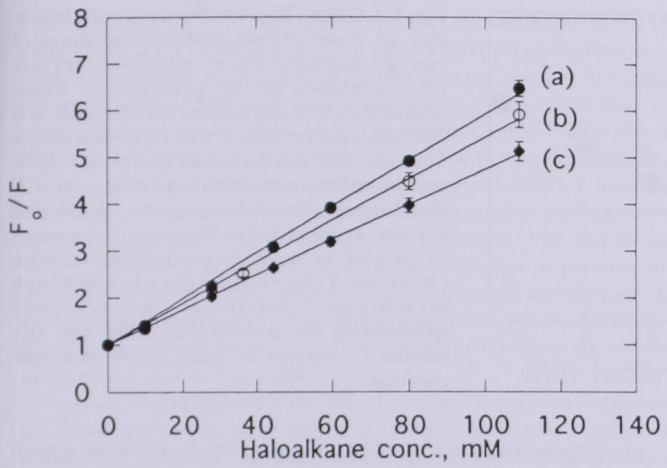

Fig. 1. Stern–Volmer plots for indole (10 μM) fluorescence quenching in methanol by (*A*) 1,2-dichlorohexafluorocyclobutane, (*B*) halothane, and (*C*) 2,3-dichlorooctafluorobutane. Excitation at 295 nm with emission peaks at 331 nm. Data plotted as $F_o/F = 1 + K_{sv} \cdot$ [Haloalkane], where F_o and F are the fluorescence intensities in the absence and presence of added haloalkane, respectively, and K_{sv} is the Stern–Volmer quenching constant. Data points are the means of three to seven experiments, with error bars representing SEM.

cence by the nonanesthetics leads to decreased energy transfer to the fluorescent acetylcholine analog, which consequently emits less of a signal. Fluorescence energy transfer[7] from tryptophan to a dansyl group is estimated to occur efficiently over distances of up to 40–50 Å (for comparison, the width of the phospholipid bilayer is 30–40 Å), suggesting that multiple tryptophan residues in the nAChR are responsible for exciting the fluorescent acetylcholine analog. The observation that the fluorescence intensity of the added acetylcholine analog increases at higher concentrations of nonanesthetic might then reflect the balance between fluorescence quenching of selected tryptophan residues and nonanesthetic-induced conformational changes in the nAChR associated with desensitization. In support of this interpretation is the finding that the relative inability of the two nonanesthetics to promote nAChR desensitization (as shown in figs. 5 and 6 of reference 1) correlates with their fluorescence quenching efficiency, and not with their potency as predicted by oil–gas solubility.[2] It is open to question whether the approach used in this study, which relies on changes in the fluorescence intensity of an added ligand, that is in turn excited by energy transfer from multiple protein tryptophan residues, rules out an effect of nonanesthetics on nAChR desensitization kinetics.

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In Reply:—The premise of Johansson's alternative interpretation is that nonanesthetics decrease energy transfer and reduce the fluorescence emission of the acetylcholine analog, Dns-C_6-Chol. However, this premise is not supported by experimental data; titrations using increasingly higher nonanesthetic concentrations under conditions of energy transfer from nAcChoRs revealed no reduction in Dns-C_6-chol fluorescence emission at equilibrium (fig. 1A). Therefore, the nonanesthetics 1,2-dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane do not significantly disrupt energy transfer from nAcChoR tryptophan residues to Dns-C_6-Chol.

Johansson assumes that indole in methanol is an adequate experimental model for tryptophan residues in intrinsic membrane proteins, and he bases his premise on the observation that anesthetic and nonanesthetic compounds quench essentially all of the fluorescence in this model system. However, a Stern-Volmer plot of halothane quenching of nAcChoR-rich membrane fluorescence is quite different from that of halothane quenching of indole fluorescence (fig. 1B). It is nonlinear, with curvature toward the concentration axis, indicating that a significant fraction of protein tryptophans are either poorly quenched or not quenched at all.[1–3]

Finally, none of the kinetic parameters reported in my study are even derived from absolute amplitudes. They are derived from amplitudes that are normalized to their final intensities (at equilibrium) or from observed rates.[4,5] These values are affected little, if at all, by quenching. However, because quenching could have reduced the signal:noise ratio and made the analysis more difficult, reason dictated

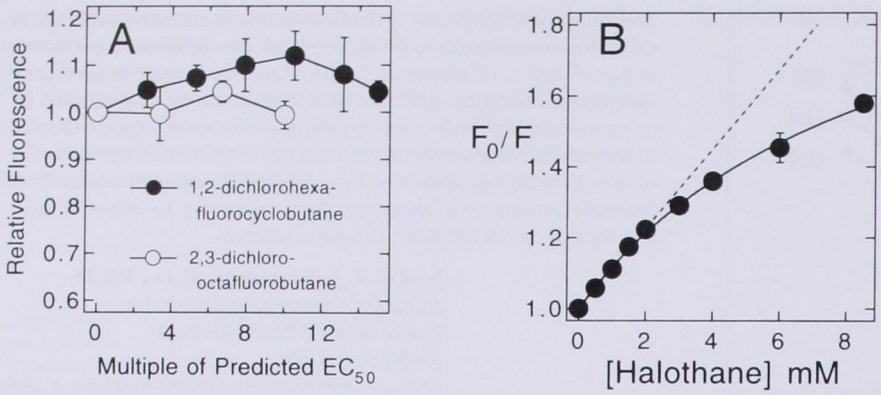

Fig. 1. (*A*) Equilibrium fluorescence intensity of Dns-C_6-Chol under conditions of energy transfer from nAcChoR tryptophan residues in the presence of nonanesthetics. The excitation wavelength was 290 nm, and emission was recorded above 560 nm. The predicted EC_{50}s for 1,2-dichlorohexafluorocyclobutane and and 2,3-dichlorooctafluorobutane are 16 μM and 4.5 μM, respectively. Data are the means (± SD) of at least three determinations. (*B*) Stern–Volmer plots for nAcChoR-rich membrane intrinsic fluorescence quenching by halothane. Data are the means (± SD) of at least three determinations.

avoiding compounds that seemed to offer the greatest probability of quenching.

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Enoxaparin and Epidural Analgesia

To the Editor:—Enoxaparin sodium (Lovenox), a low molecular weight heparin (LMWH) manufactured by Rhone-Poulenc Rorer Pharmaceuticals, has a new warning included in its recently changed package insert. This change can have significant implications for anesthesiologists. It states "Lovenox injection, like other anticoagulants, should be used with extreme caution in conditions with increased risk of hemorrhage, such as[1] . . . in patients with indwelling intrathecal or epidural catheters, . . ." Because of this warning, some orthopedic surgeons at our institution have decided against the use of an epidural catheter for postoperative pain management in patients receiving Lovenox for deep venous thrombosis (DVT) prophylaxis after total hip and knee replacement surgery.

Lovenox was released for use in Europe in 1987 and in the United States in 1993. Bergqvist *et al.*[1] reported that of more than one million patients receiving LMWH, there was one case of a spinal hematoma. In other controlled studies of at least 10,000 patients who received epidural/spinal anesthesia or analgesia during LMWH prophylaxis, this combination of treatments did not produce any catheter-related complication.[1,2] A similar safety record was observed in three other studies of 1,792 patients.[2–4] In contrast, during post-market clinical use in the United States since 1993, Rhone-Poulenc Rorer Pharmaceuticals reports that there have been seven cases of epidural hematoma formation after epidural anesthesia/analgesia in association with Lovenox use. All but two cases were associated with an epidural catheter that was inserted or removed, either before the patient was started on Lovenox. Because of this new development, the drug manufacturer recently changed the package insert to reflect the perceived increased risk of epidural hematoma formation in patients with an epidural catheter during Lovenox prophylaxis.

Epidural hematoma formation in patients receiving anticoagulation therapy is a known but rare complication irrespective of central neuraxial blockade. Is the risk of spontaneous or catheter-related spinal