study is at odds with his own findings.[2] We believe that findings from both studies can be easily reconciled by taking into account some overlapping findings as well as a few obvious methodologic differences between these studies.[1,2]

In accordance with our data, Duvaldestin *et al.* did not observe flow limitation during normal breathing. However, our data show that integrity was impaired during airway challenges.

Duvaldestin and coworkers[2] correctly point out that, in their study, volunteers were breathing *via* a mouthpiece whereas we used a nasal mask during our experiments.[1] Indeed, this is an important methodologic difference. In fact, our approach allows for analysis of the pressure–flow relationship of the whole supraglottic airway, whereas the method of Dr. Duvaldestin is restricted to the oropharyngeal airway only. However, it is clinically important to evaluate both, the retropharyngeal and retroglossal upper airway.

We have shown that the effects of partial neuromuscular blockade on the upper airway muscles are significantly greater in the retropalatal compared with the retroglossal airway.[3] In accordance, Schwab and coworkers[4] showed that the soft palate plays the predominant role in mediating airway narrowing during sleep, and this is thought to be related to a decrease in upper airway dilator muscle activity. Thus, the retropalatal area seems to be particularly susceptible to a decrease in upper airway dilator tone. Accordingly, the technique used by Dr. Duvaldestin and coworkers is not sensitive to detect upper airway collapse in its most collapsible segment.

Although in Dr. Duvaldestin's opinion this circumstance is the main difference between the two studies, we believe that further differences in methodology exist with far greater impact on the results.

First, Dr. Duvaldestin and coworkers studied six volunteers, and there is no information provided how the number of volunteers was determined. Our study was performed following a power analysis based on pilot experiments and we examined 15 volunteers. Thus, one might speculate that Dr. Duvaldestin's study lacked the power to demonstrate significant results—absence of significance does not reflect significance of absence.

Second, Dr. Duvaldestin and coworkers conducted a negative pressure challenge using a stepwise decrease in airway pressure from ambient pressure to -40 cm H_2O with a decrease in airway pressure by 5 cm H_2O implemented every three respiratory cycles. This technique is assumed to assess active dynamic responses to airway obstruction, and the critical airway pressure obtained is thus the so-called active Pcrit.[5] Depending on the volunteers' respiratory rates, the time between the onset and the nadir of the negative pressure challenge with this technique varies and occurs over time. Most likely, this results in differences in compensatory mechanisms such as airway muscle activation or changes in respiratory drive. In our study, in contrast, volunteers were exposed to short random pressure drops alternating with longer periods of breathing at a (slightly positive) holding pressure. This latter technique is suitable to assess the passive mechanical properties of the upper airway and has thus been coined the passive Pcrit.[5] This variable reflects the mechanical integrity of the upper airway and, potentially, the patient's ability to compensate for challenges such as a forced inspiration.

Although not addressed in any of the publications, upper airway muscles are likely more susceptible to neuromuscular blocking agents than the diaphragm. Whether this is due to particular resistance of the diaphragm to such drugs or to particular susceptibility of the upper airway muscles has not been elaborated.

Accordingly, although we agree with Dr. Duvaldestin that further work on the susceptibility of the airway muscles is warranted, this issue does not alter our findings or dilute their significance.

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Inhibition of Muscle Acetylcholine Receptors by Nondepolarizing Drugs: Humans Are Not Unique

To the Editor:

We have several concerns about the data and the conclusions of the article by Fagerlund *et al.*[1] that reported on block of adult human muscle acetylcholine receptors (nAChR) by nondepolarizing neuromuscular blockers (NDMBs). Overall, the study by Fagerlund *et al.*[1] confirms that nondepolarizing neuromuscular blocking drugs have both competitive and noncompetitive blocking actions at neuromuscular nicotinic receptors. However, the study does not have the resolution to define the time or receptor state dependence of the block and, hence, provides no insights into the relative roles of the mechanisms in the clinically relevant actions of

NDMBs. Accordingly, it is not possible to conclude that NDMBs have a qualitatively different mode of action for blocking the human neuromuscular junction. Indeed, the current results have a great similarity to the results obtained with similar techniques on receptors derived from other species. Given the wealth of information on receptors from other species and the strong support for the idea that the competitive mechanism underlies functional block for these species, it is still most likely that these relaxants are functionally competitive blockers in humans.

Our major concern is that their experimental protocol does not produce a known set of receptor states; moreover, these states change with time during an application. Several investigators[2,3] have noted that whole frog oocytes cannot be superfused quickly enough with agonist to make reliable determinations of receptor kinetics. As a result, the acetylcholine (ACh)-activated current at any time point represents a combination of open, closed ($\geq$3), and desensitized ($\geq$2) receptor states. Experiments performed in the presence of antagonists produce at least two additional states. Unless measurements are made after the system has equilibrated among the states or at times when the states are clearly defined, the results cannot be interpreted. The traces shown by Fagerlund *et al.*[1] are complex (*e.g.*, in fig. 1, there are multiple phases to the responses, whereas in fig. 5, the time course is slow) but suggest that the time course for solution exchange is relatively slow (seconds) and that the exchange may not be uniform over the entire oocyte surface.

A consequence of slow solution exchange is that receptor desensitization complicates the interpretation of the measured currents. Desensitization of muscle nAChR proceeds mainly from the open state,[4] and therefore, both the rate and the extent of desensitization are enhanced at higher ACh concentrations. In human adult ACh receptor, 10 μM ACh activates approximately 60% of the receptors, and fast desensitization proceeds with a time constant of approximately 100 ms, and results in 90% desensitization (Mandy Liu, Ph.D., unpublished data, November 2005).

A final kinetic factor that can affect measurements with relatively undefined concentration change time courses is the establishment of the competitive equilibrium between agonist and antagonist at the agonist-binding site. Depending on concentration and protocol (*e.g.*, preapplication *vs.* coapplication), it can take some time for equilibrium competition to be established. As exemplified by controlled perfusion protocols, the resultant time course can be biphasic.[5,6]

The idea that NDMBs exhibit noncompetitive actions is not new. The classic experiments by Colquhoun *et al.*[7] definitively demonstrated that D-tubocurarine has both competitive and noncompetitive inhibitory actions on frog muscle receptors. Furthermore, they reported that the noncompetitive mechanism was more apparent at higher agonist concentrations (*i.e.*, at higher levels of channel activation). From their careful analysis of the concentration and voltage dependence of the inhibition, they concluded that the noncompetitive mechanism reflected open-channel block. The open-channel block actually had a higher affinity than the competitive block; however (as they point out), the open-channel block is not of major functional or clinical importance. The reason for this is that the channel must be open to be blocked, whereas during normal physiologic function, the channels are open very briefly and significant block does not develop. Accordingly, the competitive block, which is established for resting receptors, provides the clinically relevant muscle relaxation. Further studies[8,9] reported similar (but less comprehensive) observations at mammalian nAChR. At normal membrane potentials, channel block by curare develops at a rate of about $10^7\ M^{-1} \cdot s^{-1}$, for a channel when it is open, and therefore, at the higher concentrations of agonist and NDMB, the block will be significant during the initial seconds of the slow applications used by Fagerlund *et al.*[1] Hence, an agonist concentration activating more than half the receptors (*e.g.*, 10 μM ACh) would be expected to show a significant contribution from open-channel block, as is observed.[1]

Fagerlund *et al.*[1] suggest that noncompetitive block is related to receptor desensitization. Possible interactions between desensitization and block do not seem to be required to explain previously published observations. In addition, other studies of the interaction between D-tubocurarine and the mouse fetal muscle nicotinic receptor have not found any indication that D-tubocurarine desensitizes nAChR.[10]

One way to determine the half maximal inhibitory concentration (IC_{50}) values of competitive antagonists is to perfuse outside-out patches rapidly with saturating concentrations of ACh and to assess the number of activatable channels before significant desensitization, channel block, or dissociation of antagonist.[5,6,11–14] This method avoids the complications of multiple receptor states: at the time of the peak current, nearly all the receptors are either in the open state or in one of the antagonist-bound (nonconducting) states. An alternative approach is to activate channels with low concentrations of agonist; this reduces the effects of both desensitization and channel block.[15] Neither method, however, directly addresses the question of whether inhibition is competitive with agonist. That question is addressed with α-bungarotoxin binding experiments. Importantly, the antagonist affinity derived from the binding experiments are fully able to predict functional block of responses[15] and are in agreement with more quantitative studies of functional block.[5,6,11–14] In other words, the functional consequences of NDMB agents can be quantitatively explained by competitive inhibition of ACh binding.

In summary, the study by Fagerlund *et al.*[1] confirms that nondepolarizing neuromuscular blocking drugs have both competitive and noncompetitive blocking actions at neuromuscular nicotinic receptors. However, the experimental protocols do not have sufficient definition to allow quantitative analysis.

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In Reply:

We appreciate the interest of Drs. Dilger and Steinbach in our work and are grateful for their comments on our recent publication.[1] To properly study the molecular mechanism behind nondepolarizing neuromuscular blocking agents' (NMBAs) inhibition of the nicotinic acetylcholine receptor (nAChR)-mediated signaling in the human neuromuscular junction, an isolated preparation of the junction is needed. For obvious reasons, such approach is not possible and in lieu of that various techniques are used, ranging from acute neuronal preparations to various heterogeneous cellular expression systems. All these methods have different shortcomings in terms of relevance. The neuronal preparations often have insufficient washing to do proper *in vitro* pharmacology, and the heterogeneous systems often overexpress the receptors studied. Yet, what we can do is comparative *in vitro* pharmacologic studies using similar methods to compare potency and efficacy *in vitro* using receptors from relevant species. The *Xenopus* oocyte two-electrode voltage clamp system is very well suited for such studies. It is a widely used and well-established technique yielding stable and comparable *in vitro* pharmacologic data from numerous laboratories and has been doing so for 3 decades.

We agree with Drs. Dilger and Steinbach that whole cell two-electrode voltage clamp recordings from *Xenopus* oocytes are not a system well suited for detailed kinetic studies of receptor and ligand interaction. However, we never claim this in our article.[1] What we describe is the whole cell functional pharmacology of a range of nondepolarizing NMBAs studied on the human muscle nAChR activated by acetylcholine and dimethylphenylpiperazinium. We show that acetylcholine desensitizes the receptor, whereas dimethylphenylpiperazinium does not: when using larger concentrations of ACh (10 *vs.* 1 μM), we increase receptor desensitization determined by a decrease in current activated by repeated applications of acetylcholine[1] (figs. 2C and D). At the higher concentration of agonist, a classic competitive antagonist will be less efficacious and we do not observe this. In fact, we generally observe an increased efficacy of inhibition by the nondepolarizing NMBAs[1] (table 2). A careful analysis of the inhibition curves[1] (figs. 2 and 3) shows that nondepolarizing NMBAs induce both right-shifted curves and a depression of maximum currents that are the hallmarks of competitive and noncompetitive inhibition, respectively. The noncompetitive mode of action is primarily observed at higher concentrations of acetylcholine, which also induces receptor desensitization. Further, when using the nondesensitizing antagonist dimethylphenylpiperazinium, the inhibition becomes more competitive[1] (fig. 5 and table 5).

In this context, we have to remember that the resolution of the nAChR family X-ray and electron microscopy structures are insufficient to determine the exact molecular interactions, neither with nondepolarizing NMBAs nor with acetylcholine itself.[2] The structure of an acetylcholine-binding protein is known,[3] but nondepolarizing NMBA binding to this crystal is not studied. Thus, we have to acknowledge the fact that it is still only a theoretical model framework describing receptor function by multiple open, closed, and desensitized states. The published structures of the nAChR do not have the resolution to dissect between multiple open, closed, or desensitized states and of course not the transition between these. To resolve the functional interaction and kinetics between the nAChRs and ligands, we agree with Drs. Dilger and Steinbach that one must use either binding studies or outside out isolated patch recordings. However, the former is unable to resolve the functional effect of the interaction (agonism or antagonism) and the latter suffers from