffeine, the parameters were remeasured. h, Heart rate; BR, breathing rate; pre, predrug application; post, 10 min after drug application.

Fig. 8. Caffeine accelerated recovery from propofol anesthesia. Adult rats were anesthetized with 3% isoflurane for 8 min at which time an intravenous line was inserted. The animals were allowed to wake and then a bolus of propofol (4 mg/kg) was injected along with either saline (control, ■) or with 25 mg/kg caffeine in saline (○). All rats became unconscious again within 5 s of propofol injection. The animals were allowed to wake. The same animals were used as controls and to test caffeine (n = 16 per group).

Forskolin signaling has also been shown to play an important role in forms of augmented synaptic plasticity, like long-term potentiation (Bliss and Collingridge 1993; Huang and Kandel 1994; Huang et al. 1994; Weisskopf et al. 1994). Furthermore, a C. elegans mutant that results in elevated levels of intracellular cAMP is resistant to isoflurane anesthesia (Saifee et al. 2011). Although it is tempting to assign a direct role for inhibition of neurotransmitter release in anesthesia, at present the link is purely circumstantial. In a similar manner, relief from anesthetic block of neurotransmitter by elevation of cAMP has not yet been causally linked to accelerated recovery from anesthesia. Additionally, elevated cAMP has important postsynaptic effects (Lee and Messing 2008); we do not yet know whether such effects play a role in accelerating emergence from anesthesia.

Forskolin is known to potently stimulate adenylyl cyclase (Laurenza et al. 1989; Simonds 1999), thereby elevating intracellular cAMP. Forskolin has been used since ancient times to treat heart disorders. More recently it has been used to treat congestive heart failure, asthma, and glaucoma. When tested in vitro, forskolin completely reversed isoflurane-mediated inhibition of the neurotransmitter release machinery. Forskolin dramatically accelerated recovery from anesthesia in rats.

Theophylline, a methylxanthine drug used in the treatment of asthma (Kips et al. 1999), infant apnea, and chronic obstructive airway disease (Jenne 1987), is structurally similar to caffeine. Theophylline relaxes bronchial smooth muscle and increases cardiac contractility. Theophylline inhibits phosphodiesterase, thereby elevating intracellular cAMP (Essayan 2001). Theophylline also blocks adenosine receptors (Ribeiro et al. 2002). In our studies theophylline completely reversed isoflurane-mediated inhibition of the neurotransmitter release machinery and it dramatically accelerated recovery from anesthesia.

The drug with the highest efficacy was caffeine. Caffeine is a stimulant drug that has a variety of effects, but the two most noteworthy are its ability to block adenosine receptors (Lazarus et al. 2011) and to elevate cytosolic cAMP levels (Rang et al. 2007) by inhibiting phosphodiesterase. Caffeine is the most commonly used psychoactive drug (Nehlig et al. 1992) and in the United States >90% of adults use it daily. Clinically, caffeine is primarily used to treat neonatal apnea and certain types of headache. Caffeine binds with similar affinity to A1 and A2A receptors and antagonizes both (Lazarus et al. 2011) at the concentrations shown in Fig. 5 (Chen et al. 2013).

Adenosine regulates sleep and waking (Huang et al. 2011; Lazarus et al. 2011). Caffeine, like theophylline, blocks adenosine receptors nonselectively, but it appears that A2A receptors mediate the arousal effects of caffeine since knocking out this receptor or blocking it pharmacologically suppresses caffeine-mediated arousal (El Yacoubi et al. 2000; Huang et al. 2005; Lazarus et al. 2011; Svenningsson et al. 1997). Although some of the accelerated recovery from anesthesia produced by caffeine may be mediated by adenosine receptors, the robust response observed to forskolin suggests that elevation of cAMP dramatically accelerates recovery from anesthesia. Nonetheless, future studies should explore the involvement of adenosine receptors in the ability of caffeine to accelerate recovery from anesthesia.

Optimal delivery times were not investigated in this study. Caffeine and forskolin were injected as a bolus 5 min before isoflurane administration was terminated. It may be that a different time, other than 5 min, would have produced a more robust result. At first theophylline was injected into rats 5 min before isoflurane anesthesia was terminated, but this combination produced a smaller effect than did injection of theophylline 30 min before discontinuing anesthesia. Our data suggest that theophylline may take somewhat longer to reach its target or that it may act more slowly or that both are true. We do not know whether 30 min provides the largest possible response to theophylline. Similarly, in the experiment where caffeine accelerated recovery from propofol anesthesia, both drugs were injected at the same time. Future studies will be required to determine whether this is the most efficacious timing possible and to determine optimal dosages, since only one concentration was tested.

Responses to forskolin and theophylline saturated at higher concentrations. However, the response to caffeine did not saturate. Larger concentrations, which may have produced even larger responses, were not tested to avoid potential side effects in the rats.

The data in Fig. 7 show no significant change in blood pressure or heart rate in rats injected with forskolin, theophylline or caffeine. Elevating intracellular cAMP can cause vasodilation of arteries (Rang et al. 2007), which should lower blood pressure, an undesirable side-effect for anesthesia. We were surprised when no significant change in blood pressure was observed after cAMP was elevated. Although fortuitous, this result is somewhat puzzling. It is possible that elevating cAMP levels also stimulated cardiac muscle, which increased contractile force thereby maintaining blood pressure. It may be that at the concentrations of drugs tested the two effects cancelled and there was no resulting change in vital signs. Alternatively, since vital signs were measured after anesthesia, blood vessels may have already been dilated. Similarly, emergence from anesthesia may also involve multiple targets, all sensitive to elevated cAMP. Both pre- and postsynaptic loci may be targeted.

It will be interesting to know whether caffeine accelerates recovery from anesthesia in human patients in a manner similar to that observed in rat. Because caffeine is so widely used in the general population and at doses similar to those outlined in this study and because the FDA categorizes caffeine as a “generally recognized as safe” drug, it might provide a relatively innocuous and inexpensive way to accelerate recovery from anesthesia in patients who are slow to “wake.” More important will be to

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identify drugs that reverse the cognitive problems associated with anesthesia. Even after emerging from anesthesia, patients can still exhibit cognitive impairment that lasts for hours. For instance 30 min after sevoflurane anesthesia patients provided 56% correct answers in a Digital Substitution Test (DSST); test subjects with a 0.1% blood alcohol level had a similar accuracy (Larsen et al. 2000). In a different study, 26% of patients were unable to complete a DSST, 2 h following termination of either sevoflurane or desflurane anesthesia, even though average eye-opening and extubation occurred within 10 min of discontinuing anesthesia (Nathanson et al. 1995). The cognitive abilities of elderly patients were impaired 3 h after anesthesia was terminated (Chen et al. 2001). Even subanesthetic doses of drugs like sevoflurane cause significant cognitive impairment (Galarkin et al. 1997; Janiszewski et al. 1999). Drugs that accelerate cognitive recovery in addition to accelerating “waking” times would be extremely useful as they would allow patients to be released more rapidly leading to better outcomes and lower costs. Future studies will be required to determine whether caffeine can reverse some of the cognitive deficits associated with anesthesia in addition to shortening waking times.

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