5. Walters RJ, Hadley SH, Morris KD, Amin J: Benzodiazepines act on GABAA receptors via two distinct and separable mechanisms. Nat Neurosci 2000; 3:1274–81
6. Chiara DC, Jayakar SS, Zhou X, Zhang X, Savechenkov PY, Bruzik KS, Miller KW, Cohen JB: Specificity of intersubunit general anesthetic-binding sites in the transmembrane domain of the human α1β3γ2 γ-aminobutyric acid type A ($GABA_A$) receptor. J Biol Chem 2013; 288:19343–57

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A Rising Tide Lifts All Boats: Increased Ventilation May Be Involved in Accelerated Recovery from Isoflurane Anesthesia after Flumazenil Administration

To the Editor:

Recently, Safavynia *et al.*[1] demonstrated that flumazenil administration upon cessation of isoflurane anesthesia hastens the reappearance of the wake-like electroencephalographic activity in rats that is suggestive of accelerated emergence. They also demonstrated that flumazenil can inhibit enhancement of isoflurane-induced γ-aminobutyric acid (GABA) receptor—mediated activity in preparations of cultured human cells.[1] Hence, the authors are inclined to think that the observed speedier recovery is a direct consequence of flumazenil antagonism of GABA receptors in the brain that results in faster restoration of cortical activity. They exclude the possibility of improved lung ventilation after flumazenil administration as a potential cause for accelerated emergence based solely on similar respiratory rates observed in their pilot studies in control and flumazenil-treated groups, which, in our view, is not sufficient. By examining only a respiratory rate, the authors leave out another critical determinant of minute ventilation, tidal volume, which can be modulated positively by flumazenil.

In this regard, enhancement of function of GABA receptor type A ($GABA_A$) in brainstem respiratory neurons has a primary role in the respiratory depression caused by all volatile and most intravenous anesthetics.[2] Under general anesthesia or non–rapid eye movement sleep, the excitatory input from the retrotrapezoid nucleus to the respiratory centers of the pontomedullary region is critical for breathing automaticity.[3] Retrotrapezoid nucleus inactivation under anesthesia inhibits breathing markedly primarily *via* a decrease in tidal volume. In rats, local microdialytic application of the $GABA_A$ receptor agonist muscimol decreases tidal volume and minute ventilation significantly,[4] whereas application of the $GABA_A$ receptor antagonist bicuculline increases them.[5] In cats, potentiation of $GABA_A$ receptors at the ventral surface of the medulla reduces tidal volume and arterial pressure, and these cardiorespiratory-depressant effects can be counteracted by flumazenil or bicuculline.[6] In humans, flumazenil reverses the reduction in tidal volume and minute ventilation caused by midazolam effectively.[7–9] The fact that Safavynia *et al.*[1] have shown that flumazenil can counteract isoflurane-mediated enhancement of function of $GABA_A$ receptors makes it very likely that its administration at cessation of isoflurane delivery could have resulted in improved ventilation and faster elimination of isoflurane through the lungs, thereby hastening postanesthetic recovery. Therefore, to exclude the possibility of improved ventilation as a reason for faster emergence after flumazenil administration, a more detailed look into cardiorespiratory parameters accompanied by blood gases analyses is required.

Additionally (and perhaps more importantly), to demonstrate unequivocally the ability of any candidate drug (including flumazenil) to reverse anesthesia, it should be administered during continuous administration of an anesthetic (and not at cessation of delivery of anesthetic gas as undertaken by the authors), and its ability to restore the righting reflex under these conditions must be demonstrated clearly. Such a protocol was introduced originally (and is used actively) by Chemali *et al.*,[10] Solt *et al.*,[11] and Taylor *et al.*[12] Without demonstrating the ability of flumazenil to restore the righting reflex during maintenance of the minimal concentration of isoflurane required for loss of consciousness, flumazenil cannot be called an "isoflurane-reversal agent" in the strict sense of the phrase. Simultaneously, demonstration of reappearance of the wake-like electroencephalographic activity after flumazenil administration during continuous administration of isoflurane would provide supportive (though not essential) evidence indicating the ability of flumazenil to reverse isoflurane anesthesia, and thus could be omitted if positioning of the electroencephalographic recording cable presented a problem for the authors.[1]

The ability of flumazenil to reverse isoflurane anesthesia must be demonstrated, and the exact mechanism underlying a wake-promoting effect of flumazenil may prove to be different from that suggested by Safavynia *et al.*[1] Nevertheless, these factors in no way depreciate the practical importance of their findings, suggesting that flumazenil may become a valuable addition to the anesthesiologist's armamentarium to facilitate postanesthetic recovery.

Competing Interests

The authors declare no competing interests.

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References

1. Safavynia SA, Keating G, Speigel I, Fidler JA, Kreuzer M, Rye DB, Jenkins A, García PS: Effects of γ-aminobutyric acid type A receptor modulation by flumazenil on emergence from general anesthesia. Anesthesiology 2016; 125:147–58

2. Stuth EA, Stucke AG, Brandes IF, Zuperku EJ: Anesthetic effects on synaptic transmission and gain control in respiratory control. Respir Physiol Neurobiol 2008; 164:151–9
3. Guyenet PG: The 2008 Carl Ludwig Lecture: Retrotrapezoid nucleus, CO_2 homeostasis, and breathing automaticity. J Appl Physiol (1985) 2008; 105:404–16
4. Nattie E, Li A: Muscimol dialysis in the retrotrapezoid nucleus region inhibits breathing in the awake rat. J Appl Physiol (1985) 2000; 89:153–62
5. Nattie E, Shi J, Li A: Bicuculline dialysis in the retrotrapezoid nucleus (RTN) region stimulates breathing in the awake rat. Respir Physiol 2001; 124:179–93
6. Gillis RA, Namath IJ, Easington C, Abrahams TP, Guidotti A, Quest JA, Hamosh P, Taveira da Silva AM: Drug interaction with gamma-aminobutyric acid/benzodiazepine receptors at the ventral surface of the medulla results in pronounced changes in cardiorespiratory activity. J Pharmacol Exp Ther 1989; 248:863–70
7. Flögel CM, Ward DS, Wada DR, Ritter JW: The effects of large-dose flumazenil on midazolam-induced ventilatory depression. Anesth Analg 1993; 77:1207–14
8. Gross JB, Weller RS, Conard P: Flumazenil antagonism of midazolam-induced ventilatory depression. Anesthesiology 1991; 75:179–85
9. Mora CT, Torjman M, White PF: Sedative and ventilatory effects of midazolam infusion: Effect of flumazenil reversal. Can J Anaesth 1995; 42:677–84
10. Chemali JJ, Van Dort CJ, Brown EN, Solt K: Active emergence from propofol general anesthesia is induced by methylphenidate. Anesthesiology 2012; 116:998–1005
11. Solt K, Cotten JF, Cimenser A, Wong KF, Chemali JJ, Brown EN: Methylphenidate actively induces emergence from general anesthesia. Anesthesiology 2011; 115:791–803
12. Taylor NE, Chemali JJ, Brown EN, Solt K: Activation of D1 dopamine receptors induces emergence from isoflurane general anesthesia. Anesthesiology 2013; 118:30–9

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In Reply:

We thank Dr. Raines for his interest in our work and thoughtful comments on the molecular pharmacology underlying our observed effects.

Our study[1] was primarily motivated by commonly encountered clinical scenarios, and our conclusions focused on network and *in vivo* actions of the combination of flumazenil in the setting of decreasing isoflurane concentration. While we are confident in concluding that flumazenil modulates emergence in rodents, there are insufficient data to completely describe the range of pharmacologic interactions between flumazenil and γ-aminobutyric acid (GABA) type A receptors ($GABA_A$Rs), including site-specific interactions. Our demonstration of "antagonistic activity" by flumazenil on $GABA_A$Rs in heterologous expression systems in the presence and absence of coapplied isoflurane mainly served to emphasize a GABA-mediated effect of flumazenil and isoflurane.

Based on our previous work[2,3] and that of several other successful laboratories[4–6] focused on GABA pharmacology, we are forced to constantly reevaluate the biophysical relationships of these compounds with the $GABA_A$R. We still struggle with a comprehensive understanding of moiety-specific interactions with regard to the effects of flumazenil on binding, gating, desensitization, and/or membrane regulation of the $GABA_A$R.

Our manuscript demonstrates that flumazenil robustly inhibits the enhancement of GABA-mediated current by isoflurane. However, in isolation, our work is insufficient to ascribe a precise mechanism of action, and we accept Dr. Raines's assertion that the antagonism may not be competitive. By the same token, however, from the dataset provided, it is not possible to confidently say that flumazenil is a noncompetitive antagonist. Nor is it possible to say at which site(s) flumazenil is acting. The intrinsic efficacy we demonstrate suggests that it is also a partial agonist. So, without a battery of new pharmacology experiments, we are in agreement that (1) the sites and mechanisms of flumazenil action are far from well understood and (2) the status of flumazenil as a simple benzodiazepine competitive antagonist should be called into question.

During the construction of the published version of this manuscript, the electroencephalogram and behavioral results were emphasized, and some of the pharmacologic descriptions were eliminated or simplified. Early versions of the manuscript described flumazenil as a "negative allosteric modulator of the $GABA_A$R at site(s) unknown." However, this wording is vague and imprecise. We decided it would be most appropriate to use similar wording to that in our human studies in this area.[3] We look forward to following the work of others in this area to improve the collective knowledge of $GABA_A$R pharmacology as applied to general anesthesia.

We also thank Drs. Petrenko and Baba for their interest in our work from a respiratory physiology perspective. In their letter, they raise the possibility that the influence of flumazenil on emergence from isoflurane anesthesia observed in our rodent model may not be entirely mediated by the neurophysiologic changes observed in cortical neurons but could be, in part, influenced by an effect of flumazenil on brainstem nuclei controlling respiration.

As evidence for influence of this alternative mechanism, they provide references to work in the *awake* rat that demonstrates respiratory changes when traditional GABA agonists[7] and antagonists[8] are applied by microdialysis to the retrotrapezoid nucleus in the ventral medulla. As discussed above, flumazenil does not affect the $GABA_A$R like the traditional agonists/antagonists, *i.e.*, muscimol and bicuculline. We are aware that GABA, in combination with adenosine, glutamate, and other neurotransmitters, is involved in regulating breathing in the retrotrapezoid nucleus, as well as other structures in the ventral respiratory group, and that a complex interplay exists between the ventral respiratory group and higher order structures (*e.g.*, pons, hypothalamus, and cortex).[9] For this reason, we carefully considered an influence of respiration on our results. Before initiation of our study, we performed a small (n = 6) pilot study on rats under near-identical conditions, and blood-gas measurements from these animals revealed