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ORIGINAL ARTICLE

Synergistic effect of sevoflurane and isoflurane on inhibition of the adult-type muscle nicotinic acetylcholine receptor by rocuronium

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Abstract
Purpose Inhaled anesthetics increase the incidence of postoperative residual neuromuscular blockade, and the mechanism is still unclear. We have investigated the synergistic effect of low-concentration inhaled anesthetics and rocuronium on inhibition of the inward current of the adult-type muscle nicotinic acetylcholine receptor (ε-nAChR).
Methods Adult-type mouse muscle ε-nAChR was expressed in HEK293 cells by liposome transfection. The inward current of the ε-nAChR was activated by use of 10 μmol/L acetylcholine alone or in combination with different concentrations of sevoflurane, isoflurane, or rocuronium. The concentration–response curves of five cells were constructed, and the data yielded the 5, 25, and 50 % inhibitory concentrations (IC_5, IC_{25}, and IC_{50}, respectively) for single-drug application. Subsequently, the functional channels were perfused by adding 0.5 IC_5 of either sevoflurane or isoflurane (aqueous concentrations 140 and 100 μmol/L, respectively) to the solution, followed by addition of IC_5, IC_{25}, or IC_{50} rocuronium. The amount of inhibition was calculated to quantify their synergistic effect.
Results The inhibitory effect of rocuronium was enhanced by sevoflurane or isoflurane in a concentration-dependent manner. Sevoflurane or isoflurane (0.5 IC_5) with rocuronium at IC_5, IC_{25}, and IC_{50} synergistically inhibited the current amplitude of adult-type muscle ε-nAChR. When the IC_5 of rocuronium was used, isoflurane had a stronger synergistic effect than sevoflurane ($p < 0.05$). When rocuronium was applied at higher concentrations (IC_{25} and IC_{50}), sevoflurane had an effect similar to that of isoflurane. For both inhaled anesthetics, the synergistic effect was more intense for rocuronium at IC_5 than for rocuronium at IC_{25} or IC_{50}.
Conclusion Residual-concentration sevoflurane or isoflurane has a strong synergistic effect with rocuronium at clinically relevant residual concentrations. A lower rocuronium concentration resulted in a stronger synergistic effect.

Keywords Anesthesia · Nondepolarizing muscle relaxants · Rocuronium · Nicotinic acetylcholine receptor · Whole-cell recording · Concentration–response relationship

Introduction

Residual neuromuscular block is one of the major risk factors of anesthesia-related morbidity and mortality in postanesthesia care [1]. Clinical studies have demonstrated that small degrees of residual paralysis are associated with impaired pharyngeal function [1, 2], obstructed airway [3], and attenuated hypoxic ventilatory response [4]. Therefore, neuromuscular management is important for patient safety and achieving desirable postoperative outcomes.

Inhaled anesthetic agents enable smooth and reliable induction and maintenance of general anesthesia, and are thus extensively used. However, inhaled anesthetics enhance the neuromuscular blocking effects of nondepolarizing neuromuscular blocking agents (NMBAs) [5] and prolong the duration of inhibitory action and recovery from neuromuscular blockade [6, 7]. Residual concentrations of inhaled

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anesthetics increase the incidence of postoperative residual neuromuscular blockade [8–10].

Several studies have indicated that the enhancement effect of inhaled anesthetics results from the synergistic effects at the neuromuscular junction [11, 12]. NMBAs block neuromuscular transmission by competitively binding at the agonist site of muscle nicotinic acetylcholine (Ach) receptors (nAChRs) [13]. Inhaled anesthetics also partially inhibit the inward current of muscle nAChR [14], although clinically relevant concentrations have not been observed to elicit muscle relaxation by nAChR inhibition [15]. Paul et al. [16] indicated that isoflurane has a synergistic effect with NMBAs on nAChRs. However, inhaled anesthetics are intraoperatively administered at a fixed concentration. Thus, the possible changes in the synergistic effects with reduced NMBA concentration remain to be elucidated.

In this study, our objective was to characterize the synergistic effect of inhaled anesthetics and rocuronium at different concentrations. We used adult-type mouse muscle nAChR (ε-nAChR) heterologously expressed in HEK293 cells by liposome transfection. Functional receptors were activated with 10 μmol/L Ach alone or in combination with solutions containing anesthetics at different concentrations. The resulting currents were recorded by use of a whole-cell two-electrode voltage-clamp technique.

Materials and methods

Cell culture and transfection

The recombinant vectors pSP65α, pSP65β, pSP64δ, and pBssk(+)ε, whose cDNAs encoded the α, β, δ, and ε subunits, respectively, of adult-type mouse nAChR were obtained from FocusBio Institute (Guangzhou, China). The objective gene fragments were cut and subcloned into a pcDNA3.1 plasmid (Invitrogen Life Technologies, Carlsbad, CA, USA) carrying the neomycin-resistance gene. The recombinant plasmid sequences (pcDNA3.lα, pcDNA3.lβ, pcDNA3.1δ, and pcDNA3.1ε) were identified by Sanger's dideoxy chain termination method. The recombinant plasmids were transformed into *Escherichia coli* for amplification.

HEK293 cells were obtained from the Molecular Biology Laboratory, Chongqing Medical University (Chongqing, China). The cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Grand Island, NY, USA) supplemented with 10 % bovine calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin incubated at 37 °C in a humidified atmosphere containing 5 % CO_2.

For transfection, the recombinant vectors were mixed with 10 μL lipofectamine 2000 (Invitrogen Life Technologies) in a 2:1:l:1 ratio. The reaction temperature was maintained at 22–24 °C. The total quantity of α, β, δ, and ε plasmids was <1.5 μg, and the plasmid-to-liposome ratio was maintained between 1:3 and 1:5 (μg:μL).

All transfections were maintained for 2 weeks in a selection medium composed of growth medium containing 500 mg/mL G418 (Gibco, BRL) or 100 mg/mL G418. Antibiotic-resistant colonies were isolated and maintained in the presence of the selection medium. Cells that survived the 2 week selection period in 100 mg/mL G418 were then used to identify positively transfected cells. These transfected cells were then incubated for 24 h before analysis.

Drugs and materials

Ach was purchased from Sigma (St Louis, MO, USA), rocuronium was from Merck (St Whitehouse, NJ, USA), and isoflurane and sevoflurane were from Abbott (Princeton, NJ, USA). All drugs were dissolved in an extracellular solution containing 140 mmol/L NaCl, 2.5 mmol/L KCl, 2 mmol/L $CaCl_2$, 0.8 mmol/L $MgCl_2$, 10 mmol/L HEPES–NaOH, and 10 mmol/L glucose. The solution was adjusted to pH 7.4 with NaOH. At the time of measurements, the cell culture medium was replaced with the extracellular solution.

The solutions were diluted to the experimental concentrations immediately before use. Saturated stock solutions of sevoflurane were prepared by adding 50 mL inhaled anesthetic to an airtight balance system with 500 mL extracellular solution. The inhaled anesthetics at saturated concentrations were maintained at 36–37 °C and atmospheric pressure. The solutions were stored for 24 h at 37 °C for equilibration. A four-tube rapid-perfusion system that rapidly activates ligand ion channels was used to apply Ach alone or in combination with sevoflurane, isoflurane, or rocuronium to the whole-cell patches. The drugs were administered by a liquid filament and discharged by a tube connected to an injector pump. Rocuronium was diluted to 5, 10, 20, 50, and 100 nmol/L. The inhaled anesthetic solutions were sealed to prevent evaporation. The anesthetic concentrations in the perfuser apparatus before application were measured by gas chromatography (HP6890, USA). The concentrations of sevoflurane in the extracellular solution were 480, 673, 1010, 1324, 2084, and 3079 μmol/L. The concentrations of isoflurane were 179, 299, 731, 894, 2095, and 2611 μmol/L. Patches were placed at the interface between the filament and a bathing solution, and the compounds were applied repeatedly for 2 s or 1 min with 1–2 min intervals to guarantee full recovery of the channels from desensitization.

Electrophysiological recording

A conventional high-impedance seal of the whole-cell patch technique recorded the Ach-activated currents in HEK293

cells at room temperature (23–25 °C). A tight electrical seal with a resistance of several GΩ was formed between the membrane, and a patch-clamp electrode enabled this transmembrane current to resolve within the pA range.

The recombinant HEK293 cells were placed in a continuous flow recording chamber (25 μL) and superfused with 1–2 mL/min extracellular solution. Pipettes were pulled from borosilicate glass tubes with a 1–5 MΩ-resistance PC-10 electrode puller (Narishige Instrument, Tokyo, Japan). The pipette electrode was filled with a solution containing 140 mmol/L CsCl, 0.8 mmol/L $MgCl_2$, 10 mmol/L HEPES-CsOH, 0.5 mmol/L EGTA, and 4 mmol/L Na-ATP (pH 7.3). The cells were voltage-clamped at a holding potential of −80 mV (Axopatch 200B; Axon Instruments, USA). The signals were transformed to Digidata 1200B (Axon Instruments) then digitized and stored in a computer using PCLAMP 10.0 (USA).

The experiments were conducted to fit the concentration–response curves of the agonists (Ach) and antagonists (rocuronium and inhaled anesthetics). Recombinant cells were perfused using extracellular solutions containing different drug concentrations for 2 s (rocuronium or Ach) or 1 min (inhaled anesthetics). The peak current was obtained as a measure of receptor activity. The control responses of the cells to Ach alone were determined before and after each application of antagonist, and the mean value of these two results was taken as the "average control current", to which the antagonist response was normalized as follows:

$$\%\,\text{inhibition} = 100 \times [1 - (\text{Current in presence of antagonist}/\text{Average control current})] \quad (1)$$

These data were collected to establish the concentration–response curves for inhibition of Ach-induced current and to calculate the 5, 25, and 50 % inhibitory concentrations (IC_5, IC_{25}, and IC_{50}, respectively).

$$Y = \text{START} + (\text{END} - \text{START})/[x^n/(IC_{50})^n + 1] \times 100\% \quad (2)$$

where Y is the fraction of inhibition, START is the value of Y for the minimal response, END is the value of Y for the maximum response, n is the Hill coefficient (steepness factor), and x is the antagonist concentration.

For the combination experiments, the recombinant cells were randomly divided into three groups: roc (rocuronium alone), roc + iso (rocuronium + isoflurane), and roc + sev (rocuronium + sevoflurane). The cells of each group were further randomly divided into three subgroups ($n = 5$) on the basis of rocuronium concentration: IC_5, IC_{25}, and IC_{50}. First, receptors on the cell were activated by use of 10 μmol/L Ach alone for 2 s. The peak current of the ε-nAChR was recorded as the control current. The corresponding drugs in each group were then added to the extracellular solution. The roc group served as the control and was treated with IC_5, IC_{25}, or IC_{50} of rocuronium alone without co-application of inhaled anesthetic. The cells of the roc + iso and roc + sev groups were perfused with an extracellular solution containing 0.5 IC_5 of inhaled anesthetics and IC_5, IC_{25}, or IC_{50} of rocuronium for 1 min. A longer exposure time did not have any additional effect on the current in the cells. The Ach-induced currents were recorded, and each data point represented measurements from five cells. Cells from at least two different batches were used for each experiment. The washout time between each drug application was at least 1–2 min to minimize the amount of desensitization throughout the course of the experiment. The control current in response to Ach alone was recorded again after washout of the antagonist. The responses to Ach alone were determined before and after each antagonist application. The mean value of these two Ach applications was selected as the "average control current" and used to calculate percentage inhibition.

Statistical analysis

The concentration–response curves for rocuronium, isoflurane, and sevoflurane were fitted to the Hill equation by use of Origin software version 8.0 (Origin-Lab, Northampton, MA, USA), and the IC_{50} values were determined. Results are expressed as mean ± SEM or 95 % confidence interval. Statistical significance was assessed by one-way repeated-measures ANOVA followed by ANOVA with Scheffé F testing. $P < 0.05$ was considered statistically significant.

Data analysis in the combination experiments was performed by use of repeated-measures ANOVA followed by ANOVA. Results are expressed as the mean ± SD. Statistical significance groups were compared by use of the *SNK* method. $P < 0.05$ was considered statistically significant.

Results

Concentration of Ach for activating ε-nAChR

Ach at different concentrations was applied for 2 s to HEK293 cells. The data were fitted to the Hill equation. The Ach-induced inward currents were found to be concentration-dependent, and the Ach concentration producing 50 % of the maximum response (EC_{50}) was 13.05 ± 3.14 μmol/L (Fig. 1). For all experiments, the Ach concentration used was 10 μmol/L, which was close to EC_{50} of Ach in ε-nAChR. This concentration ensured robust baseline responses and diminished receptor desensitization caused by repeated Ach application.

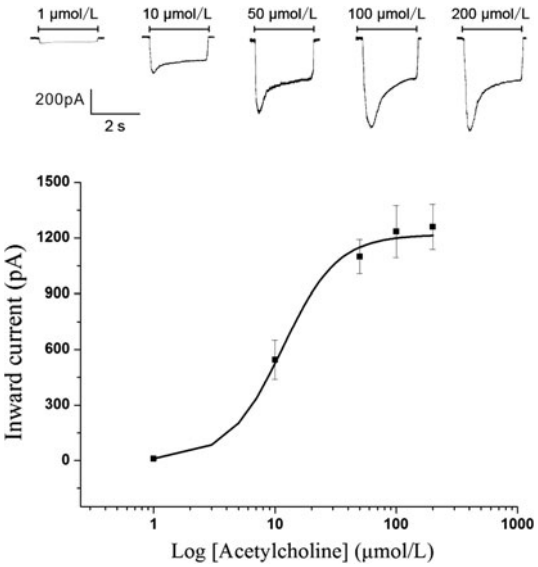

Fig. 1 Acetylcholine produces concentration-dependent inward currents in the recombinant HEK293 cells expressing adult muscle-type acetylcholine receptors. Concentration–response curve for acetylcholine on adult muscle-type acetylcholine receptor is shown. Data points show the mean ± SD for 5 HEK293 cells. Recordings of inward currents from a single HEK293 cell were elicited by 1, 10, 50, 100, and 200 μmol/L acetylcholine

Concentration–response curves for rocuronium, sevoflurane, and isoflurane

The concentration–response curve for rocuronium is shown in Fig. 2a. Rocuronium caused reversible, concentration-dependent inhibition of 10 μmol/L Ach-induced currents. Fitting the concentration responses for rocuronium to the Hill equation yielded IC_{50} values. IC_{50} of rocuronium was 0.023 ± 0.004 μmol/L.

Isoflurane and sevoflurane also inhibited Ach-induced currents in a concentration-dependent manner (Figs. 2b, c). Fitting the concentration responses for isoflurane and sevoflurane to the Hill equation yielded IC_{50} values of 1059.61 ± 62.91 μmol/L and 823.85 ± 48.67 μmol/L, respectively.

The IC_{50} values of isoflurane and sevoflurane were significantly higher than that of rocuronium ($P < 0.01$) (Table 1). At the IC_{50}, inhibition of adult nAChR by sevoflurane was stronger than by isoflurane ($P < 0.05$). The IC_5 and IC_{25} values were calculated from the concentration–response curves (Table 1).

Interaction between isoflurane or sevoflurane and rocuronium on adult muscle-type ε-nAChR

To determine the quality of interaction between inhaled anesthetics and rocuronium on the function of the ε-nAChR, we compared the observed inhibition with the predicted additive inhibition. Table 2 shows that the observed inhibition was far higher than the additive inhibition at any inhaled anesthetic concentration. Both inhaled anesthetics exerted a synergistic effect with rocuronium on inhibition of inward currents. Representative recordings of the raw data for both rocuronium and inhaled anesthetics are shown in Fig. 3.

The inward currents from ε-nAChR induced by 10 μmol/L Ach in the presence of rocuronium at different concentrations are shown in Fig. 4. The 0.5 IC_5 of inhaled anesthetics resulted in strong enhancement of rocuronium-induced inhibition ($P < 0.05$). The peak inward currents under control conditions depended on the concentration of rocuronium. When rocuronium was administered at IC_5, inhibition was stronger with isoflurane than with sevoflurane ($P < 0.05$). In the presence of IC_{25} or IC_{50}, no significant difference was observed between sevoflurane and isoflurane ($P > 0.05$; Fig. 4).

Synergistic effect between isoflurane or sevoflurane and rocuronium on ε-nAChR

To quantify the synergistic effect between inhaled anesthetics and rocuronium on the function of ε-nAChR, we used the previously described ratio of observed inhibition to predicted inhibition [17, 18] to assess the strength of synergism:

$$\text{Ratio of synergism} = \text{observed inhibition} \,/\, \text{predicted inhibition}$$

The predicted response was calculated as the response to rocuronium × response to inhaled anesthetics. If IC_5 of inhaled anesthetics and IC_{25} of rocuronium were used, the response would be $0.95 \times 0.75 = 0.72$. The predicted inhibition was approximately 28 %, and the observed inhibition was obtained from the combination experiments.

The results are shown in Table 2. The inhaled anesthetics synergistically enhanced the rocuronium-mediated inhibition at the lowest concentration (IC_5). The ratio of synergism was the highest in the groups. The synergistic effect was less intense when rocuronium was used at higher concentrations (IC_{25} and IC_{50}) ($P < 0.05$). Comparison of the roc + sev and roc + iso groups reveals that the synergistic effect was stronger with isoflurane than with sevoflurane when rocuronium was administered at IC_5 ($P < 0.05$). However, no difference was observed between isoflurane and sevoflurane when rocuronium was administered at IC_{25} and IC_{50} ($P > 0.05$).

Discussion

Our results showed that both 0.5 IC_5 of sevoflurane and isoflurane had strong synergistic effects with rocuronium on

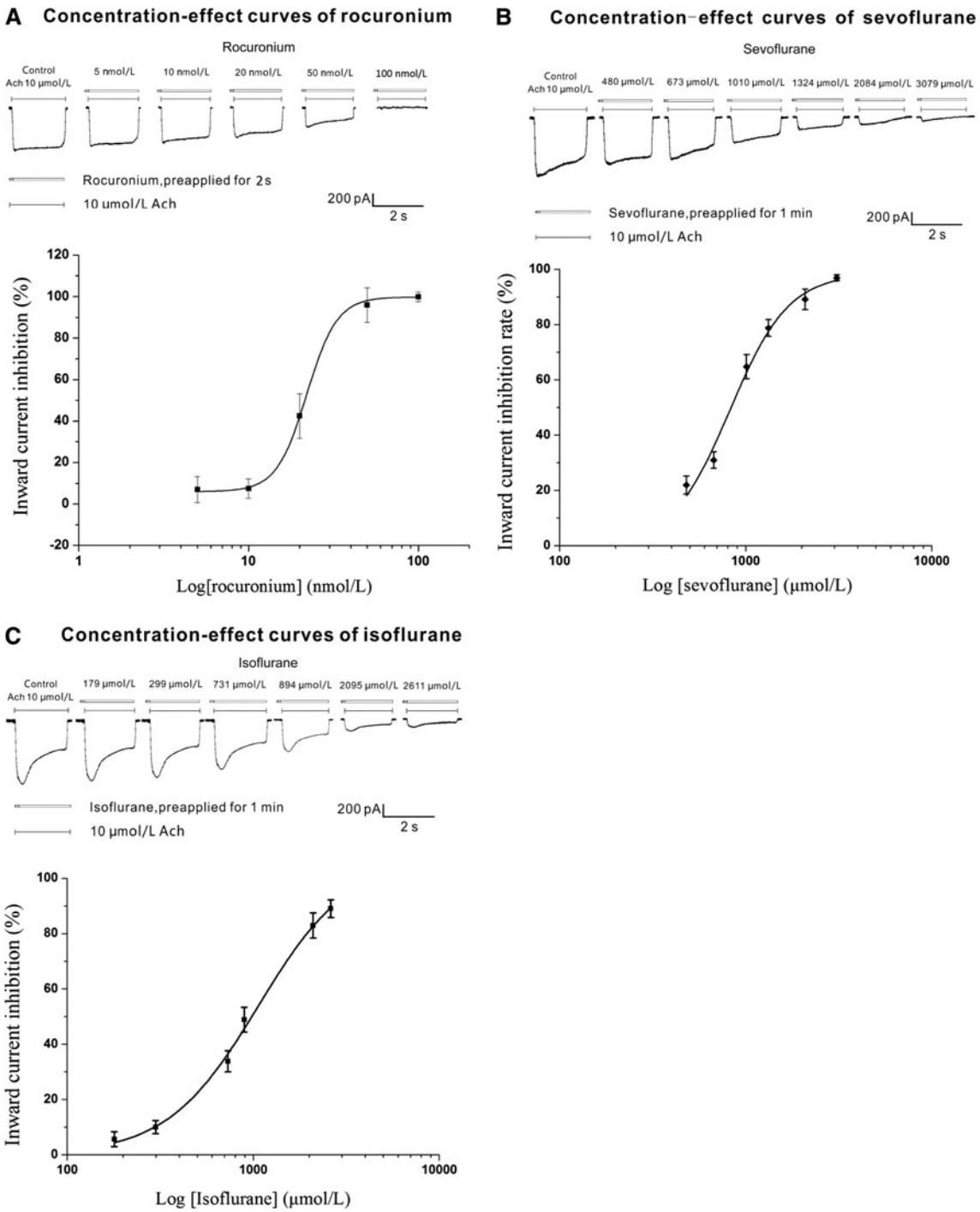

Fig. 2 Concentration–response curves for rocuronium (**a**), sevoflurane (**b**), and isoflurane (**c**) for inhibition of 10 μmol/L acetylcholine-induced currents in HEK293 cells expressing adult-type muscle nicotinic acetylcholine receptors. Each point represents the mean ± SD of the relative peak amplitude (relative peak amplitude activated by 10 μmol/L acetylcholine = 1) from 5 cells. The lines are unweighed least-square fits of the mean peak currents to a Hill equation (Eq. 2)

the inward-current inhibition of nAChR. The synergistic effects were also more intense with low-concentration rocuronium, and isoflurane was more potent than sevoflurane.

We first determined the effects of sevoflurane, isoflurane, and rocuronium individually on ε-nAChR function and established the concentration–response curves for inhibition of the Ach-induced current. Isoflurane and sevoflurane reversibly reduced the peak current amplitude in a concentration-dependent manner. Isoflurane and sevoflurane at the IC_{50} inhibited adult nAChRs. These

Table 1 Results from fitting the equilibrium concentration–response curves (Fig. 2) to the Hill equation

	IC_{50} (μmol/L), mean ± SE	95 % Confidence interval for IC_{50} (μmol/L)		Hill coefficient (*n*), mean ± SE	IC_5 (μmol/L)	IC_{25} (μmol/L)
		Lower bound	Upper bound			
Rocuronium	0.023 ± 0.004	0.018	0.028	2.41 ± 0.40	0.004	0.015
Sevoflurane	823.85 ± 48.67[a]	783.36	865.17	2.75 ± 0.39	281.83	558.75
Isoflurane	1059.61 ± 62.91[a, b]	856.85	1206.22	1.77 ± 0.18	203	547.48

The total number of data for the drug series (*n*) were collected from 5 cells. Best fit values are given as mean ± SD. The IC_{50} of rocuronium, sevoflurane, and isoflurane were tested for statistical significance (see "Materials and methods") with the corresponding data from the drugs. The IC_{50} of rocuronium was significantly smaller than those of the inhaled anesthetics (vs. roc group, $^{a}P < 0.01$). The value for sevoflurane was significantly different from that for isoflurane (vs. sev group, $^{b}P < 0.05$; vs. iso group, $^{c}P < 0.05$)

Table 2 The ratio of synergism between inhaled anesthetics and rocuronium on the function of the adult muscle-type acetylcholine receptor

Concentration of rocuronium	0.5 × IC_5 sevoflurane + rocuronium				0.5 × IC5 isoflurane + rocuronium			
	Additive inhibition (%)	Observed inhibition (A, %)	Predicted inhibition (B, %)	Ratio (=A/B)	Additive inhibition (%)	Observed inhibition (A, %)	Predicted inhibition (B, %)	Ratio (=A/B)
IC_5	9	51.46 ± 3.70	9	5.21 ± 0.04	9	57.63 ± 3.11[a]	9	6.10 ± 0.06[a]
IC_{25}	29	73.37 ± 2.83[b]	28	2.73 ± 0.03[b]	29	71.22 ± 5.16[b]	28	2.42 ± 0.21[b]
IC_{50}	54	71.70 ± 4.08[b]	52	1.27 ± 0.08[b,c]	54	74.46 ± 4.64[b]	52	1.43 ± 0.13[b,c]

The ratios of synergism were tested for statistical significance (see "Results") with the corresponding data from the drugs. Values are given as mean ± SD. The synergistic effect was stronger with isoflurane than with sevoflurane when the rocuronium was administered at the IC_5 (vs. sevoflurane group; $^{a}P < 0.05$). 0.5 × IC_5 of inhaled anesthetics induced the largest synergistic effect with IC_5 of rocuronium (vs. IC_5 group $^{b}P < 0.05$; vs. IC_{25} group $^{c}P < 0.05$). 0.5 × IC_5 sevoflurane ≈ 4 % inhibitory concentration; 0.5 × IC_5 isoflurane ≈ 4 % inhibitory concentration vs. sevoflurane group

results were similar to those reported for clonal BC3H-1 cells [19, 20]. The inhaled anesthetics were much less potent inhibitors of the Ach-induced current than rocuronium, as reflected by their IC_{50} values, which were several times higher than that of rocuronium. The concentration–response curves for sevoflurane and isoflurane had a point of intersection. As calculated from the fitting equation, the intersection had approximate coordinates of (522.87, 28.27). Thus, when the sevoflurane and isoflurane concentrations were below 522.87 μmol/L, the potential of isoflurane was higher than that of sevoflurane. By contrast, when the concentrations were above 522.87 μmol/L, the potential of sevoflurane was stronger.

To compare the synergistic effects among the different drugs, we applied equipotent concentrations with regard to their nAChR-blocking ability. A 0.5 IC_5 concentration of inhaled anesthetics was used in the combination experiments, and resulted in approximately 4 % inhibition of the receptors (calculated from the Hill equation). About 1 MAC isoflurane and sevoflurane, similar aqueous concentrations of 270 and 300 μmol/L, respectively, were achieved [21]. The applied 0.5 IC_5 concentrations of isoflurane (100 μmol/L) and sevoflurane (140 μmol/L) in aqueous solution corresponded to approximately 0.3 and 0.4 MAC isoflurane and sevoflurane, respectively. The residual inhaled anesthetic concentration was considered to be less than 0.4 MAC [9]. Therefore, 0.5 IC_5 of sevoflurane and isoflurane was equivalent to the residual inhaled anesthetic concentrations in patients in a postanesthesia care unit. However, according to a previous study, less than 0.4 MAC of inhaled anesthetics cannot induce neuromuscular blockade [22]. On the other hand, only 25 % of functional nicotinic receptors are required to activate an action potential by the end-plate potential according to the safe-margin theory [23, 24]. In this study, the function of neuromuscular transmission began to decline significantly when >75 % of Ach receptors on the postsynaptic membrane were blocked. Therefore, IC_{75} of drug in an in-vitro model can be regarded as the minimum effective dose inducing neuromuscular blockade. Similarly, three concentrations of rocuronium (4, 15, and 23 nmol/L) inhibited <75 % of the receptors, and consequently induced slight neuromuscular blockade. Nevertheless, when rocuronium was combined with isoflurane or sevoflurane at 0.5 IC_5, the inhibitory effect was strongly enhanced. The number of occupied receptors was almost 75 % for this drug combination (Fig. 4), which was sufficient to induce neuromuscular blockade.

The mechanism of the synergistic effect between inhaled anesthetics and NMBAs remains unknown. The different synergistic effects were related to the allosteric modulation of receptor induced by the interaction among different

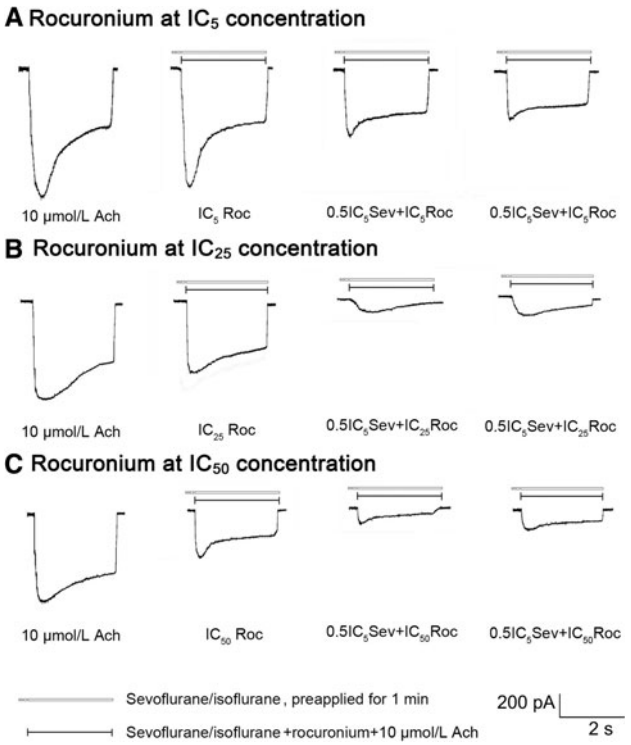

Fig. 3 Inhibition of acetylcholine-induced currents of the adult-type muscle nicotinic acetylcholine receptors expressed in HEK293 cells by rocuronium alone and by rocuronium in combination with isoflurane or sevoflurane. Traces represent the raw currents observed during application of 10 μmol/L Ach for 2 s, either alone (as control) or in combination with IC_5, IC_{25}, or IC_{50} of rocuronium with or without 0.5 IC_5 of the inhaled anesthetics (co-applied for 1 min) as indicated

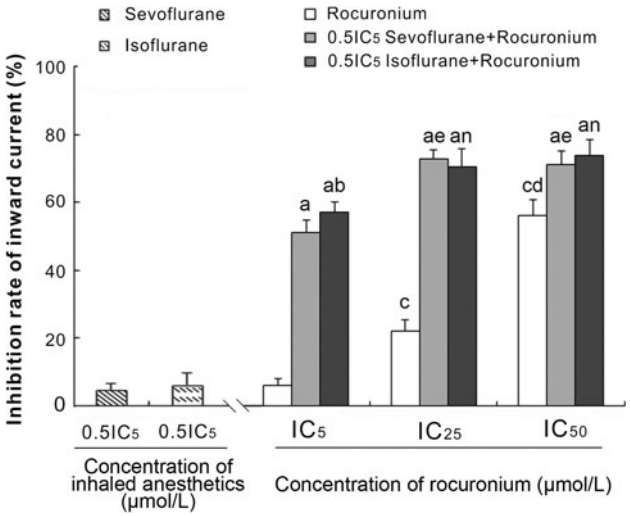

Fig. 4 Sevoflurane and isoflurane-associated enhancement of the percentage inhibition of 10 μmol/L acetylcholine-induced currents by rocuronium. Three concentrations of rocuronium (IC_5 4 nmol/L, IC_{25} 15 nmol/L, and IC_{50} 23 nmol/L) were administered alone (*white bars*) and in the presence of 0.5 IC_5 of isoflurane (100 μmol/L; *black bars*) or sevoflurane (140 μmol/L; *grey bars*). Isoflurane or sevoflurane significantly enhanced the current-blocking effect of rocuronium (vs. roc group in same IC level; $^{a}P < 0.01$). Isoflurane had a stronger enhancing effect than sevoflurane for the IC_5 of rocuronium (vs. sev group at the same IC level; $^{b}P < 0.05$). Rocuronium alone had an inhibitory effect in a concentration-dependent manner (vs. IC_5 subgroup in roc group $^{c}P < 0.05$; vs. IC_{25} subgroup in roc group $^{d}P < 0.05$). Combined with sevoflurane or isoflurane, IC_5 of rocuronium had a slightly weaker inhibitory effect than IC_{25} and IC_{50} of rocuronium (vs. IC_5 subgroup in sev group, $^{e}P < 0.05$; vs. IC_5 subgroup in iso group, $^{n}P < 0.05$)

binding regions of drugs [25]. NMBAs are reportedly located at the interface of the α–δ and α–ε subunits in the extracellular domain of the receptor [26]. In fact, more than one binding site for inhaled anesthetics exist in the nAChR. Specifically, isoflurane persistently binds to three classes of site in the nAChR transmembrane domain:

1. an isoflurane dimer occluding the pores;
2. several nAChR subunit interfaces; and
3. subunit centers of both nAChR α chains [27].

The allosteric modulation of receptors induced by inhaled anesthetics can enhance the binding affinity of NMBAs with nAChRs [16, 28]. In our study, when rocuronium was administered at IC_5, the inhibitory effect was stronger in combination with isoflurane than with sevoflurane. This result indicated that the activity of isoflurane, which induced allosteric modulation of the receptor, was stronger than that of sevoflurane. Nevertheless, with increased rocuronium concentration, the synergistic effect was gradually hidden by the effect of rocuronium. Thus, the differences between sevoflurane and isoflurane were not observed at higher concentrations of rocuronium.

Our results partially provide the reasons for the occurrence of residual neuromuscular blockade in the postanesthesia care of patients intraoperatively administered inhaled anesthetics. During the clinical postanesthesia recovery period, even if the concentrations of inhaled anesthetic and NMBA decreased, the synergistic effect of inhaled anesthetics and NMBAs can block a large number of receptors, inducing muscle relaxation. Postanesthetic hyperpnea is beneficial because it shortens the postanesthesia care unit stay after anesthesia inhalation [29, 30]. This phenomenon can be attributed to acceleration of the complete exclusion of inhaled anesthetics. Therefore, monitoring and pharmacological reversal of NMBAs should be routinely practiced, particularly for patients intraoperatively administered inhaled anesthetics.

In conclusion, we quantified the effects of sevoflurane and isoflurane on the binding of rocuronium with skeletal muscle ε-nAChR. Our findings showed that inhaled anesthetics strongly enhanced the inhibitory effect of rocuronium. Thus, rocuronium below effective concentrations in combination with low-concentration inhaled anesthetics can induce intense neuromuscular blockade. These findings

also provide the reason for the increased incidence of residual neuromuscular blockade in postanesthesia care patients intraoperatively administered inhaled anesthetics.

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