extensively studied. To claim that the effects reported in the current study occur at a "clinically relevant concentration" misrepresents this body of research and can easily lead to erroneous conclusions. We hope that this letter can serve as a reminder of the fundamental importance of knowing where the field has been to avoid the pitfalls of the past.

Competing Interests

Dr. Hemmings serves as an editor of Anesthesiology and the *British Journal of Anaesthesia*, he has received research funding from the National Institutes of Health (Bethesda, Maryland) and TEM International (München, Germany), and he has served as a consultant for Elsevier (Amsterdam, The Netherlands). Dr. Goldstein receives funding from the U.S. Department of Defense (Washington, DC) and Mallinckrodt Pharmaceuticals (Dublin, Ireland). Dr. Riegelhaupt declares no competing interests.

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(Accepted for publication June 23, 2016.)

In Reply:

We thank Dr. Riegelhaupt and colleagues for their thoughtful comments on our article.[1] We stated that our concentrations (10 to 100 μM) of propofol are "clinically relevant," because the total concentration of propofol in the blood of anesthetized humans is typically in the range of approximately 10 to 40 μM. Dr. Riegelhaupt and colleagues correctly point out that "free" propofol concentration in blood of anesthetized humans is estimated to be lower (approximately 0.4 μM),[2] because more than 97% of total propofol is bound to blood constituents, such as albumin, and thus is pharmacologically sequestered.[3] They expressed concerns that our statement of clinical relevance is misleading.

There has been a long-standing debate about the concentrations necessary to cause effects *in vitro* and lower concentrations of free propofol in the blood of anesthetized humans. For instance, the best-studied effects of propofol are its actions on γ-aminobutyric acid receptor type A ($GABA_A$) receptors. Here, the concentration of propofol to potentiate GABA-induced chloride currents of freshly dissociated neurons is in the range of 1 to 100 μM.[4–6] This is significantly higher than the concentration of estimated "free" propofol. Therefore, it had been argued that the effect of propofol on $GABA_A$ receptors of neurons may not be clinically relevant.[7] However, this concern was put to rest by the finding that a point mutation in the β_3 subunit of the receptor in a knock-in mouse was sufficient to abolish the actions of propofol in preventing a response to a painful stimulus *in vivo*.[8] It is now almost unequivocally believed that a major target for propofol is the $GABA_A$ receptor, and using 1 to 100 μM of propofol *in vitro* has been validated.

Similarly, the critical mechanisms that explain propofol-induced hypotension have been investigated using high concentrations of propofol applied to *in vitro* preparations of cardiac cells or arterial rings. These studies have led to important insights into the propofol actions, which include direct myocardial depression by inhibition of Ca^{2+}-induced excitation–contraction coupling, reduced Ca^{2+} influx,[9,10] and acetylcholine-induced pulmonary vasodilation.[11] It is probably unproductive to dismiss these results as clinically irrelevant simply because the nominal propofol concentration used *in vitro* was higher than the estimated free propofol concentration in patients.

Why is the nominal concentration of propofol used *in vitro* higher than the estimated "free" propofol in the blood? First, we would like to point out that even in the case of *in vitro* studies, the nominal concentration is higher than the concentration available for biologic action. Drugs bind nonspecifically biologic preparations and to surfaces of the dish in tissue cultures, or to superfusion lines, filter material, and chambers in synaptosomal experiments, reducing free drug concentrations. Furthermore, propofol is an oil and is insoluble in physiologic buffers. A liquid emulsion formulation is used clinically, or an aqueous formulation, fospropofol, which itself is inactive. In our experiments, we made propofol stocks in dimethyl sulfoxide (DMSO) and limited the final concentration of DMSO to 0.1% to avoid potential side effects of DMSO. In our synaptosome experiments, we applied DMSO-based stock solution in small increments to agitated physiologic buffer to make final solutions of propofol. This prevented precipitation of propofol. Occasionally we observed some precipitation once we stopped agitation of the

buffer with oxygen/carbon dioxide at the end of the experiment. These factors and observations suggest that the effective *in vitro* concentrations may be lower than the calculated/nominal concentration in our experiments, as is the case, for partially overlapping reasons, in clinical applications.

In general, it is extremely difficult to accurately reproduce the clinical setting by *in vitro* experiments. For instance, the binding affinity and kinetics between propofol and albumin *versus* propofol and $GABA_A$ receptors or other ion channels remain unknown. As such, the direct comparison of free concentration of propofol in the blood and the nominal concentration of propofol used *in vitro* may be impossible. It would be important to test how or whether our findings are affected by increasing concentrations of albumin, which sequester propofol. Such an attempt has been made just recently.[12]

In summary, we agree that the concentration we used is significantly higher than the concentration of "free" propofol estimated to be present in anesthetized humans. At the same time, "free" concentration of propofol is likely to be lower than the nominal concentration, even in our experiments. More pertinent for potential clinical relevance is that the concentrations we used are in line with numerous previous *in vitro* studies, which led to the discovery of the clinically relevant, important actions of propofol, including its actions on $GABA_A$ receptors.

Competing Interests

The authors declare no competing interests.

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(Accepted for publication June 23, 2016.)