J Anesth (2007) 21:171–175
DOI 10.1007/s00540-006-0478-6

Journal of
Anesthesia

Succinylcholine potentiates acetylcholine-induced contractile and phosphatidylinositol responses of rat trachea

Kenji Nishioka, Osamu Shibata, Masakazu Yamaguchi, Maki Yoshimura, Tetsuji Makita, and Koji Sumikawa

Department of Anesthesiology, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Abstract

Purpose. Although succinylcholine (SCh) is often used as a muscle relaxant in electroconvulsive therapy, its influence on airway reactivity has not been fully investigated. We examined the effects of SCh on acetylcholine (ACh)-, carbachol (CCh)-, and electrical field stimulation (EFS)-induced contractions, and on the ACh-induced phosphatidylinositol (PI) response of rat trachea.

Methods. Thirty-two male Wistar rats weighing 250–350 g were used. The trachea was rapidly isolated and cut into 3-mm-wide rings. The resting tension was adjusted periodically to 1.0 *g* during the equilibration period. ACh, 1 μM; carbachol (CCh), 0.05 μM; or neither of them, was added, and SCh was then added at 1–300 μM final concentrations, and ring tension was examined. Contractions were elicited by EFS in the presence or absence of 100 μM SCh. Tracheal slices were incubated with [^{3}H] *myo*-inositol, 1 μM ACh, and various concentrations of SCh. The accumulation of [^{3}H] inositol monophosphate (IP_1) was measured.

Results. SCh did not affect the tension by itself without ACh, or with CCh, but SCh potentiated the ACh-induced contraction of rat trachea at concentrations of 10 μM or more (50% effective concentration [EC_{50}]; 43.6 μM). SCh produced a significant increase in the amplitude and duration of EFS-induced contractions. SCh, at concentrations of 10 μM and 100 μM, potentiated ACh-induced IP_1 accumulation.

Conclusion. SCh potentiated ACh-induced, but not CCh-induced, contractile and PI responses, and enhanced EFS-induced contraction of rat trachea, suggesting that competition for butyrylcholinesterase (BChE) in airway smooth muscle could be involved in the potentiation by SCh of ACh-induced airway smooth muscle contraction.

Key words Airway smooth muscle · Contractile response · Succinylcholine

Address correspondence to: O. Shibata
Received: March 15, 2006 / Accepted: November 1, 2006

Introduction

In electroconvulsive therapy (ECT), complications arise chiefly from the activation of the sympathetic nervous system [1], whereas the parasympathetic nervous system does not seem to be affected predominantly. However, some reports suggest that bronchospasm could occur, possibly resulting from parasympathetic stimulation, during ECT [2]. Although succinylcholine (SCh) is often used as a muscle relaxant in ECT [1], its influence on airway reactivity has not been fully investigated. Kaise et al. [3] studied a canine model, and reported that SCh probably enhanced airway reactivity to intravenous acetylcholine (ACh). Although the mechanism was not clear, they suggested that SCh might compete for plasma butyrylcholinesterase (BChE) against ACh, resulting in enhancement of ACh's action. We hypothesized that the enhancement by SCh of ACh's action could occur at the airway level, because ACh is subjected to the activities of acetylcholinesterase (AChE) and BChE. It has been demonstrated that BChE also exists on the surface of airway smooth muscle, and that the total cholinesterase activity in tracheal smooth muscle consists of BChE and AChE activities [4]. The physiological role of BChE on the surface of airway smooth muscle is unclear [4–7]. SCh may also modify BChE activity, resulting in the potentiation of airway reactivity to ACh. Thus, we examined the effects of SCh on ACh-induced and electrical field stimulation (EFS)-induced contractions, and on the ACh-induced phosphatidylinositol (PI) response of rat trachea.

Materials and methods

The studies were conducted under guidelines approved by the Institutional Animal Care Committee. Thirty-two male Wistar rats, weighing 250–350 g, were used in the experiments. The rats were exsanguinated under

anesthesia with 50 mg·kg^{-1} intraperitoneal pentobarbital, and the trachea was rapidly isolated.

Contractile responses

The trachea was chopped into 3-mm-wide rings with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, UK). The tracheal rings were placed between two stainless steel hooks in a 5-ml water-jacketed organ chamber (Kishimotoika, Kyoto, Japan) containing Krebs-Henseleit (K-H) solution (composition in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 1.3; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11; Na_2-ethylenediameine tetraacetic acid [EDTA] 0.05). The solution was continuously aerated with 95% O_2 and 5% CO_2, and the pH was maintained at approximately 7.4 and the temperature at 37ºC. Isometric tensions were measured using an isometric transducer (Kishimotoika) and recorded using a MacLab system (Milford, MA, USA). The resting tension was adjusted periodically to 1.0 *g* during the equilibration period. The rings were washed every 15 min and re-equilibrated to resting tension for 60 min. First, the ring tension was examined by stepwise cumulative additions of SCh, at 1–300 µM final concentrations. The tracheal rings were divided into three groups; each group received ACh, 1 µM; CCh, 0.05 µM; or neither of them, 10 min before SCh administration. The dose of ACh was determined from the concentration required to cause almost 30% of maximum contraction, and that of CCh was determined from the potency nearly equal to 1 µM ACh to induce a contractile response. Second, after the equilibration period, contractions were elicited by EFS in the presence or absence of 100 µM SCh, to examine the effect of SCh on the contraction induced by endogenous ACh. Electrical stimuli were generated by a SEN-7203 stimulator (Nihon Kohden, Tokyo, Japan) and applied between two platinum electrodes. Pulses of 2 ms and 50 V were delivered at frequencies of 5–20 Hz for 10 s, and a 5-min recovery period was allowed between successive trains.

Phosphatidylinositol (PI) response

The technique of Brown et al. [8] was used to measure PI response. Inositol 1,4,5-trisphosphate (IP_3) is rapidly degraded into inositol monophosphate (IP_1) and subsequently recycled back to PI via free inositol. Lithium inhibits the conversion of IP_1 to inositol. In the presence of Li^+, the accumulation rate of IP_1 reflects the extent of the PI response. We measured [^{3}H] IP_1 in tracheal slices incubated with [^{3}H] *myo*-inositol (Amersham, Tokyo, Japan) [9–11]. The trachea was cut longitudinally and chopped into 1-mm-wide slices with McIlwain tissue chopper. Three tracheal slices were placed in small flat-bottomed tubes and pre-incubated for 15 min in K-H solution containing 5 mM LiCl. The solution was continuously aerated with 95% O_2 and 5% CO_2. An aliquot of 0.5 µCi [^{3}H] *myo*-inositol was then added to each tube (final concentration of 0.1 µM in 300-µl incubation volume) and the tubes were flushed with 95% O_2 and 5% CO_2, capped, set in a shaking bath at 37ºC, and then incubated for 30 min (time 0). To confirm the activation by SCh of the PI response mediated through muscarinic receptors, the effects of SCh on the ACh-induced IP_1 accumulation in rat trachea were examined. At time 0, the tracheal slices were incubated with varying doses (0–100 µM) of SCh for 15 min. Then, ACh, at 1 µM final concentration, was added. The tubes were re-aerated with 95% O_2 and 5% CO_2, re-capped, and re-incubated. After an additional 60-min incubation, the reaction was stopped with 940 µl of chloroform/methanol (1:2; v/v). Chloroform and water were then added (300 µl each) and the phases were separated by centrifugation at 90 *g* for 10 min. The [^{3}H] IP_1 was separated from [^{3}H] *myo*-inositol in the 750-µl water phase by column chromatography, using Dowex AG 1-X8 resin (Bio-Rad, Richmond, CA, USA) in the formate form. The [^{3}H] IP_1 formed in the tracheal slices was counted with a liquid scintillation counter and measured in becquerels (Bq). The scintillation counts of the blank values (no slices present) were subtracted to obtain the experimental data.

Statistical analysis

Values were expressed as means ± SD. The results for the EFS data were analyzed by paired *t*-test. The other results were analyzed by one-way analysis of variance. A value of $P < 0.05$ was considered statistically significant.

Results

Contractile responses

Figure 1a shows a typical recording of the effects of SCh on ACh- or CCh-induced contraction of rat trachea. A typical recording of the effects of SCh on the EFS-evoked contraction of rat trachea is shown in Fig. 1b. SCh, without ACh or with CCh, did not affect the tension, but SCh potentiated the ACh-induced contraction of rat trachea at concentrations of 10 µM or more (EC_{50}, 43.6 µM; Fig. 2). Figure 3a, b shows the effect of SCh on the EFS-evoked contraction of rat tracheal rings. SCh produced a significant increases in the tension (Fig. 3a) and the half-relaxation time (Fig. 3b), of the EFS-induced contraction; the half-relaxation

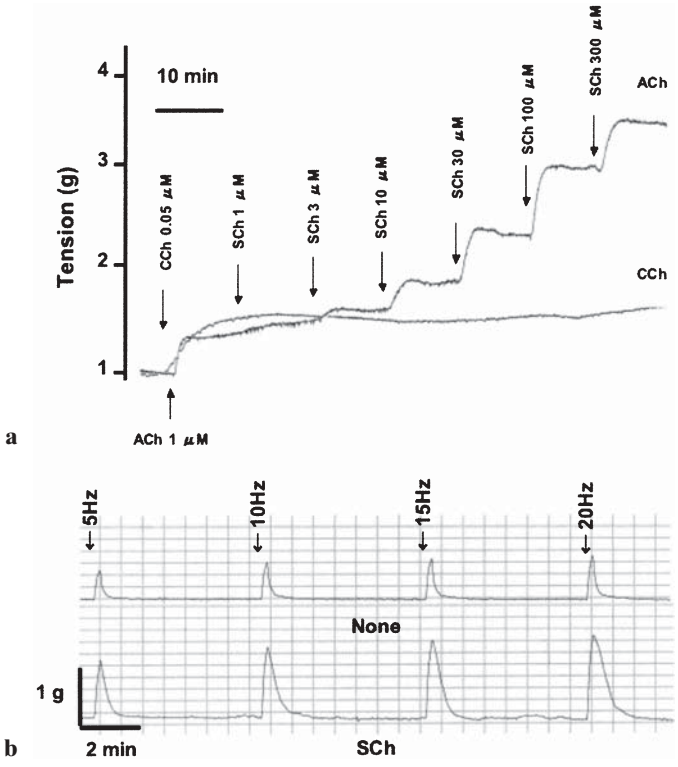

Fig. 1. **a** A typical recording of the effects of succinylcholine (*SCh*) on acetylcholine (*ACh*)- or carbachol (*CCh*)-induced contraction of rat trachea. **b** A typical recording of the effect of SCh on electrical field stimulation (EFS)-induced contraction of rat trachea. SCh, at 100 μM, was added in the second train

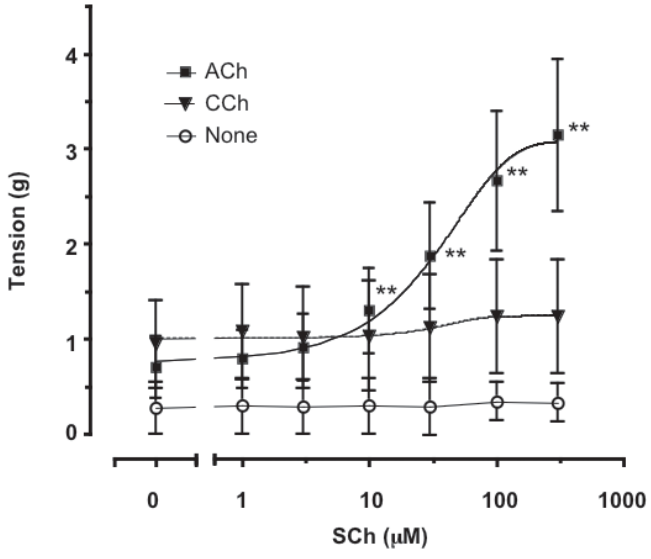

Fig. 2. Effects of succinylcholine (*SCh*) on acetylcholine (*ACh*)- and carbachol (*CCh*)-induced contractions (mean ± SD; $n = 6$, ** $P < 0.001$ vs SCh 0)

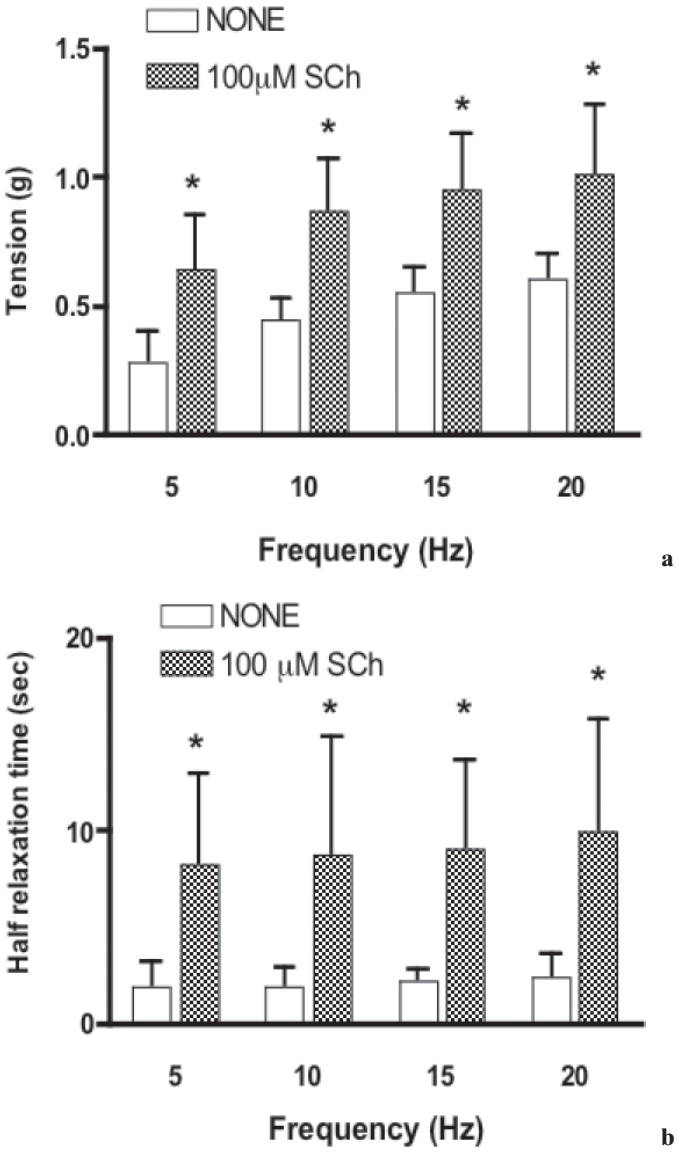

Fig. 3a,b. Effects of succinylcholine (*SCh*) on electrical field stimulation (EFS)-evoked contraction of rat trachea. SCh produced significant increases in the amplitude (**a**) and duration (**b**) of the EFS-induced contraction at each frequency (mean ± SD; $n = 7$ or 8; *$P < 0.05$, by paired *t*-test). *Half-relaxation time*, the time from the peak to half-maximum tension

time being the time from the peak to half-maximum tension.

Phosphatidylinositol (PI) response

The basal IP_1 accumulation, in which no drug was administered, was 3.24 ± 0.29 Bq. ACh, at 1 μM, increased the IP_1 accumulation to 4.76 ± 0.47 Bq, and SCh potentiated the ACh-induced IP_1 accumulation to 6.87 ± 1.05 Bq and 7.95 ± 0.81 Bq at concentrations of 10 μM and 100 μM, respectively (Fig. 4).

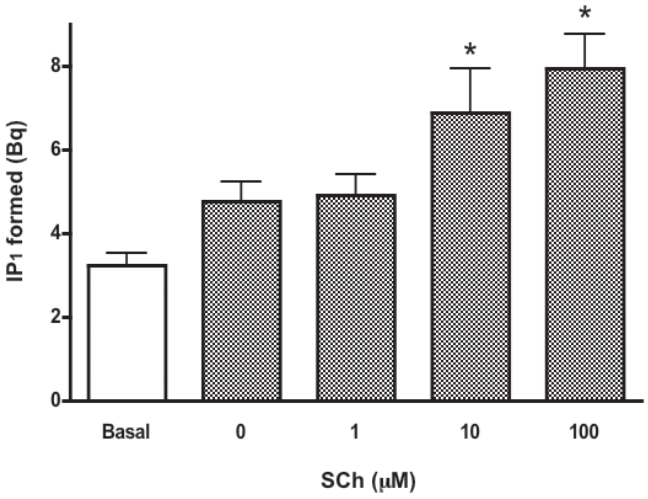

Fig. 4. Effects of succinylcholine (SCh) on acetylcholine-induced inositol monophosphate (IP_1) formation (mean ± SD; $n = 8$; * $P < 0.05$ vs SCh 0). *Bq*, becquerel

Discussion

The present results show that SCh potentiates the ACh-induced contractile and PI responses of rat trachea. Although the mechanism involved in this potentiation has not been fully clarified, the following explanations could be advanced.

Koga et al. [12] reported that SCh produced large increases in endotracheal tube cuff pressure in anesthetized dogs. Either vagotomy or intravenous atropine or hexamethonium prevented this response, and SCh did not constrict canine tracheal smooth muscle in vitro. Thus, they concluded that the tracheal contraction induced by SCh could result from stimulation of the parasympathetic nerves rather from than a direct action on the tracheal smooth muscle. Kaise et al. [3], using a fiberoptic bronchoscope technique, showed that SCh increased the canine tracheal smooth muscle contraction induced by intravenous ACh, and that this increase was not completely abolished by hexamethonium. In addition, SCh did not increase the contraction induced by intravenous methacholine or aerosolized ACh. These findings suggest that the mechanism of SCh-induced tracheal smooth muscle contraction is via both stimulation of the parasympathetic nervous system, and inhibition of plasma BChE.

However, the physiological role of BChE is not fully clarified. BChE and AChE could complement each other, and recent reports suggest that the physiological role of BChE is greater than previously supposed [6,7]. Li et al. [6] proposed that BChE has a natural physiological function at certain sites and, more generally, serves as a backup to AChE in supporting and regulating cholinergic transmission. In fact, BChE was shown to modulate synaptic transmission in the smooth muscle of the canine trachea [13] and the human lung [5]. Adler et al. [4] reported that the total cholinesterase activity in canine tracheal smooth muscle consisted of BChE and AChE at a ratio of 3:1, and that most of these enzymes were distributed on the muscle surface. BChE is also present on the smooth muscle and coregulates the degradation of ACh in human trachea [5]. The present results show that SCh potentiates the ACh-induced, but not the CCh-induced contractile response of rat trachea. This potentiation was confirmed by the increase in the PI response, which represents muscarinic receptor activation. Because CCh is not broken down by cholinesterase, SCh probably enhances airway reactivity to exogenous ACh by competing for BChE on the smooth muscle.

Furthermore, in the present study, SCh also produced a significant increase in the amplitude and duration of EFS-induced contractions. Adler et al. [14] examined the role of AChE and BChE in the regulation of ACh hydrolysis, by evaluating the effects of BW284C51 (a selective AChE inhibitor) and tetraisopropylpyrophosphoramide (iso-OMPA) (a selective BChE inhibitor) on the contractile responses induced by EFS. They found that the amplitude and duration of EFS-induced contractions were significantly increased in the presence of these inhibitors. The degree of enhancement was greatest in the presence of both inhibitors, as compared to each inhibitor alone. Thus, it is likely that ACh is subjected to both AChE and BChE in the airway.

The doses of SCh used in ECT in clinical practice are 0.5–1.5 mg·kg^{-1} [2]. It has been reported that the plasma concentration of SCh is in a range of 5–10 μg·ml^{-1} (13–25 μM) at 60 s after intravenous administration of 1–2 mg·kg^{-1} in humans [15]. In the present study, the concentration of SCh required to cause the potentiation of ACh-induced contraction did not deviate from the clinical range. Thus, it should be noted that SCh may potentiate bronchoconstriction during ECT in patients with bronchial asthma.

In conclusion, SCh potentiated ACh-induced, but not CCh-induced contractile and PI responses, and enhanced EFS-induced contraction in rat trachea, suggesting that competition for BChE in airway smooth muscle could be involved in the potentiation by SCh of the ACh-induced airway smooth muscle contraction.

Acknowledgments. This study was supported in part by Grant 15591638 for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

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