Anesthesiology 2006; 104:724–33

Activation and Inhibition of Human Muscular and Neuronal Nicotinic Acetylcholine Receptors by Succinylcholine

Malin Jonsson, M.D., Michael Dabrowski, Ph.D.,† David A. Gurley, M.S.,‡ Olof Larsson, Ph.D.,§ Edwin C. Johnson, Ph.D.,‡ Bertil B. Fredholm, M.D., Ph.D.,‖ Lars I. Eriksson, M.D., Ph.D.#*

Background: **Succinylcholine is one of the most widely used muscle relaxants in clinical anesthesia and emergency medicine. Although the clinical advantages and cardiovascular side effects are well known, its mechanism of action within the human nicotinic cholinergic receptor system remains to be understood. The aim of this study was to investigate the effect of succinylcholine on human muscle and neuronal nicotinic acetylcholine receptor (nAChR) subtypes.**

Methods: ***Xenopus laevis*** **oocytes were injected with human messenger RNA for muscle and neuronal nAChR subunits. Receptor activation, desensitization, and inhibition induced by the natural ligand acetylcholine or by succinylcholine was studied using a multichannel two-electrode voltage clamp setup. Responses were measured as peak current and net charge.**

Results: **Succinylcholine concentration-dependently activated the muscle-type nAChR with an EC_{50} value of 10.8 μM (95% confidence interval, 9.8–11.9 μM), and after the initial activation, succinylcholine desensitized the muscle-type nAChR. Succinylcholine did not activate the neuronal nAChR subtypes $\alpha3\beta2$, $\alpha3\beta4$, $\alpha4\beta2$, or $\alpha7$ at concentrations up to 1 mM and was a poor inhibitor at these receptor subtypes, with IC_{50} values above 100 μM.**

Conclusion: **Succinylcholine activates the muscle-type nAChR followed by desensitization. The observation that succinylcholine does not inhibit the presynaptic $\alpha3\beta2$ autoreceptor at clinically relevant concentrations provides a possible mechanistic explanation for the typical lack of tetanic fade in succinylcholine-induced neuromuscular blockade. Finally, cardiovascular side effects (*e.g.*, tachyarrhythmias) of succinylcholine are not mediated *via* direct activation of the autonomic ganglionic $\alpha3\beta4$ subtype because succinylcholine does not activate the neuronal nAChRs.**

This article is accompanied by an Editorial View. Please see: Martyn J, Durieux ME: Succinylcholine: New insights into mechanisms of action of an old drug. ANESTHESIOLOGY 2006; 104:633–4.

SUCCINYLCHOLINE has been used for more than 50 yr in clinical anesthesia and emergency medicine, and it is still one of the most widely used muscle relaxants. Succinylcholine is composed of two acetylcholine molecules, linked end to end at the acetyl side, and has the potential to exert acetylcholine-like effects at nicotinic acetylcholine receptors (nAChRs).

The nAChRs are members of a neurotransmitter-gated ion channel superfamily. They are composed of five transmembrane subunits with a central cation pore, and the stoichiometry of subunits gives each receptor its unique properties.[1] To date, 17 nicotinic subunits have been cloned in vertebrates: the muscle $\alpha1$, $\beta1$, δ, γ, and ε subunits and the neuronal $\alpha2$–10 and $\beta2$–4 subunits.[2] The fetal muscle nAChR consists of two $\alpha1$, one $\beta1$, one δ, and one γ subunit, but in the adult muscle nAChR, the γ subunit is replaced by ε.[3,4] The neuronal nAChRs include both of homomeric and heteromeric receptors, with the $\alpha7$–9 subunits forming homomeric nAChRs. The heteromeric receptors are formed by a combination of $\alpha2$–6 and $\beta2$–4.[1] Although there are many potential combinations of heteromeric neuronal nAChRs, to date, only a few have been found to be of biologic importance.[5,6]

The muscle nAChR is present at the postsynaptic muscle membrane in the neuromuscular junction, whereas the neuronal nAChRs are found both presynaptically and postsynaptically in the central and peripheral nervous system as well as in extraneuronal tissues and cells, such as keratinocytes, muscle, lymphocytes, macrophages, carotid bodies, and neurosecretory cells.[1,2,7] There is evidence for nicotinic autoreceptors at the presynaptic terminal in the neuromuscular junction,[8,9] and it has recently been shown that a block of $\alpha3\beta2$ nicotinic receptors produce tetanic fade by interruption of nicotine-mediated autofacilitation of acetylcholine release.[10,11] The major autonomic ganglionic receptor is $\alpha3\beta4$, but functional $\alpha3\beta2$ and, to a lesser extent, $\alpha7$ receptors have been detected in ganglionic neurons.[6,12,13] In the central nervous system, nAChRs are widespread: $\alpha4\beta2$ is the most common nAChR, but $\alpha3\beta2$ and $\alpha7$ are also present and functional.[6,14]

In clinical anesthesia, administration of succinylcholine activates the muscle-type nAChR, which is seen as fasciculations of skeletal muscle. This is followed by a block or desensitization, seen as flaccidity. Succinylcholine-induced paralysis lasts for only 5–10 min after a single dose, because of the rapid degradation of succinylcholine to succinylmonocholine and choline by

* Resident, # Professor, Department of Anesthesiology and Intensive Care Medicine, Karolinska University Hospital and Karolinska Institutet. † Senior Research Scientist, § Associate Professor, Molecular Pharmacology, AstraZeneca R&D, Södertälje, Sweden. ‡ Target Biology, AstraZeneca Pharmaceuticals, Wilmington, Delaware. ‖ Professor, Department of Physiology and Pharmacology, Karolinska Institutet.

Received from the Department of Anesthesiology and Intensive Care Medicine, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden. Submitted for publication September 15, 2005. Accepted for publication December 1, 2005. Supported by The Medical Research Council (project No. K2001-73X-10401-09A), Olof Norlander Travel Grant, the Stockholm County Council, the Swedish Society of Medicine, and KI travel grants, all in Stockholm, Sweden. Presented in part as a poster discussion at "Frontiers in Neuromuscular Physiology and Pharmacology 2005," Stockholm, Sweden, June 16–18, 2005, and as a poster discussion at the Annual Meeting of the American Society of Anesthesiologists, Atlanta, Georgia, October 21–26, 2005.

Address correspondence to Dr. Jonsson: Department of Anesthesiology and Intensive Care Medicine, Karolinska University Hospital and Karolinska Institutet, SE-171 76 Stockholm, Sweden. malin.jonsson@karolinska.se.

plasma butyrylcholinesterase.[15] In contrast to nondepolarizing neuromuscular blocking agents, succinylcholine does not produce tetanic fade and therefore seems to lack interaction with presynaptic feedback control of transmitter release mediated *via* the $\alpha3\beta2$ nAChR subtype.[8,10]

Succinylcholine has many side effects, of which the cardiovascular effects are more serious.[16] It has been suggested that some cardiovascular side effects caused by succinylcholine arise from an interaction with neuronal nAChRs in autonomic ganglia[17]; however, this has not been confirmed at the molecular level. Furthermore, the mechanism of action of succinylcholine within the neuromuscular junction is not clear, and the putative interaction with the presynaptic $\alpha3\beta2$ nAChR has not been studied previously.

Our aim was to study the effect of succinylcholine on human muscle and neuronal nAChRs expressed in *Xenopus* oocytes, to describe activation, desensitization, and inhibition of the nAChRs in interaction with the natural ligand, acetylcholine.

Materials and Methods

Clones

The human nAChR subunits $\alpha1$, $\alpha3$–4, $\alpha7$, $\beta1$, $\beta2$, $\beta4$, δ, and ε were cloned from a human complementary DNA (cDNA) library. GenBank (Bethesda, MD) access numbers for the cDNA nucleotide sequences are as follows: NM 000079 ($\alpha1$), NM 000747 ($\beta1$), NM 000751 (δ), NM 000080 (ε), HSU62432 ($\alpha3$), L35901 ($\alpha4$), Y08420 ($\alpha7$), Y08415 ($\beta2$), and NM 000750 ($\beta4$). The cDNAs were subcloned into different expression vectors, pBluescript II SK (−) (Stratagene, La Jolla, CA; $\alpha7$), pKGem (AstraZeneca, Wilmington, DE; $\alpha1$, $\alpha3$, $\beta1$, $\beta2$, δ, and ε), and pBSTA (University of California, Irvine, CA; $\alpha4$ and $\beta4$). Messenger RNA (mRNA) was transcribed *in vitro* using the mMessage mMachine® T7 kit (Ambion, Austin, TX) and analyzed using a bioanalyzer (Agilent Technologies, Palo Alto, CA).

Xenopus *Oocyte Injection*

The study was approved by the local animal ethics committee at Karolinska Institutet, Stockholm, Sweden. *Xenopus laevis* oocytes were isolated by partial ovariectomy from frogs anesthetized with 0.2% tricaine (3-aminobenzoic acid ethyl ester, 2 g/l added to the water). The incision was sutured, and the animals were monitored during the recovery period before being returned to their tank. The ovaries were mechanically dissected to smaller lumps and digested in OR-2 buffer (82.5 mM NaCl, 2 mM KCl, $_2$ 1 mM MgCl, 5 mM HEPES, pH adjusted to 7.5 with NaOH) containing 1.5 mg/ml collagenase (type 1A; Sigma, St. Louis, MO) for 90 min to remove the follicular epithelia from the oocytes. Injection electrodes were made from 3.5-in Drummond tubes (#3-000-203-G/X; Drummond Scientific Company, Broomall, PA) and pulled using a DMZ-Universal Puller (Zeitz-Instrumente GmbH, Munich, Germany). After 1–24 h, the oocytes were injected with 0.2–18 ng mRNA in a total volume of 30–40 nl/oocyte. Multiple subunit combinations were injected at a 1:1 ratio ($\alpha1\beta1\delta\varepsilon$ or $\alpha_x\ \beta_y$), except for $\alpha4\beta2$, where the injection ratio was 1:9. The oocytes were maintained in Leibovitz L-15 medium (Sigma) diluted 1:1 with Millipore (Billerica, MA) filtered double distilled water and 80 μg/ml gentamicin, 100 units/ml penicillin and 100 μg/ml streptomycin added. Oocytes were incubated at 18°–19°C for 3–7 days after injection before being studied.

Electrophysiologic Recordings

All recordings were performed at room temperature (20°–22°C). During recording, the oocytes were continuously perfused with ND-96 (96.0 mM NaCl, 2.0 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.0 mM HEPES, pH 7.4 adjusted with NaOH). Oocyte recordings were performed using an integrated system that provides automated impalement of up to eight oocytes studied in parallel with two-electrode voltage clamp, and current measurements were automatically coordinated with fluid delivery throughout the experiment (OpusXpress™ 6000A; Molecular Devices, Union City, CA). Electrodes made from 1.5-mm borosilicate tubes (World Precision Instruments Inc, Sarasota, FL) were pulled using a micropipette puller (PP-83; Narishige Scientific Instrument Lab, Tokyo, Japan) and filled with 3 M KCl (0.5–2.5 MΩ resistance). The oocytes were voltage clamped at a holding potential of −60 mV.

Protocol

Oocytes were continuously perfused with ND-96 at a rate of 2 ml/min in a 150-μl chamber. Drugs were delivered from a 96-well plate using disposable tips and administrated at a rate of 2 ml/min for the first 2 s, and thereafter at 1 ml/min. In activation experiments, acetylcholine and succinylcholine were applied for 20 s. To determine whether succinylcholine inhibited acetylcholine-induced currents, succinylcholine was coapplied and preapplied with acetylcholine for 55 s before a 20-s application of both acetylcholine and succinylcholine. Between each drug application, there was a 6-min washout period to allow clearance of the drugs and to avoid desensitization of the channels. Before and after each concentration–response experiment, three control responses were recorded using an EC_{50} acetylcholine concentration for each receptor subtype to exclude desensitization (precontrol and postcontrol). Experiments were rejected if the postcontrol response was less than 80% of the precontrol response. To adjust for the level of channel expression, the responses in agonist concentration–response experiments were normalized to peak response in each oocyte. For inhibition experiments, re-

sponses in each oocyte were normalized to the mean of the second and third acetylcholine precontrols.

Drugs

Succinylcholine, acetylcholine, and butyrylcholinesterase were purchased from Sigma. Chemicals used in buffers were purchased from Sigma or Merck (Nottingham, United Kingdom) unless otherwise stated. Stock solutions of 1 mM acetylcholine and succinylcholine were prepared in ND-96 buffer and frozen. All drugs were then diluted in ND-96 immediately before use.

Data Analysis and Statistics

Off-line analyses were made using Clampfit 9.2 (Molecular Devices). Changes in currents were studied both as peak and net charge responses (area under the curve); however, for $\alpha 7$, only net charge analysis was used, as previously described.[18,19] The baseline current immediately before drug application was subtracted from the response, and the analysis region for peak and net charge analysis was 20 s, *i.e.*, during the time of agonist application. Concentration–response relations for agonists (acetylcholine and succinylcholine) were fitted by nonlinear regression (Prism 4.0; GraphPad, San Diego, CA) to the four parameter logistic equation: Y = Bottom + (Top − Bottom)/(1 + 10^((Log EC_{50} − X) * Hill Slope)), wherein Y is the normalized response, X is the logarithm of concentration, and EC_{50} is the concentration of agonist eliciting half-maximal response. When succinylcholine-induced inhibition was studied, the same equation was used, and EC_{50} was replaced by IC_{50}, which is the concentration of antagonist eliciting half maximal inhibition. Unless otherwise stated, data are given as mean ± SEM or 95% confidence interval (CI). Differences in IC_{50} values were compared by using paired or unpaired two-tailed Student *t* test as appropriate. A *P* value of less than 0.05 was considered significant.

Results

Acetylcholine Concentration–Response Relations for Muscular and Neuronal nAChRs

Acetylcholine produced a concentration-dependent inward current in oocytes injected with muscle- and neuronal-type nAChRs and voltage clamped at −60 mV (fig. 1), whereas uninjected oocytes did not respond to acetylcholine (data not shown). The responses to acetylcholine of the nAChR subtypes are consistent with previous reports[20–22] in terms of kinetics and EC_{50} values (fig. 1 and table 1), thus confirming the expression of the individual nAChRs in our receptor model. However, there is a lack of published data for comparison of net charge in human nAChRs, the only exception being for the $\alpha 7$ nAChR subtype.[19] As shown in figure 1B, the $\alpha 1\beta 1\delta\varepsilon$, $\alpha 3\beta 4$, and $\alpha 4\beta 2$ nAChR concentration–response relations based on net charge analysis correlate well with peak currents, with almost identical EC_{50} and Hill coefficients (table 1). However, the $\alpha 7$-subtype nAChR displays unique properties, with very fast desensitization kinetics (fig. 1A), which gives a different concentration–response relation depending on whether peak response or net charge was measured (fig. 1B). Therefore, EC_{50} is significantly lower if calculated from net charge analysis (table 1; $P < 0.0001$), in agreement with Papke *et al.*[18,19]

Succinylcholine and Activation of Muscular nAChRs

Succinylcholine produced a concentration-dependent inward current in voltage clamped oocytes expressing adult muscle-type ($\alpha 1\beta 1\delta\varepsilon$) nAChR (fig. 1). Succinylcholine caused an increasing activation of the $\alpha 1\beta 1\delta\varepsilon$ nAChR in concentrations up to 100 μM; however, higher concentrations resulted in a reduced response to succinylcholine, likely because of a more rapid channel desensitization by higher succinylcholine concentrations (fig. 1B). In most of these succinylcholine concentration–response experiments, we never saw full recovery despite trying both extended washout periods and application of the succinylcholine degrading enzyme, butyrylcholinesterase. Butyrylcholinesterase did not seem to have any effect by itself in the range of 100–500 μM (data not shown). The maximal response to succinylcholine tended to be 30–40% of that produced by acetylcholine, but detailed comparisons were not performed to calculate efficacy, because the controls did not return at the end of the experiments.

Succinylcholine and Inhibition of Muscular nAChRs

To investigate succinylcholine as a blocker at the $\alpha 1\beta 1\delta\varepsilon$-subtype nAChR, succinylcholine was applied before (preapplication) and together with acetylcholine (coapplication). Two concentrations of acetylcholine were studied, 1 μM and 5 μM. As shown in figure 2A, the concentration–response curve for succinylcholine in the presence of 1 μM acetylcholine was similar to the concentration–response curve for succinylcholine alone (fig. 1B), but slightly shifted to the right with an EC_{50} of 19.3 μM (95% CI, 5.1–73.2 μM) and a Hill coefficient of 1.12 ± 0.59 (n = 8). In contrast, the succinylcholine concentration–response curve in presence of 5 μM acetylcholine (fig. 2B) was bell shaped. The responses to low added concentrations of succinylcholine (0.1–1 μM) were not different from the 5 μM acetylcholine control response, whereas 1–100 μM succinylcholine caused a concentration-dependent potentiation of the acetylcholine current. However, at succinylcholine concentrations above 100 μM, the potentiation decreased similar to the activation curve elicited by succinylcholine alone. The kinetics were unchanged at succinylcholine up to

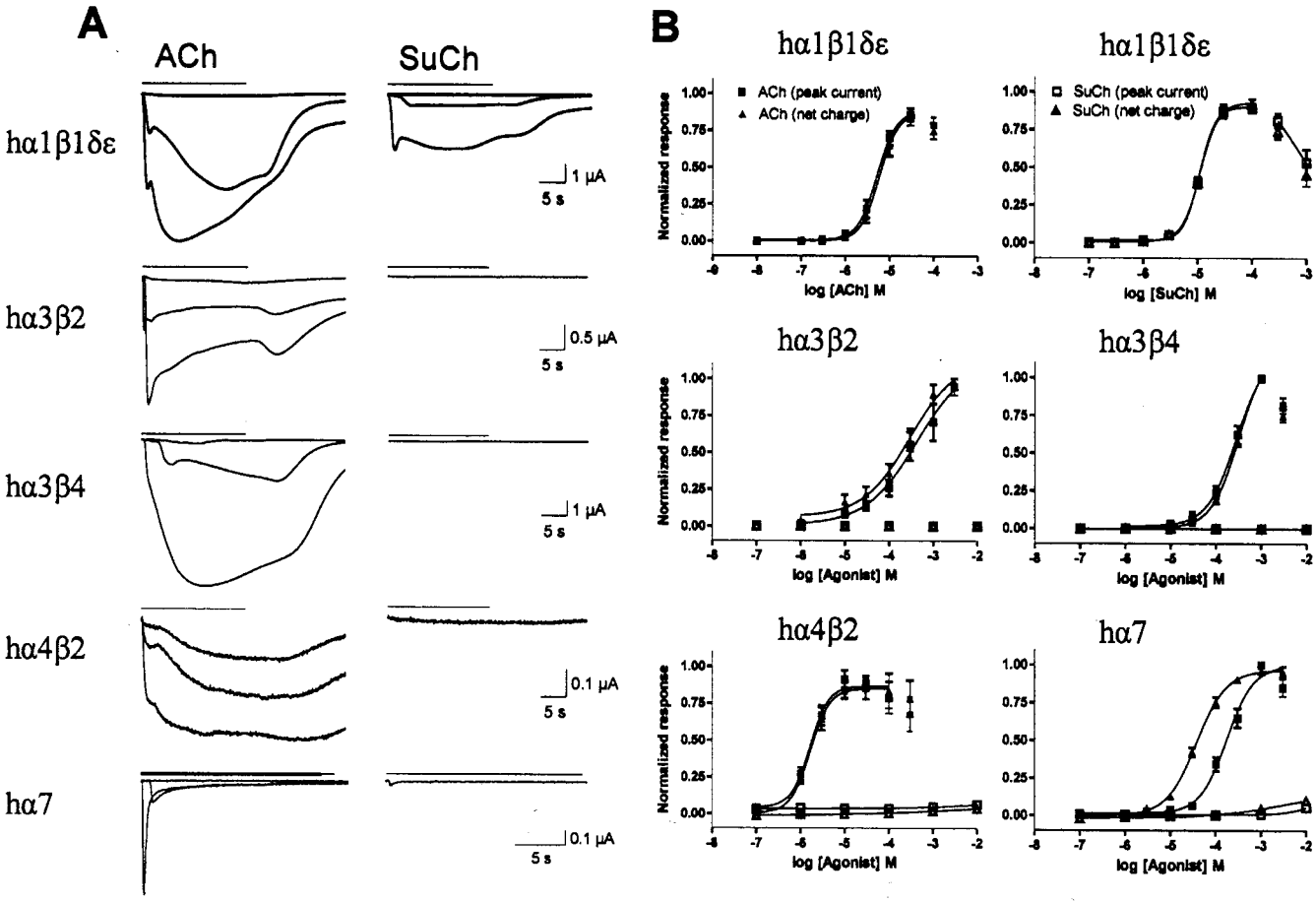

Fig. 1. Activation by acetylcholine (ACh) and succinylcholine (SuCh) in voltage clamped (−60 mV) *Xenopus* oocytes expressing human muscle and neuronal nicotinic ACh receptors (nAChRs). (*A*) Representative currents activated by 1, 10, and 100 μM ACh and SuCh for α1β1δε; 1, 100, and 1,000 μM ACh and 1 mM SuCh for α3β2 and α3β4; 1, 3, and 100 μM ACh and 1 mM SuCh for α4β2; and 1, 300, and 3,000 μM ACh and 10 mM SuCh for α7 nAChR. All cells were perfused for 20 s with agonist as indicated by the *horizontal bar*. Current traces from a single oocyte for each receptor subtype are superimposed. (*B*) Concentration–response curves for ACh and SuCh on muscular (α1β1δε) and neuronal (α3β2, α3β4, α4β2, α7) nAChRs expressed in *Xenopus* oocytes. Current responses in each oocyte were normalized to the peak current and maximal net charge response to ACh in each oocyte. For the α1β1δε nAChR, SuCh-induced responses were normalized to peak SuCh response within each oocyte. Peak and net charge analysis are displayed. Each *symbol* represents mean ± SEM of 7–17 oocytes. When no error bars are seen, they are smaller than the symbols.

100 μM for both 1 and 5 μM acetylcholine. Preapplication of succinylcholine for 55 s before 5 μM acetylcholine concentration-dependently inhibited the receptor acetylcholine response (fig. 2C). To calculate an IC_{50} for the succinylcholine-dependent decrease in acetylcholine activation, the peak acetylcholine response in presence of succinylcholine minus the base current after 54 s of succinylcholine incubation was normalized to the acetylcholine preapplication current. This yielded an IC_{50} of 126 μM (95% CI, 47.8–334 μM) and a Hill coefficient of −1.23 ± 0.93 (n = 11).

As for the succinylcholine activation concentration–response relations, the 5 μM acetylcholine controls did not recover fully at the end of the experiments in either the coapplication or the preapplication protocol. In contrast, in succinylcholine concentration–response experiments with 1 μM acetylcholine, the acetylcholine controls displayed full recovery, indicating receptor desensitization at only repeated higher acetylcholine applications. In previous studies investigating the effect of succinylcholine on the α1β1δε nAChR, precontrol and postcontrol responses to the agonist have not been measured,[23] or a mean of the controls before and after succinylcholine has been used as reference, and it has not been stated whether the controls where stable during the experiment.[24]

Succinylcholine and Activation of Neuronal nAChRs

In contrast to the muscle-type nAChR, the neuronal types did not respond to succinylcholine in concentrations up to 1 mM (fig. 1). In the range of 1–10 mM succinylcholine, the neuronal α3β2 and α3β4 displayed no succinylcholine activated current, and the α4β2 and α7 displayed very small inward currents, with a very low efficacy compared with acetylcholine (fig. 1A). The lack of activation of the neuronal nAChRs is not explained by receptor desensitization, inhibition, or oocyte deteriora-

Table 1. Pharmacologic Properties of Human Muscle ($\alpha1\beta1\delta\varepsilon$) and Neuronal nAChRs Activated by Acetylcholine and, for the Muscle Subtype, Also Succinylcholine

Human nAChR	EC_{50} (95% CI), μM	$n_H \pm$ SEM	*n*	*P* Value
$\alpha1\beta1\delta\varepsilon$				
ACh peak	5.23 (4.14–6.60)	2.00 ± 0.33	10	NS
ACh net charge	5.95 (4.56–7.73)	2.06 ± 0.38	10	
SuCh peak	10.82 (9.81–11.94)	3.01 ± 0.75	17	NS
SuCh net charge	11.20 (10.13–12.38)	2.62 ± 0.44	17	
$\alpha3\beta2$				
ACh peak	431 (76–2,453)	0.75 ± 0.11	7	NS
ACh net charge	290 (123–681)	0.83 ± 0.06	7	
$\alpha3\beta4$				
ACh peak	277 (187–412)	1.36 ± 0.26	7	NS
ACh net charge	313 (251–390)	1.46 ± 0.16	7	
$\alpha4\beta2$				
ACh peak	1.63 (1.11–2.38)	2.08 ± 0.60	7	NS
ACh net charge	1.67 (1.11–2.52)	1.98 ± 0.61	7	
$\alpha7$				
ACh peak	174 (139–217)	1.47 ± 0.21	8	*
ACh net charge	37.3 (32.3–43.1)	1.32 ± 0.11	8	

Concentration–response curves were constructed from the four-parameter logistic equation (see Materials and Methods) using peak current and area under the curve (net charge).

* $P < 0.0001$ by paired two-tailed Student *t* test, comparing peak current *vs.* net charge for each receptor subtype.

ACh = acetylcholine; CI = confidence interval; EC_{50} = half activation concentration; nAChR = neuronal nicotinic acetylcholine receptor; n_H = Hill coefficient; NS = not significant; SuCh = succinylcholine.

tion, because we obtained identical EC_{50} acetylcholine control values before and after the experiments.

Succinylcholine and Inhibition of Neuronal nAChRs

To investigate whether succinylcholine inhibits acetylcholine-induced nAChR responses by an interaction with the nAChR site or by succinylcholine-induced desensitization, succinylcholine was given both as a preapplication for 55 s before acetylcholine and as a coapplication with acetylcholine. Concentration–response relations were established with increasing succinylcholine concentrations for each nAChR subtype. For each receptor subtype a fixed acetylcholine concentration was applied (approximately EC_{50}), being 300 μM for $\alpha3\beta2$ and $\alpha3\beta4$, 5 μM for $\alpha4\beta2$, and 100 μM for $\alpha7$. Succinylcholine reversibly and concentration-dependently inhibited acetylcholine-induced currents in the neuronal nAChRs tested (fig. 3). There was no significant difference in IC_{50} values whether succinylcholine was preapplied or coapplied with acetylcholine (table 2). The kinetics of the activated current were unchanged with increased succinylcholine concentrations.

To determine the possible mechanism of succinylcholine-induced inhibition of the $\alpha3\beta4$ nAChR, we investigated the effect of 150 μM succinylcholine on the acetylcholine concentration–response curve. As shown in figure 4, 150 μM succinylcholine reduced acetylcholine-induced currents and peak responses to approximately 70% of the maximal acetylcholine response, and the EC_{50} was 304 μM (95% CI, 200–463 μM) for acetylcholine and 529 μM (95% CI, 83–3,359 μM), for acetylcholine plus 150 μM succinylcholine ($P < 0.05$). Because the succinylcholine-induced inhibition on the $\alpha3\beta4$ subtype seems not to be a pure competitive displacement of acetylcholine at the acetylcholine-binding site, we cannot rule out inhibition by channel block or allosteric inhibition.

Discussion

This study demonstrates that succinylcholine desensitizes the muscular $\alpha1\beta1\delta\varepsilon$ nAChR after initial activation. In contrast, succinylcholine does not activate or desensitize neuronal $\alpha3\beta2$, $\alpha3\beta4$, $\alpha4\beta2$, or $\alpha7$ nAChRs and is moreover a low potency inhibitor of these receptor subtypes.

Succinylcholine concentration-dependently activates the human $\alpha1\beta1\delta\varepsilon$ nAChR heterologously expressed in *Xenopus* oocytes with an EC_{50} of 10.8 μM. This indicates that the human receptor may be slightly more sensitive compared with the mouse receptor, for which an EC_{50} value of 33 μM [24] has been reported. Succinylcholine has been described as a partial agonist at the muscle nAChR,[23] but because of receptor desensitization after repeated applications of acetylcholine at EC_{50}, we were unable to properly compare the potency of succinylcholine with acetylcholine. Coapplication of succinylcholine with 1 and 5 μM acetylcholine slightly right shifted the concentration–response activation curve of succinylcholine, and in addition, 5 μM acetylcholine produced a decidedly bell-shaped curve. Preapplication of succinylcholine before 5 μM acetylcholine concentration-dependently inhibited the response of the $\alpha1\beta1\delta\varepsilon$ receptor subtype to acetylcholine with an IC_{50} of 126 μM. Our results support the hypothesis that succinylcholine prevents receptor activation at least partly by desensitization of the $\alpha1\beta1\delta\varepsilon$ nAChR and are in line with a previous report.[25]

We also demonstrate that succinylcholine does not activate the human neuronal nAChRs, $\alpha3\beta2$, $\alpha3\beta4$, $\alpha4\beta2$, or $\alpha7$, in concentrations up to 1 mM and furthermore is a low-potency antagonist at these receptor subtypes. Succinylcholine has previously been shown to be a weak agonist at the rat $\alpha7$ subtype, and furthermore, it has been reported that the rat $\alpha3\beta4$ nAChR is not activated by succinylcholine in concentrations up to 1 mM.[23] Interestingly, insertion of a point mutation in the second transmembrane domain of the $\alpha7$ receptor, T244F (Thr to Phe), dramatically changes both biophysical and pharmacologic properties toward those of the muscle receptor.[23] The mutation results in succinylcholine having increased potency and efficacy: Although being a partial agonist at the wild-type $\alpha7$ receptor, succinylcholine is a full agonist at the T244F mutant $\alpha7$ receptor.[23]

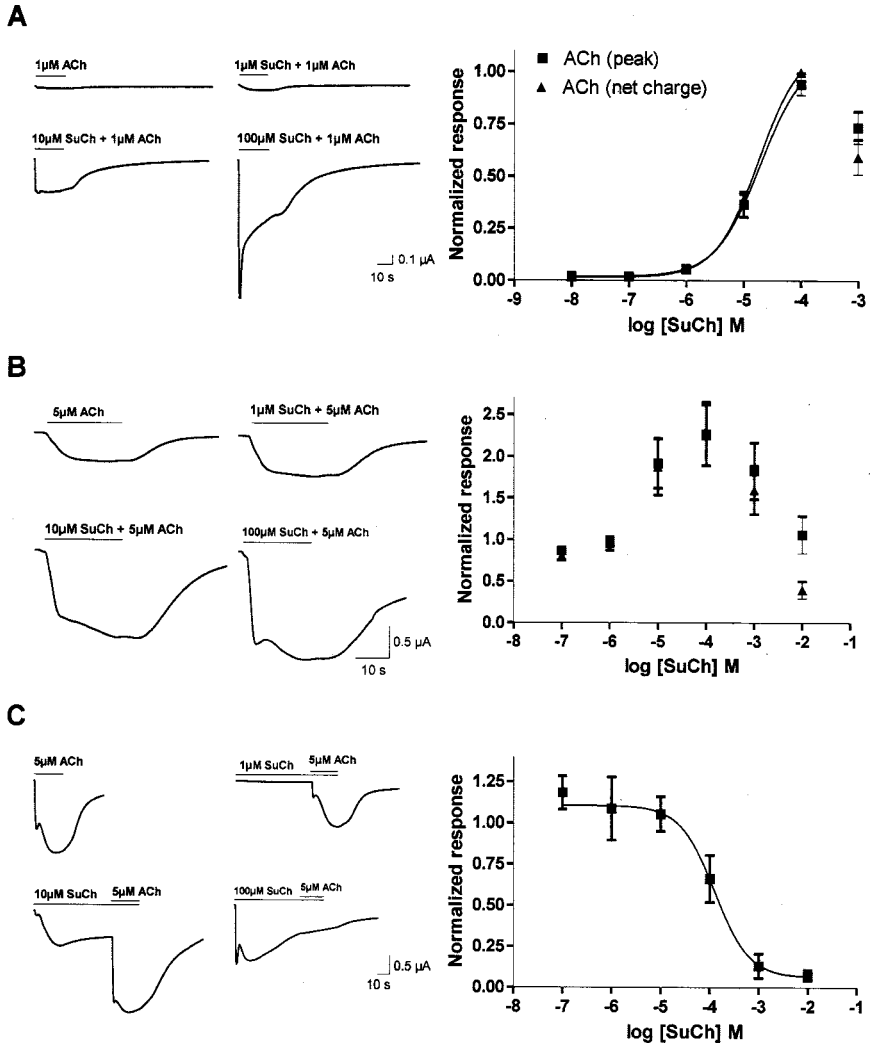

Fig. 2. The effect of succinylcholine (SuCh) on acetylcholine (ACh)–mediated responses in human $\alpha1\beta1\delta\varepsilon$ nicotinic ACh receptor expressed in *Xenopus* oocytes voltage clamped at −60 mV. (*A*) ACh, 1 μM, was coapplied with various concentrations of SuCh. Current responses in each oocyte were normalized to the peak current and maximal net charge response to ACh in each oocyte yielding the concentration–response relations shown on the *right*. ACh, 5 μM, was coapplied (*B*) or preapplied (*C*) for 55 s with SuCh. The ACh responses in each oocyte were normalized to the ACh precontrols (see Materials and Methods). The preapplication SuCh current was subtracted from the ACh current in preapplication experiments. Representative current traces from a single oocyte are shown on the *left* with ACh and SuCh added as indicated by the *horizontal bars*. For concentration–response curves, data are presented as mean ± SEM. When no error bars are seen, they are smaller than the symbol.

There is more than 80% homology between human and rodent DNA for a given nAChR subunit.[1] However, a small difference in amino acid sequence at some regions can cause significant changes in biophysical and pharmacologic properties of the receptors.[18,20,26]

Here, we show that succinylcholine is a partial agonist at the human muscle nAChR; however, we could not calculate the exact efficacy because of apparent receptor desensitization. In contrast, either succinylcholine does not act as an agonist or it is a very weak partial agonist at the neuronal nAChR subtypes. The efficacies were extremely low at these receptor subtypes. Furthermore, no desensitization was seen, because the acetylcholine control responses after the succinylcholine concentration–response curve were preserved, and there were no differences in inhibition whether succinylcholine was preapplied or coapplied with acetylcholine. Altogether, succinylcholine does not desensitize the neuronal nAChRs in contrast to the muscle subtype, and the succinylcholine-induced inhibition on the $\alpha3\beta4$ subtype seems not to be a pure competitive displacement of acetylcholine at the acetylcholine-binding site. We speculate that inhibition by channel block or allosteric inhibition may also be involved in the inhibition. This has obviously to do with binding site, but this is beyond the scope of this article.

For the neuronal nAChRs, a stoichiometry of 2 α and 3 β has been found when DNA/RNA have been injected into *Xenopus* oocytes at a ratio of 1:1 (α:β).[27–29] However, it has recently been shown that the $\alpha4\beta2$ subtype, when injected in a subunit ratio of 1:9, displays a subtype that is more sensitive to activation and desensitizes more slowly compared with the 1:1 subtype.[30] In addition, human embryonic kidney cells transfected with the human cDNA for the $\alpha4$ and $\beta2$ subunits displayed the more sensitive subtype if transfected with additional $\beta2$ subunits or if they were exposed to nicotine or low temperature.[31] In the current study, we investigated this more sensitive $\alpha4\beta2$ subtype, and the EC_{50} (1.63 μM) was similar to those reported in previous studies (1.8 μM).[30,32]

We analyzed both peak current and net charge after acetylcholine-induced nAChR activation and restricted the analysis to 20 s (during agonist application) because we believe that this better reflects the effects of agonist

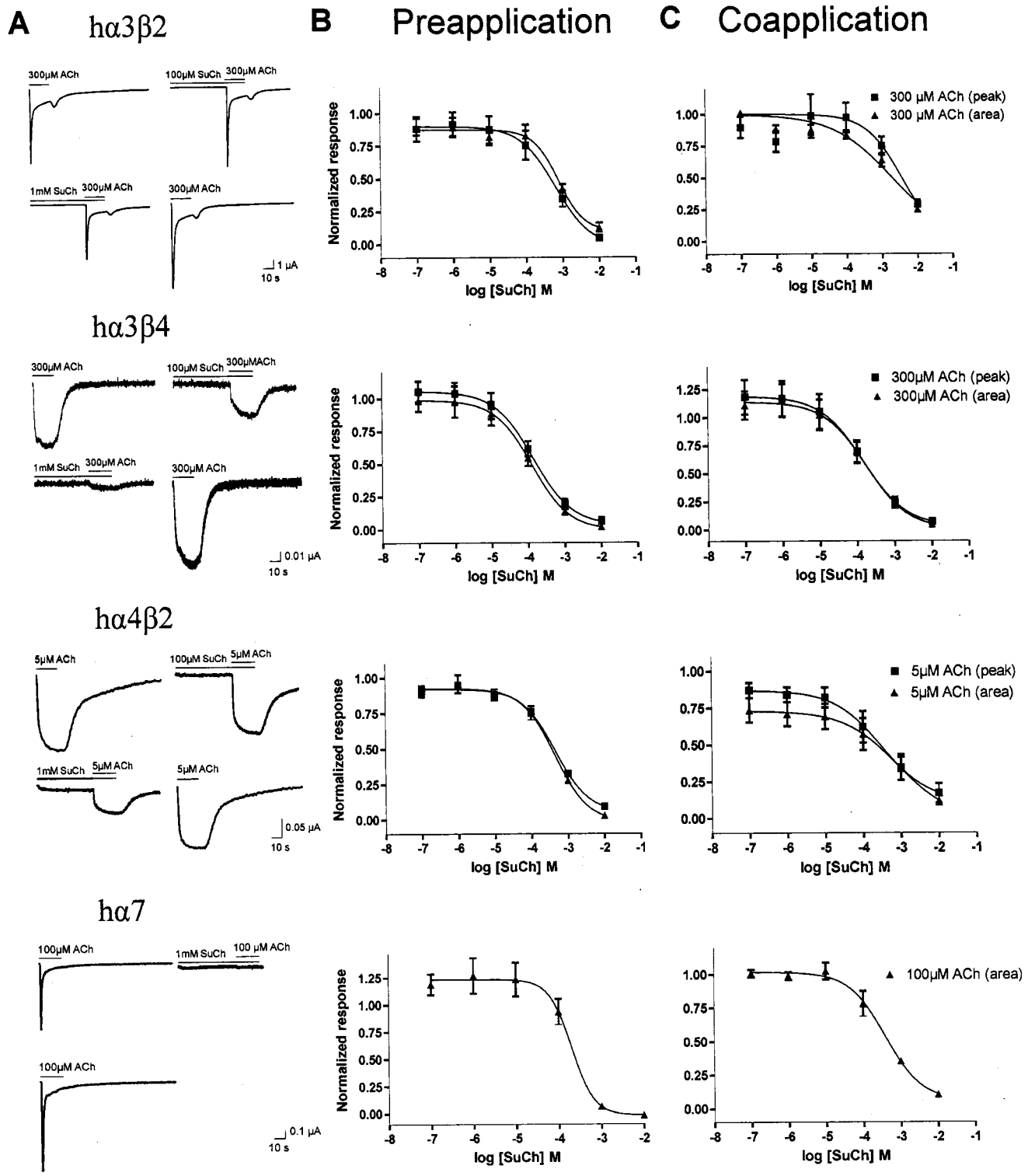

Fig. 3. Concentration–response curves of succinylcholine (SuCh)–induced inhibition of acetylcholine (ACh)–mediated response in human $\alpha3\beta2$, $\alpha3\beta4$, $\alpha4\beta2$, and $\alpha7$ nicotinic ACh receptor expressed in *Xenopus* oocytes, voltage clamped at −60 mV. For each receptor subtype, an ACh concentration around the EC_{50} was applied: 300 μM for $\alpha3\beta2$ and $\alpha3\beta4$, 5 μM for $\alpha4\beta2$, and 100 μM for $\alpha7$ as indicated by the *horizontal bars* above the traces. (*A*) Representative traces from oocytes expressing the individual receptors showing the response to various concentrations of ACh and SuCh. Inhibition curves for SuCh-induced inhibition of ACh responses when SuCh was either preapplied for 55 s (*B*) or coapplied with ACh (*C*). Current and net charge responses in each oocyte were normalized to the ACh precontrols (see Materials and Methods). For each receptor subtype, 5–16 oocytes were studied. Data are presented as mean ± SEM. When no error bars are seen, they are smaller than the symbols.

Table 2. Pharmacologic Properties of Succinylcholine as an Inhibitor of Acetylcholine-induced Activation of Human Neuronal nAChRs Expressed in *Xenopus* Oocytes

Human nAChR	IC_{50} (95% CI), μM	n_H ± SEM*	*n*
α3β2 ACh 300 μM			
Preapplication			
Peak	638 (178–2,292)	−0.90 ± 0.45	9
Net charge	786 (406–1,522)	−1.26 ± 0.67	
Coapplication			
Peak	3,613 (1,553–8,405)	−0.86 ± 0.29	16
Net charge	1,925 (1,275–2,905)	−0.52 ± 0.06	
α3β4 ACh 300 μM			
Preapplication			
Peak	138 (59.3–323)	−0.83 ± 0.27	5
Net charge	128 (52.2–313)	−0.86 ± 0.32	
Coapplication			
Peak	147 (35–616)	−0.76 ± 0.39	7
Net charge	171 (46–636)	−0.78 ± 0.36	
α4β2 ACh 5 μM			
Preapplication			
Peak	455 (332–624)	−0.96 ± 0.11	7
Net charge	446 (265–752)	−0.97 ± 0.19	
Coapplication			
Peak	300 (48.9–1,842)	−0.70 ± 0.37	7
Net charge	805 (19.9–32,570)	−0.62 ± 0.48	
α7 ACh 100 μM			
Preapplication			
Net charge	201 (78.8–513)	−1.67 ± 0.88	7
Coapplication			
Net charge	385 (193–769)	−0.90 ± 0.21	7

Concentration–response curves were constructed using both peak current and net charge analysis.

* Negative Hill coefficient is a result of inhibition.

ACh = acetylcholine; CI = confidence interval; nAChR = neuronal nicotinic acetylcholine receptor; n_H = Hill coefficient.

and eliminates confounding factors such as rundown and washout conditions. Net charge analysis has convincingly been shown to be a more accurate method to analyze activation of the α7 nAChR subtype, which desensitizes very rapidly.[18,19] For the α3β2, α3β4, α4β2, and α1β1δε subtypes, the pharmacologic properties are almost identical independent of the method used. The

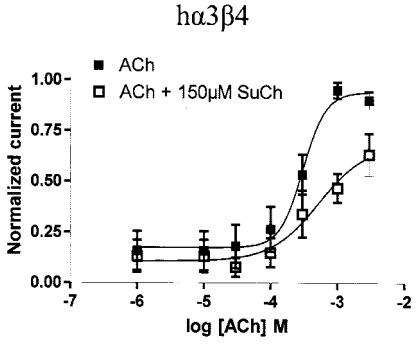

Fig. 4. Concentration–response curve for acetylcholine (ACh) current responses in oocytes expressing the human α3β4 nicotinic ACh receptor in the absence and presence of 150 μM succinylcholine (SuCh) (n = 5). Oocytes were first exposed to ACh and thereafter to 150 μM SuCh and ACh. Data were normalized to maximal ACh current within each oocyte. Data are presented as mean ± SEM.

very small difference between peak current and net charge present in the α3β2 nAChR subtype might reflect the rapid initial desensitization of this receptor subtype.

There is a long-standing controversy regarding the mechanism of action of succinylcholine, *i.e.*, whether succinylcholine blocks presynaptic or postsynaptic nAChRs or other structures in the neuromuscular junction.[33] Here, we confirm that succinylcholine initially activates the adult muscle nAChR with an EC_{50} of 10.8 μM, in reasonable accordance with the clinical EC_{50} of 2.6 μM.[34] Thereafter, succinylcholine causes a desensitization of the receptor with an apparent IC_{50} of 126 μM on the acetylcholine response. However, we found that succinylcholine is not an effective competitive antagonist at the human muscle-type nAChR, because succinylcholine, when coapplied with acetylcholine, does not block the receptor. There is a discrepancy between the clinical EC_{50} of 2.6 μM and the IC_{50} in our oocyte preparation, and in addition, nerve stimulation using an *in vitro* nerve-muscle preparation indicates an EC_{50} of 21.3 μM for succinylcholine.[35] The latter preparation, however, involves activation through direct innervation rather than through application of exogenous acetylcholine. Based on this, we believe that succinylcholine after initial receptor activation shifts the receptor population toward a desensitized state, rendering fewer receptors available for acetylcholine activation. Because of the high receptor density in the oocytes, there will still be receptors in both open and closed states available for acetylcholine activation, and what we actually measure after succinylcholine preapplication is the "nondesensitized" receptor population in the oocytes. Alternatively, in the neuromuscular junction, activation of approximately 25% of the muscle nAChRs may be sufficient to depolarize and subsequently desensitize the muscle tissue.[36] Given this view, the EC_{50} of 10.8 μM at the recombinant adult muscle nAChR agrees very closely with the clinical determined EC_{50} of 2.6 μM. In addition, desensitization of a part of the receptor population on the muscle tissue could be sufficient for inducing a neuromuscular block due to subthreshold muscle depolarization. Finally, we cannot rule out the possibility that accessory proteins associated with the muscle nAChR might be involved, yielding a more potent succinylcholine neuromuscular inhibition *in vivo*.

Tetanic fade is caused by an interaction with cholinergic presynaptic autoreceptors mediating acetylcholine release from the motor nerve end.[8] For many years, measurement of twitch tension and tetanic fade has served as the base for clinical monitoring of neuromuscular blockade.[37] In contrast to nondepolarizing neuromuscular agents, succinylcholine-induced neuromuscular block is characterized by a lack of tetanic fade. The mechanism behind tetanic fade is not known, and furthermore, it is not known how neuromuscular blocking agents interact with this receptor at a molecular level. It

has recently been shown that a block of the presynaptic $\alpha3\beta2$ nAChR produces tetanic fade due to inhibition of an acetylcholine-mediated $\alpha3\beta2$ autofacilitation.[10] Here, we clearly demonstrate that succinylcholine does not activate the human $\alpha3\beta2$ nAChR subtype and, furthermore, blocks the receptor only at very high concentrations of succinylcholine ($IC_{50} > 700$ μM). The finding that succinylcholine has a very low affinity to the $\alpha3\beta2$ nAChR is in accordance with the clinical observation that succinylcholine does not cause tetanic fade, and we can, for the first time, provide a molecular explanation and experimental evidence for the lack of tetanic fade in succinylcholine-induced neuromuscular blockade. In addition, the high IC_{50} value for succinylcholine at the $\alpha3\beta2$ nAChR might also explain the clinical "phase II block," which is seen when succinylcholine has been overdosed and is characterized by tetanic fade and a partial or complete reversal of the block by anticholinesterase.[38,39] We speculate that this phase II block might in part be due to a block of presynaptic $\alpha3\beta2$ nAChRs at high succinylcholine concentrations, because peak succinylcholine plasma concentrations at normal dosage have been shown to be approximately 75.5–143.2 μM,[34] and a "phase II block" may develop when succinylcholine is given three to five times the normal dose.[39] However, other receptors as well as other components of the presynaptic nerve terminal can modulate the tetanic fade response,[8,40,41] and therefore, involvement of other mechanisms in the "phase II block" in addition to the inhibition of the presynaptic $\alpha3\beta2$ nAChR is possible and must be further evaluated. Altogether, our results support the hypothesis that succinylcholine blocks the transmission in the neuromuscular junction by activation and desensitization of the $\alpha1\beta1\delta\varepsilon$ nAChR, not by an antagonistic effect or block at the presynaptic $\alpha3\beta2$ nAChR subtype at normal dosage.

Tachyarrhythmias, mainly of ventricular origin, as a side effect of succinylcholine have been suggested to arise as a result of catecholamine release from the adrenal medulla after stimulation of ganglionic nAChRs.[17] The nAChRs present in ganglion are mainly the $\alpha3\beta4$ type, but $\alpha3\beta2$ and $\alpha7$ nAChR subtypes are also present.[5,14] Here, we show that none of the $\alpha3\beta2$ or $\alpha3\beta4$ nAChR subtypes were activated by succinylcholine, and the $\alpha7$ nAChR subtype was activated only at succinylcholine concentrations above 1 mM.

Because succinylcholine is rapidly hydrolyzed to succinylmonocholine and choline, we cannot exclude a possible effect of these breakdown products on neuronal nAChRs. Conformational analysis, however, reveals that succinylmonocholine does not fit very well into the nAChR binding sites.[42] The other breakdown product, choline, is known as a selective $\alpha7$ nAChR agonist,[43,44] but as such has low potency, with an EC_{50} of 400–500 μM, and inhibits the receptor with an IC_{50} of greater than 1–10 mM.[18,45] Interaction of succinylcholine with the M2 muscarinic AChR has also been suggested to cause cardiovascular interactions, but a recent binding study shows that succinylcholine has low affinity for both the M2 and M3 muscarinic AChRs.[46] Interestingly, succinylcholine increases the afferent activity in the cerebral cortex as a result of a stimulation of muscle spindles, leading to an arousal effect on the electroencephalogram and increased cerebral blood flow.[47,48] The arousal effects on the electroencephalogram have been shown to be similar to those during surgical incision.[49] Therefore, it is possible that some succinylcholine-induced cardiovascular side effects are due to an indirect central activation of the sympathetic nervous system, rather than by a direct interaction with cholinergic receptors in peripheral ganglia.

In conclusion, succinylcholine activates the muscle-type nAChR followed by desensitization. Our observation that succinylcholine does not inhibit the presynaptic $\alpha3\beta2$ autoreceptor at clinically relevant concentrations provides a possible mechanistic and molecular explanation for the typical lack of tetanic fade in succinylcholine-induced neuromuscular blockade. Finally, cardiovascular side effects (*e.g.*, tachyarrhythmias) of succinylcholine are not mediated *via* direct activation of the ganglionic $\alpha3\beta4$ subtype because succinylcholine does not activate the neuronal nAChRs.

The authors thank Alan Robbins, M.S. (Scientist), Jodi D. Bradley (Associate Scientist), Robert Manning (Scientist), Rich Hastings (Associate Scientist), Geeta Iyer (Contractor), and Tirell Norris, B.S. (Scientist), from AstraZeneca Pharmaceuticals, Wilmington, Delaware, and Wendy Gion (Scientist) from AstraZeneca Pharmaceuticals, Boston, Massachusetts, for DNA cloning and generation of messenger RNA.

References

1. Lukas RJ, Changeux JP, Le Novere N, Albuquerque EX, Balfour DJ, Berg DK, Bertrand D, Chiappinelli VA, Clarke PB, Collins AC, Dani JA, Grady SR, Kellar KJ, Lindstrom JM, Marks MJ, Quik M, Taylor PW, Wonnacott S: International Union of Pharmacology: XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. Pharmacol Rev 1999; 51:397–401

2. Gotti C, Clementi F: Neuronal nicotinic receptors: From structure to pathology. Prog Neurobiol 2004; 74:363–96

3. Schuetze SM, Role LW: Developmental regulation of nicotinic acetylcholine receptors. Annu Rev Neurosci 1987; 10:403–57

4. Salpeter MM, Marchaterre M, Harris R: Distribution of extrajunctional acetylcholine receptors on a vertebrate muscle: Evaluated by using a scanning electron microscope autoradiographic procedure. J Cell Biol 1988; 106:2087–93

5. Lindstrom JM: Nicotinic acetylcholine receptors of muscles and nerves: Comparison of their structures, functional roles, and vulnerability to pathology. Ann N Y Acad Sci 2003; 998:41–52

6. Hogg RC, Raggenbass M, Bertrand D: Nicotinic acetylcholine receptors: From structure to brain function. Rev Physiol Biochem Pharmacol 2003; 147: 1–46

7. Iturriaga R, Alcayaga J: Neurotransmission in the carotid body: Transmitters and modulators between glomus cells and petrosal ganglion nerve terminals. Brain Res Brain Res Rev 2004; 47:46–53

8. Wessler I: Control of transmitter release from the motor nerve by presynaptic nicotinic and muscarinic autoreceptors. Trends Pharmacol Sci 1989; 10: 110–4

9. Bowman WC, Prior C, Marshall IG: Presynaptic receptors in the neuromuscular junction. Ann N Y Acad Sci 1990; 604:69–81

10. Faria M, Oliveira L, Timoteo MA, Lobo MG, Correia-De-Sa P: Blockade of neuronal facilitatory nicotinic receptors containing alpha 3 beta 2 subunits contribute to tetanic fade in the rat isolated diaphragm. Synapse 2003; 49:77–88

11. Tsuneki H, Kimura I, Dezaki K, Kimura M, Sala C, Fumagalli G: Immunohistochemical localization of neuronal nicotinic receptor subtypes at the pre- and postjunctional sites in mouse diaphragm muscle. Neurosci Lett 1995; 196:13–6

12. Bibevski S, Zhou Y, McIntosh JM, Zigmond RE, Dunlap ME: Functional nicotinic acetylcholine receptors that mediate ganglionic transmission in cardiac parasympathetic neurons. J Neurosci 2000; 20:5076–82

13. Hogg RC, Miranda LP, Craik DJ, Lewis RJ, Alewood PF, Adams DJ: Single amino acid substitutions in alpha-conotoxin PnIA shift selectivity for subtypes of the mammalian neuronal nicotinic acetylcholine receptor. J Biol Chem 1999; 274:36559–64

14. Paterson D, Nordberg A: Neuronal nicotinic receptors in the human brain. Prog Neurobiol 2000; 61:75–111

15. Goedde HW, Held KR, Altland K: Hydrolysis of succinyldicholine and succinylmonocholine in human serum. Mol Pharmacol 1968; 4:274–87

16. Ostergaard D, Engbaek J, Viby-Mogensen J,: Adverse reactions and interactions of the neuromuscular blocking drugs. Med Toxicol Adverse Drug Exp 1989; 4:351–68

17. Naguib M, Lien CA: Pharmacology of muscle relaxants and their antagonists, Miller's Anesthesia. Edited by Miller RD. Philadelphia, Elsevier, 2005, pp 481–572

18. Papke RL, Porter Papke JK: Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. Br J Pharmacol 2002; 137: 49–61

19. Papke RL, Thinschmidt JS: The correction of alpha7 nicotinic acetylcholine receptor concentration-response relationships in *Xenopus* oocytes. Neurosci Lett 1998; 256:163–6

20. Chavez-Noriega LE, Crona JH, Washburn MS, Urrutia A, Elliott KJ, Johnson EC: Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors h alpha 2 beta 2, h alpha 2 beta 4, h alpha 3 beta 2, h alpha 3 beta 4, h alpha 4 beta 2, h alpha 4 beta 4 and h alpha 7 expressed in *Xenopus* oocytes. J Pharmacol Exp Ther 1997; 280:346–56

21. Garland CM, Foreman RC, Chad JE, Holden-Dye L, Walker RJ: The actions of muscle relaxants at nicotinic acetylcholine receptor isoforms. Eur J Pharmacol 1998; 357:83–92

22. Hatton CJ, Shelley C, Brydson M, Beeson D, Colquhoun D: Properties of the human muscle nicotinic receptor, and of the slow-channel myasthenic syndrome mutant epsilonL221F, inferred from maximum likelihood fits. J Physiol 2003; 547:729–60

23. Placzek AN, Grassi F, Papke T, Meyer EM, Papke RL: A single point mutation confers properties of the muscle-type nicotinic acetylcholine receptor to homomeric alpha7 receptors. Mol Pharmacol 2004; 66:169–77

24. Yost CS, Winegar BD: Potency of agonists and competitive antagonists on adult- and fetal-type nicotinic acetylcholine receptors. Cell Mol Neurobiol 1997; 17:35–50

25. Marshall CG, Ogden DC, Colquhoun D: The actions of suxamethonium (succinyldicholine) as an agonist and channel blocker at the nicotinic receptor of frog muscle. J Physiol 1990; 428:155–74

26. Peng X, Katz M, Gerzanich V, Anand R, Lindstrom J: Human alpha 7 acetylcholine receptor: Cloning of the alpha 7 subunit from the SH-SY5Y cell line and determination of pharmacological properties of native receptors and functional alpha 7 homomers expressed in *Xenopus* oocytes. Mol Pharmacol 1994; 45:546–54

27. Anand R, Conroy WG, Schoepfer R, Whiting P, Lindstrom J: Neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes have a pentameric quaternary structure. J Biol Chem 1991; 266:11192–8

28. Cooper E, Couturier S, Ballivet M: Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor. Nature 1991; 350: 235–8

29. Boorman JP, Groot-Kormelink PJ, Sivilotti LG: Stoichiometry of human recombinant neuronal nicotinic receptors containing the b3 subunit expressed in *Xenopus* oocytes. J Physiol 2000; 529(pt 3): 565–77.

30. Zwart R, Vijverberg HP: Four pharmacologically distinct subtypes of alpha4beta2 nicotinic acetylcholine receptor expressed in *Xenopus laevis* oocytes. Mol Pharmacol 1998; 54:1124–31

31. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J: Alternate stoichiometries of alpha4beta2 nicotinic acetylcholine receptors. Mol Pharmacol 2003; 63:332–41

32. Chiodini F, Charpantier E, Muller D, Tassonyi E, Fuchs-Buder T, Bertrand D: Blockade and activation of the human neuronal nicotinic acetylcholine receptors by atracurium and laudanosine. ANESTHESIOLOGY 2001; 94:643–51

33. Bowman WC: Block by depolarization. Acta Anaesthesiol Scand 1994; 38:529–32

34. Roy JJ, Donati F, Boismenu D, Varin F: Concentration-effect relation of succinylcholine chloride during propofol anesthesia. ANESTHESIOLOGY 2002; 97: 1082–92

35. Fortier LP, Robitaille R, Donati F: Increased sensitivity to depolarization and nondepolarizing neuromuscular blocking agents in young rat hemidiaphragms. ANESTHESIOLOGY 2001; 95:478–84

36. Tuba Z, Maho S, Vizi ES: Synthesis and structure-activity relationships of neuromuscular blocking agents. Curr Med Chem 2002; 9:1507–36

37. Ali HH, Utting JE, Gray C: Stimulus frequency in the detection of neuromuscular block in humans. Br J Anaesth 1970; 42:967–78

38. Lee C: Dose relationships of phase II, tachyphylaxis and train-of-four fade in suxamethonium-induced dual neuromuscular block in man. Br J Anaesth 1975; 47:841–5

39. Lee C, Katz RL: Neuromuscular pharmacology: A clinical update and commentary. Br J Anaesth 1980; 52:173–88

40. Timoteo MA, Faria M, Correia-de-Sa PM: Endogenous adenosine prevents post-tetanic release facilitation mediated by alpha3beta2 nicotinic autoreceptors. Eur J Pharmacol 2003; 464:115–25

41. Prior C, Tian L, Dempster J, Marshall IG: Prejunctional actions of muscle relaxants: Synaptic vesicles and transmitter mobilization as sites of action. Gen Pharmacol 1995; 26:659–66

42. Lee C: Conformation, action, and mechanism of action of neuromuscular blocking muscle relaxants. Pharmacol Ther 2003; 98:143–69

43. Papke RL, Bencherif M, Lippiello P: An evaluation of neuronal nicotinic acetylcholine receptor activation by quaternary nitrogen compounds indicates that choline is selective for the alpha 7 subtype. Neurosci Lett 1996; 213:201–4

44. Alkondon M, Pereira EF, Cortes WS, Maelicke A, Albuquerque EX: Choline is a selective agonist of alpha7 nicotinic acetylcholine receptors in the rat brain neurons. Eur J Neurosci 1997; 9:2734–42

45. Fuentealba J, Olivares R, Ales E, Tapia L, Rojo J, Arroyo G, Aldea M, Criado M, Gandia L, Garcia AG: A choline-evoked [Ca2+]c signal causes catecholamine release and hyperpolarization of chromaffin cells. FASEB J 2004; 18:1468–70

46. Hou VY, Hirshman CA, Emala CW: Neuromuscular relaxants as antagonists for M2 and M3 muscarinic receptors. ANESTHESIOLOGY 1998; 88:744–50

47. Lanier WL, Milde JH, Michenfelder JD: Cerebral stimulation following succinylcholine in dogs. ANESTHESIOLOGY 1986; 64:551–9

48. Brunner MD, Nathwani D, Rich PA, Thornton C, Dore CJ, Newton DE: Effect of suxamethonium on the auditory evoked response in humans. Br J Anaesth 1996; 76:34–7

49. Oshima E, Shingu K, Mori K: E.E.G. activity during halothane anaesthesia in man. Br J Anaesth 1981; 53:65–72