

## Succinylcholine Metabolite Succinic Acid Alters Steady State Activation in Muscle Sodium Channels

Gertrud Haeseler, M.D.,\* Jacqueline Petzold,† Hartmut Hecker, M.D.,‡ Axel Würz, M.D.,§ Reinhard Dengler, M.D.,|| Siegfried Piepenbrock, M.D.,# Martin Leuwer, M.D.\*\*

**Background:** Animal experiments revealed that succinylcholine produced masseter muscle rigidity and activated myotonic discharges despite neuromuscular blockade with a nondepolarizing blocker. These results suggest that either succinylcholine or its metabolites might interfere directly with voltage-operated ion channels of the sarcolemma. The aim of this study was to examine effects of one product of succinylcholine hydrolysis, succinic acid, on voltage-gated muscle sodium ( $\text{Na}^+$ ) channels.

**Methods:** Alpha subunits of human muscle sodium channels were heterologously expressed in HEK293 cells. Activation of  $\text{Na}^+$  currents was examined applying standard whole-cell voltage-clamp protocols in the absence (control and washout) and presence of succinic acid in different concentrations (0.05–10 mM).

**Results:** Succinic acid shifted the midpoints of steady state activation plots in the direction of more negative test potentials, indicating that channels open during smaller depolarizations in the presence of the drug. The maximum amount of the negative shift in 10 mM succinic acid was  $-6.3 \pm 1.7$  mV; the  $\text{EC}_{50}$  for this

effect was 0.39 mM. In addition, succinic acid (10 mM) significantly enhanced maximum currents after depolarizations with respect to a series of control experiments.

**Conclusion:** Succinic acid facilitates voltage-dependent activation in muscle sodium channels *in vitro*. This might lead to muscle hyperexcitability *in vivo*. (Key words: Depolarizing muscle relaxant; muscle hyperexcitability.)

WHEN rapid insertion or change of an endotracheal tube in emergency situations is required, the depolarizing muscle relaxant succinylcholine is still a drug of choice. Nevertheless, some of the complications associated with the use of the drug are immediately life-threatening and range from myotonic reactions that obstruct adequate ventilation<sup>1</sup> to massive hyperkalemia and cardiac arrest.<sup>2,3</sup> Both myotonia and hyperkalemia reflect muscle hyperexcitability in response to succinylcholine. Analogous to the initial hyperkalemia observed at the onset of muscle exercise,<sup>4,5</sup> the amount of drug-induced hyperkalemia is probably related to the degree of muscle activity. Initial serum  $\text{K}^+$  increase after depolarization is attributed to  $\text{K}^+$  release from delayed rectifier  $\text{K}^+$  channels during the repolarization phase of each sarcolemmal action potential along with the failure of the  $\text{Na}^+$ ,  $\text{K}^+$ -adenosine triphosphatase to keep pace with the  $\text{K}^+$  loss.<sup>4</sup> Muscle hyperexcitability in response to succinylcholine is generally attributed to up-regulation of acetylcholine receptors expanding the area of chemosensitivity. Although this mechanism seems to play the crucial role in patients with neurologic disorders of immobilization (see review by Martyn *et al.*<sup>3</sup>), aberrant response to succinylcholine in some patients with massive trauma<sup>6,7</sup> and exsanguinating hemorrhage<sup>8</sup> cannot be fully explained. In addition, animal experiments revealed that succinylcholine produced masseter muscle rigidity<sup>9</sup> and activated myotonic discharges<sup>10</sup> despite neuromuscular blockade. Elevation of serum  $\text{K}^+$  in humans was related to the dose of succinylcholine administered, independent of pretreatment with a nondepolarizing blocker.<sup>11</sup> These results suggest that either succinylcholine or the products of its hydrolysis might

\* Resident, Department of Anesthesiology, Hannover Medical School.

† Postgraduate Trainee, Department of Anesthesiology, Hannover Medical School.

‡ Professor, Department of Biometrics, Hannover Medical School.

§ Resident, Department of Neurology and Neurophysiology, Hannover Medical School.

|| Professor and Chair, Department of Neurology and Neurophysiology, Hannover Medical School.

# Professor and Chair, Department of Anesthesiology, Hannover Medical School.

\*\* Professor and Chair, Department of Anaesthesia, The University of Liverpool.

Received from the Departments of Anesthesiology, Neurology and Neurophysiology, and Biometrics, Hannover Medical School, Hannover, Germany; and University Department of Anaesthesia, The University of Liverpool, Liverpool, United Kingdom. Submitted for publication July 2, 2000. Accepted for publication December 17, 1999. Support was provided solely from institutional and/or departmental sources. Presented in part at the annual meeting of the Anaesthetic Research Society, London, United Kingdom, November 20–21, 1997, and was published in abstract form (Br J Anaesth 1998; 80:561P).

Address reprint requests to Dr. Haeseler: Department of Anesthesiology, OE8050, Hannover Medical School, 30623 Hannover, Germany. Address electronica mail to: Haeseler.Gertrud@MH-Hannover.de

interfere directly with voltage-operated ion channels of the sarcolemma, modulating the response to drug-induced depolarization. Because no such effects could be shown for succinylcholine,<sup>12</sup> the aim of this study was to investigate possible excitatory effects of the succinylcholine metabolite succinic acid on human muscle sodium ( $\text{Na}^+$ ) channel function.

## Methods

### Molecular Biology

Wild-type  $\alpha$  subunits of human skeletal muscle sodium channels (hSkM1<sup>13</sup>) were heterologously expressed in human embryonic kidney (HEK293) cells (American Tissue Culture Collection CRL 1573). The plasmid pRc (Invitrogen, San Diego, CA) was used for mammalian transfection. Plasmids containing the  $\alpha$  subunits were transfected into HEK293 cells using the calcium phosphate precipitation method.<sup>14</sup> Permanent expression was achieved by selection for resistance to the aminoglycoside antibiotic geneticin G418 (Life Technology, Eggenstein, Germany).<sup>15</sup> Transfected cells were a gift from Professor Lehmann-Horn, Ulm, Germany. The clone has been used in several investigations.<sup>15,16</sup> Successful transfection and expression was confirmed electrophysiologically.

### Solutions

Each experiment consisted of recordings in bath solution (control and washout) and in test solution containing succinic acid (Sigma Chemicals, Deisenhofen, Germany) in different concentrations (0.05–10 mM) derived from a 10-mM stock in bath solution. Patch electrodes contained 130 mM  $\text{CsCl}_2$ , 2 mM  $\text{MgCl}_2$ , 5 mM EGTA, and 10 mM Hepes; bath solution contained 140 mM  $\text{NaCl}$ , 1 mM  $\text{MgCl}_2$ , 4 mM  $\text{KCl}$ , 2 mM  $\text{CaCl}_2$ , 5 mM Hepes, and 5 mM dextrose. Succinic acid-containing solutions were adjusted to pH 7.4 by addition of  $\text{CsOH}$ , and osmolality was at 290 mOsm. Temperature during data acquisition was maintained at 20°C (Temperature Control System, List Medical, Darmstadt, Germany).

### Current Recordings and Analysis

HEK cells also express endogenous  $\text{Na}^+$  channels that conduct with amplitudes ranging from 50 to 350 pA (mean,  $112 \pm 12$  pA).<sup>15</sup> To minimize a possible contribution of endogenous  $\text{Na}^+$  channels, but also to avoid series resistance errors, we only analyzed currents with peaks ( $I_{\text{peak}}$ ) ranging between 1.5 and 6 nA.

Standard whole-cell voltage-clamp current recordings<sup>17</sup> were obtained at 20°C. Each patched cell was exposed to one concentration of succinic acid only; for each concentration, experiments derived from at least six patches were evaluated. For data acquisition and further analysis, we used the EPC9 digitally controlled amplifier in combination with Pulse and Pulse Fit software (HEKA Electronics, Lambrecht, Germany). The EPC9 provides automatic subtraction of capacitive and leakage currents by means of a prepulse protocol. Cell capacities ranged from 9 to 15 pF, and residual series resistance (after 50% compensation) ranged from 1.2 to 2.5 M $\Omega$ . Experiments during which an increase in series resistance occurred were rejected. The time constant of voltage settling in the membrane (residual series resistance  $\times$  cell capacitance) was generally  $< 35 \mu\text{s}$ .

For steady state voltage dependence of activation, peak currents ( $I$ ) elicited by families of depolarizing pulses from a prepulse potential of  $-120$  mV (15 ms duration, holding potential  $-100$  mV) to the indicated test potentials ( $-50$  mV,  $-45$  mV, and so on, in 5-mV increments to  $+45$  mV) were plotted against the test potentials; conductance ( $g$ ) was obtained using  $g = I/(V - V_{\text{rev}})$ , where  $V$  is the test potential and  $V_{\text{rev}}$  is the reversal potential. The reversal potential (or zero-current potential)  $V_{\text{rev}}$  was obtained by extrapolation assuming that the decline in peak current amplitude after depolarization to more positive test potentials (with respect to the test potential at which maximum current was observed) is related to the difference between test potential and reversal potential according to Ohm's law. Thus,  $V_{\text{rev}}$  is the zero current point on the abscissae derived from the linear peak current-voltage relationship after depolarizations to test potentials ranging from  $+15$  to  $+45$  mV in 5-mV increments according to  $I_{V_{\text{test}}} = (dI_{+15:+45 \text{ mV}}/dV_{+15:+45 \text{ mV}}) \times V_{\text{test}} + I_0 \text{ mV}$ . For  $V_{\text{test}} = V_{\text{rev}}$ ,  $I_{V_{\text{test}}} = 0$ , and  $V_{\text{rev}} = -I_0 \text{ mV}/(dI_{+15:+45 \text{ mV}}/dV_{+15:+45 \text{ mV}})$ .

Boltzmann fits [ $g/g_{\text{max}} = (1 + \exp(-zF(V - V_{0.5})/RT))^{-1}$ ] to the conductance-voltage plots yielded the voltage at half-maximum conductance ( $V_{0.5}$ ) and the slope factor  $z_a$ . Concentration-response curves for drug effects on voltage dependence of activation were obtained by plotting the mean difference in  $V_{0.5}$  in the indicated test concentration and  $V_{0.5}$  in the corresponding control experiment [ $\delta V_{0.5} = V_{0.5}(\text{test}) - V_{0.5}(\text{control})$ ] against the applied concentration of succinic acid. Small time-dependent shifts in  $V_{0.5}$  occurring in a series of control experiments in bath solution ( $n = 12$ ) were subtracted from the shifts observed in the presence of the drug. Hill fits [ $\delta V_{0.5} = \delta V_{0.5 \text{ max}}/(1 + (\text{EC}_{50}/[C])^{nH})$ ]

to the data yielded the concentration for half-maximum effect on voltage-dependent activation and the Hill coefficient  $n^H$  describing stoichiometry of drug binding to the channel;  $\delta V_{0.5 \text{ max}}$  is the maximum  $\delta V_{0.5}$  in the highest concentration of succinic acid applied (10 mM).

The voltage dependence of  $\text{Na}^+$ -channel inactivation was obtained from a double-pulse protocol, in which the cell membrane was first conditioned by a 20-ms prepulse starting at  $-150$  mV to prepulse potentials ranging from  $-150$  mV to  $-5$  mV, immediately followed by a 4-ms test pulse to  $0$  mV. The peak current ( $I_{\text{Na}}$ ) in response to this step was normalized to  $I_{\text{Na}}$  elicited by the test pulse at the most negative prepulse potential ( $-150$  mV). Boltzmann fits to the resulting current-voltage plots yielded the voltage at half-maximum inactivation ( $V_{0.5}$ ) and the slope factor  $z$ :  $I/I_{\text{max}} = [1 + \exp(-zF(V - V_{0.5})/RT)]^{-1}$ .

Time constants of inactivation,  $\tau_h$ , at  $0$  mV were obtained from single exponential fits to the decaying currents after 40-ms voltage jumps from  $-100$  mV to  $0$  mV:  $I(t) = a_0 + a_1 \exp(-t/\tau_{h1})$ .

#### Data Presentation and Statistical Evaluation

Data are presented as mean  $\pm$  SD. Because small time-dependent shifts in steady state activation and inactivation occur independent of drug administration, the main goal was to demonstrate the significance of succinic acid-induced effects with respect to a series of control experiments ( $n = 12$ ), exposed to bath solution only in the same time interval after seal formation (3–5 min). Changes in parameters of interest ( $V_{0.5}$  of activation and inactivation, slope factor, maximum current and conductance, time constant of inactivation) derived from single experiments ( $n = 6$ ) in each drug concentration were tested for statistical significance. The data partly showed positive skew indicative of non-normal distribution. The small sample size in each group mandated the use of the nonparametric Mann–Whitney U test;  $P < 0.05$  was considered significant. To avoid problems related to multiple hypotheses testing, we applied the method of multiple comparisons with *a priori* ordered hypotheses.<sup>18</sup> The test is based on the assumption that, if the null hypothesis is rejected, there is a positive monotonic relation between concentration and effect. As a consequence, the hypotheses to be tested could be ordered in advance, starting with the highest concentration of succinic acid. If the test results differed significantly from the control data, the effects of the next lower concentration were evaluated. The evaluation was stopped as soon as the first insignificant result was obtained. The advantage of this procedure, compared with other ap-

proaches to multiple testing, is that the level of type I error is kept at  $\alpha = 0.05$  for each statistical test. Furthermore, if the procedure starts with a significant result, the global null hypothesis is also rejected. Because of the time-dependent shifts in steady state activation, the starting values before drug application were not always reached during washout. Thus, washout was considered successful when the test results during washout did not differ significantly from the results obtained from the control experiment in bath solution in the same time interval after seal formation (5–8 min).

## Results

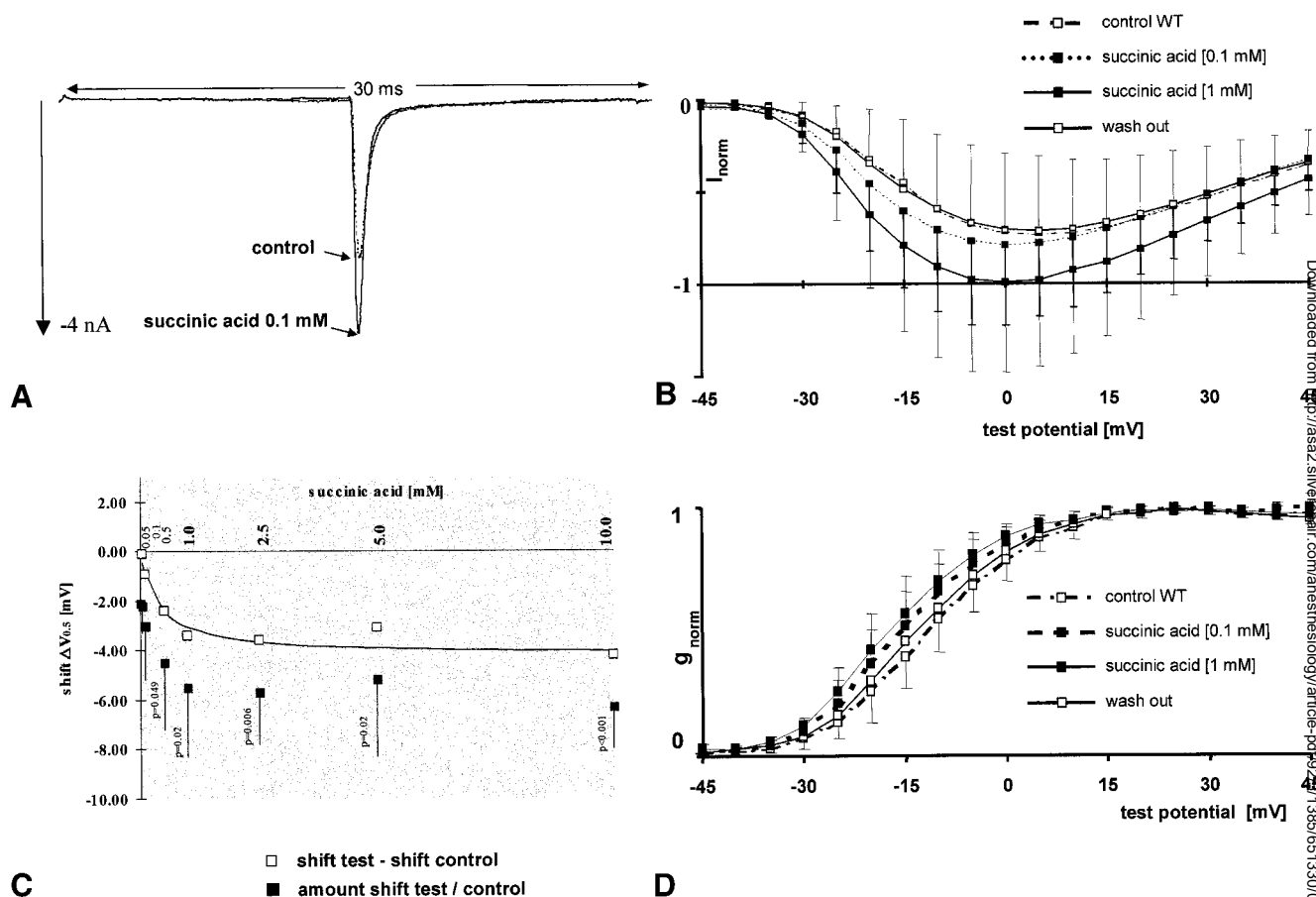
### Basic Gating Properties and Time-dependent Shifts under Control Conditions

Control values for  $V_{0.5}$  of channel activation were  $-14.8 \pm 5.3$  mV ( $n = 57$ ), and the slope factor  $z_a$  was  $3.7 \pm 0.4$ . Time constant of inactivation ( $\tau_h$ ) in the controls before succinic acid (10 mM) administration ( $n = 9$ ) was  $0.46 \pm 0.09$  ms,  $V_{0.5}$  of channel inactivation was  $-59.2 \pm 3.6$  mV, and the slope factor  $z_i$  was  $2.5 \pm 0.2$ . During application of bath solution only ( $n = 12$ ) the time-dependent shift in  $V_{0.5}$  was  $-2.1 \pm 1.6$  mV for steady state activation and  $-0.6 \pm 0.9$  mV for steady state inactivation.

### Succinic Acid Effects on Voltage-dependent Activation

Peak currents elicited by families of depolarizing voltage steps from a prepulse potential of  $-120$  mV were enhanced in the presence of succinic acid compared with the corresponding starting values (figs. 1A and 1B). Enhancement of maximum current ranged from 13% to 37% of control currents (table 1). Statistical significance of this effect with respect to the time-dependent control experiments could be shown for 10 mM succinic acid.

Conductance-voltage plots obtained from the peak currents during each depolarizing voltage step represent the fraction of available sodium channels (with respect to the maximum of open channels in the indicated test solution) that open at each test potential. The test potential at half-maximum activation ( $V_{0.5}$ ) reflects the position of the curve along the voltage axis. Succinic acid shifted the midpoints of steady state activation in the direction of more negative test potentials, indicating that a greater proportion of channels open during smaller depolarizations in the presence of the drug (fig. 1C). The



**Fig. 1.** (A) Representative current traces following depolarizations from  $-120$  mV to  $0$  mV in the absence (control) and presence of  $0.1$  mM succinic acid. (B) Current-voltage plots following families of depolarizing voltage steps from a prepulse potential of  $-120$  mV (15-ms duration, holding potential  $-100$  mV) to families of test potentials ranging from  $-50$  mV to  $+45$  mV in bath solution (control and washout) and in two representative concentrations of succinic acid ( $0.1$  mM and  $1$  mM). Each data line represents mean peak currents derived from  $n \geq 6$  patches for each concentration normalized to the peak currents in the highest concentration of succinic acid depicted; error bars indicate SDs. Currents in bath solution (control and washout) were pooled for the two different series of experiments. Succinic acid induced an enhancement of peak currents that was reversible during washout. (C) Conductance transforms of the current-voltage plots depicted in (B). Conductance was normalized to maximum conductance in the indicated solution (bath solution or succinic acid in the indicated concentration). Succinic acid shifted the midpoints of the conductance-voltage plots ( $V_{0.5}$ ) derived from Boltzmann fits to the data in the direction of more negative test potentials. This effect showed concentration dependence and was reversible during washout. (D) Concentration-effect curves for succinic acid-induced shifts in the midpoints of the conductance-voltage plots ( $V_{0.5}$ ) in the direction of hyperpolarized membrane potentials. Filled symbols represent mean data derived from at least six different patches. Error bars are SDs. Empty symbols are shifts in  $V_{0.5}$ , from which the small shift occurring in a series of control experiments ( $n = 12$ ) has been subtracted. The solid line represents a Hill fit to data yielding an  $EC_{50}$  of  $0.39$  mM for drug effects on steady state activation when the small shift in the control experiments was regarded as point 0. *P* values for significant effects refer to a series of control experiments in bath solution ( $n = 12$ ).

maximum amount of this shift was  $-6.3 \pm 1.7$  mV. Taking into account the small negative shift in the controls, a drug-related shift of  $-4.2$  mV was observed in  $10$  mM succinic acid. Washout was positive for concentrations  $> 0.5$  mM. The concentration-effect curves are depicted in figure 1D. A Hill fit to data yielded an  $EC_{50}$  value of  $0.39$  mM succinic acid (the small shift of  $-2.1$  mV observed in the control experiments was considered

to be point 0); the Hill coefficient  $n^H$  was  $1.02$ . The slope factor  $z_a$  remained unchanged in the presence of  $10$  mM succinic acid ( $3.8 \pm 0.5$ ;  $P = 0.6$ ) with respect to the starting value ( $3.7 \pm 0.4$ ).

Extrapolated values for  $V_{rev}$  were in good agreement between control ( $77.3 \pm 9.4$  mV), test ( $72.9 \pm 12$  mV) and washout ( $76.5 \pm 8.5$  mV) experiments ( $n = 57$ ). No significant results were obtained for the enhancement of



## SUCCINIC ACID AFFECTS ACTIVATION OF MUSCLE SODIUM CHANNELS

**Table 1. Succinic Acid-induced Enhancement of Maximum Currents  $I_{\max}$  [nA]**

Succinid Acid [mM]	$I_{\max}$ Control	$I_{\max}$ Test	$I_{\max}$ Wash-out
10	3.4 ± 1.7 3.8 ± 1.9	4.2 ± 1.8† 4.7 ± 2.1	* 4.1 ± 2.0§
5	2.5 ± 1.3	2.8 ± 1.5‡	2.5 ± 1.4
2.5	2.7 ± 1.3	3.1 ± 1.2	2.6 ± 1.0
1	2.2 ± 1.5	2.6 ± 1.3	2.0 ± 1.0
0.1	1.8 ± 2.1	2.1 ± 1.1	1.9 ± 1.0
0.05	5.7 ± 3.3	6.1 ± 3.5	5.8 ± 3.1

Maximum currents (mean ± SD) elicited by families of depolarizing voltage pulses from a prepulse potential of  $-120$  mV to test potentials ranging from  $-50$  mV to  $+45$  mV in 5 mV-increments in the absence (control and wash-out) and presence of different concentrations of succinic acid. At least 6 experiments were performed for each test concentration. Enhancement of peak currents reached statistical significance with respect to the time-dependent control experiments in bath solution only in 10 mM succinic acid.

\* In 10 mM succinic acid, a total of  $n = 9$  experiments were performed. In  $n = 3$  cases, the lifetime of a stable patch was too short to allow wash-out, so the mean currents derived from the 6 out of 9 experiments with positive wash-out were depicted in the second line.

†  $P = 0.002$ .

‡  $P = 0.7$ .

§  $n = 6$ .

maximum conductance in the presence of succinic acid (table 2).

### Succinic Acid Effects on Voltage-dependent Inactivation and Time Course of Inactivation

Succinic acid (10 mM) did not affect voltage-dependent inactivation or time course of channel inactivation:  $V_{0.5}$  for steady state inactivation in 10 mM succinic acid was shifted by  $-2.3 \pm 2.0$  mV into hyperpolarizing direction (to  $-61.5 \pm 2.8$  mV); this effect was not significant with

**Table 2. Succinic Acid-induced Changes in Maximum Conductance  $g_{\max}$  [ $10^{-8}$  S]**

Succinid Acid (mM)	$g_{\max}$ Control	$g_{\max}$ Test	$g_{\max}$ Wash-out
10	5.0 ± 2.3 5.4 ± 2.7	5.9 ± 2.3† 6.4 ± 2.7	* 5.8 ± 2.5‡
5	3.6 ± 2.0	4.1 ± 2.3	3.7 ± 2.0
2.5	3.9 ± 1.4	4.4 ± 1.4	3.8 ± 1.2
1	3.1 ± 2.2	3.9 ± 2.0	2.9 ± 1.4
0.1	2.8 ± 1.2	3.3 ± 1.6	2.9 ± 1.6
0.05	7.7 ± 4.6	7.8 ± 4.6	7.6 ± 4.4

Maximum conductance (mean ± SD) obtained from the experiments depicted in Table 1 and figure 1b–d, applying families of depolarizing voltage pulses from a prepulse potential of  $-120$  mV to test potentials ranging from  $-50$  mV to  $+45$  mV.

\*  $n = 9$ , no wash-out in  $n = 3$ .

†  $P = 0.1$ .

‡  $n = 6$ .

respect to the  $-0.6 \pm 0.9$  mV shift in control solution ( $P = 0.08$ ). The slope factor  $z_i$  in the presence of drug was  $2.3 \pm 0.2$  versus  $2.5 \pm 0.2$  in the controls ( $P = 0.7$ ). Time constant of inactivation ( $\tau_h$ ) in 10 mM succinic acid was  $0.36 \pm 0.05$  ms versus  $0.45 \pm 0.09$  ms in the controls ( $P = 0.2$ ).

## Discussion

Our results show that succinylcholine metabolite succinic acid facilitates voltage-dependent activation of human muscle sodium channels in a concentration-dependent manner without affecting the rate and voltage dependence of inactivation. The control data we obtained from heterologously expressed  $\alpha$  subunits of human skeletal muscle sodium channel confirm gating kinetics previously described for those channels.<sup>15,16</sup>

The observed effect of succinic acid on voltage-dependent activation is qualitatively comparable to the effect of certain anions on the voltage dependence of gating in frog skeletal muscle: For frog muscle fibers, sodium salts added to the external medium have been shown to potentiate the strength of a twitch in the order  $\text{Cl}^- > \text{Br}^- < \text{NO}_3^- < \text{I}^- < \text{SCN}^-$ .<sup>19,20</sup> The primary cause of twitch potentiation by anions such as nitrate or thiocyanate was attributed to a shift of the voltage dependence of contractile activation toward more negative potentials.<sup>21,22</sup> Dani *et al.*<sup>23</sup> showed that anions in the external solution shifted the voltage dependence of gating in frog skeletal muscle with little effect on the steepness of maximum sodium permeability. The sequence of effectiveness was acetate  $< \text{Br}^- < \text{NO}_3^- < \text{SO}_4^{2-} < \text{SCN}^- < \text{ClO}_4^-$ . However, those anions shifted activation less than inactivation with an order of magnitude ranging from  $-3.7 \pm 0.4$  mV for  $\text{Br}^-$  to  $-12.7 \pm 0.7$  mV for  $\text{ClO}_4^-$  when all the chloride in the bath was replaced by the given anion. Removal of negatively charged sialic acid residues from cells expressing rSkM1 sodium channels showed opposite effects, shifting the voltage dependence of gating into depolarizing direction.<sup>24</sup> Thus, we assume that the acidic moiety of succinic acid is not important, and the observed effects should rather be attributed to the succinate anion. In addition, decreasing the external pH to 4.5 would shift  $\text{Na}^+$  channel activation into depolarizing direction, whereas increasing the pH to 10 itself would cause a  $-8$  mV hyperpolarizing shift.<sup>25</sup> Nevertheless, the effects of succinic acid/succinate are far more specific: For the hyperpolarizing shift of steady state activation, we found an  $\text{EC}_{50}$  value of 0.4

mm, whereas the anion effects described previously were observed at  $> 100\times$  higher concentrations. In the concentration range examined, succinic acid/succinate did not affect voltage-dependent inactivation. Thus, we speculate that the substance interferes more specifically with the voltage sensor of activation. The amino acid sequences of the known voltage-operated cation channels ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ) show striking similarities. One of the transmembrane  $\alpha$  helices that is highly conserved in all known voltage-gated cation channels contains regularly spaced, positively charged amino acid residues. This helix has been implicated as the voltage sensor in these channels.<sup>26</sup> It is thus tempting to speculate that succinic acid might exert similar effects on those channels.

#### Limitations of the Model

For this study we used  $\alpha$  subunits of the human skeletal muscle sodium channel heterologously expressed in a mammalian cell line (HEK293). Despite the lack of  $\beta$  subunits, the suitability of this preparation for studying channel gating kinetics has been verified experimentally: The  $\alpha$  subunit is the primary pore-forming subunit of the channel and functions as a ion channel when expressed alone.<sup>27</sup> Expression of hSkM1  $\alpha$  subunits in *Xenopus* oocytes resulted in  $\text{Na}^+$  currents with abnormally slow inactivation. In contrast, expression of the same  $\alpha$  subunit in a mammalian cell line (tsA201, a transformed HEK293 cell line) resulted in channels exhibiting normal (with respect to experiments in native tissue) rapid activation and inactivation and retaining sensitivity to tetrodotoxin and  $\mu$ -conotoxin.<sup>28</sup> Other factors that might modify channel function *in vivo*, such as post-translational modification or phosphorylation of channel proteins,<sup>27</sup> are beyond the scope of this model.

#### Implications for Effects on Muscle Sodium Channels In Vivo

The results obtained for succinic acid at the molecular level are difficult to frame into clinical terms because they probably do not translate directly into the same effect at the tissue or organismal levels. First, small increases in sodium channel conductance have been shown to result in substantial changes in the action potential firing threshold of the squid giant axon, rendering the membrane hyperexcitable.<sup>29</sup> Thus, a relatively small effect at the molecular level might be amplified *in vivo*. Second, gating processes are highly temperature-sensitive, and effects of a substance at  $20^\circ\text{C}$  might be accelerated at body temperature. To get an idea

of the clinical relevance *in vivo*, we can compare the effects observed in the presence of succinic acid to the effect of decreasing of the  $\text{Ca}^{2+}$  concentration in bath solution on voltage-dependent activation: Lowered  $[\text{Ca}^{2+}]_o$  causes nerve and muscle hyperexcitability, a condition seen in the clinic in patients with hypoparathyroidism.<sup>25</sup> The amount of hyperpolarizing shift observed in high concentrations of succinic acid is comparable to the effect that lowering external  $[\text{Ca}^{2+}]_o$  from 2 mM to 0.5 mM in frog Ringer's solution exerts on the voltage dependence of activation in frog skeletal muscle.<sup>25</sup>

#### Possible Clinical Implications

On application of a routine dose of succinylcholine succinylcholine blood levels of 62  $\mu\text{g/ml}$  (0.17 mM) have been reported.<sup>30</sup> Thus, equimolar concentrations of succinic acid may be reached,<sup>31</sup> given that cholinesterase activity is not impaired. Furthermore, in addition to being the product of succinylcholine hydrolysis, succinic acid is an endogenous dicarboxylic acid (citric acid cycle intermediate) ubiquitous in all body tissues. Tissue and serum levels of succinic acid show a pronounced increase after ischemia<sup>32</sup> and hypoxia, up to serum levels of 0.1–0.2 mM.<sup>33</sup> Hypoxic tissue is considered to be the source of succinate due to a reversal of the oxidative pathway from succinate to oxaloacetate. Serum levels are supposed to increase slowly, secondary to a breakdown of the permeability barrier to succinate between the cell and the circulation.<sup>33</sup> Thus, we have to assume that succinic acid concentrations at the sarcolemma might even exceed serum levels during systemic hypoxia.

Despite the lack of direct evidence at the present time it is tempting to speculate that elevated tissue and serum levels of succinic acid might have mediated the aberrant response to succinylcholine seen in patients with massive trauma<sup>6,7</sup> and exsanguinating hemorrhage,<sup>8</sup> as well as succinylcholine-provoked hyperkalemia in rabbits subject to hemorrhage.<sup>34</sup>

In conclusion, our finding that activation of voltage-operated muscle sodium channels is facilitated in the presence of succinic acid now yields a new hypothetical explanation for succinylcholine-induced muscle hyperexcitability despite complete neuromuscular blockade and hypersensitivity to succinylcholine-induced depolarization in cases of severe hypoxia and hypotension. Further studies, however, are required to elucidate the potential role of succinic acid causing membrane hyperexcitability *in vivo*, i.e., a quantitative assessment of succinic

## SUCCINIC ACID AFFECTS ACTIVATION OF MUSCLE SODIUM CHANNELS

acid in tissues and serum during shock and hypoxia preceding succinylcholine-provoked hyperkalemia.

The authors thank Professor Lehmann-Horn (Ulm) for providing transfected cells, Birgitt Nentwig (Department of Anesthesiology) for taking care of the cell culture, and Jobst Kilian (Department of Neurology) for technical support.

## References

- Vita GM, Olckers A, Jedlicka AE, George AL, Heimann-Patterson T, Rosenberg H, Fletcher JE, Levitt RC: Masseter muscle rigidity associated with glycine<sup>1306</sup>-to-alanine mutation in the adult muscle sodium channel  $\alpha$ -subunit. *ANESTHESIOLOGY* 1995; 82:1097-103
- Azar I: The response of patients with neuromuscular disorders to muscle relaxants: A review. *ANESTHESIOLOGY* 1984; 61:173-87
- Martyn JAJ, White DA, Gronert GA, Jaffe RS, Ward JM: Up-and-down regulation of skeletal muscle acetylcholine receptors. *ANESTHESIOLOGY* 1992; 76:822-43
- Lindinger M: Potassium regulation during exercise and recovery in humans: Implications for skeletal and cardiac muscle. *J Mol Cell Cardiol* 1995; 27:1011-22
- Medbo J, Sejersted O: Plasma potassium changes with high intensity exercise. *J Physiol* 1990; 421:105-22
- Kopriva C, Ratcliff J, Fletcher JR, Van Tassel P, Stout R: Serum potassium change after succinylcholine in patients with acute massive muscle trauma. *ANESTHESIOLOGY* 1971; 34:246-9
- Mazze RI, Escue HM, Houston JB: Hyperkalemia and cardiovascular collapse following administration of succinylcholine to the traumatized patient. *ANESTHESIOLOGY* 1969; 31:540-7
- Schwartz DE, Kelly B, Caldwell JE, Carlisle AS, Cohen NH: Succinylcholine-induced hyperkalemic arrest in a patient with severe metabolic acidosis and exsanguinating hemorrhage. *Anesth Analg* 1992; 75:291-3
- Jantzen JP, Eberle B, Gaida BJ, Hennes HJ, Otto S, Schäfer M: Zur Wirkung von Muskelrelaxanzien auf den Masseter-tonus. *Anaesthesist* 1992; 41:248-53
- Brumback RA, Bertorini TE, Engel WK, Trotter JL, Oliver KL, Zirzow GC: The effect of pharmacologic acetylcholine receptor on fibrillation and myotonia in rat skeletal muscle. *Arch Neurol* 1978; 35:8-10
- Stoelting R, Peterson C: Adverse effects of increased succinylcholine dose following d-tubocurarine pretreatment. *Anesth Analg* 1975; 54:282-8
- Leuwer M, Haeseler G, Weckbecker K, Piepenbrock S, Dengler R: Direct effects of suxamethonium on one paramyotonia congenita: Mutant (R1448H) of voltage-gated muscle sodium channels (abstract)? *Br J Anaesth* 1997; 78:470P
- George A, Komisarof J, Kallen R, Barchi R: Primary structure of the adult human skeletal muscle voltage-dependent sodium channel. *Ann Neurol* 1992; 31:131-7
- Graham FL, Van der Eb AJ: A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 1973; 52:456-67
- Mitrovic N, George AL, Heine R, Wagner S, Pika U, Hartlaub U, Zhou M, Lerche H, Fahlke C, Lehmann-Horn F: K<sup>+</sup>-aggravated myotonia: Destabilization of the inactivated state of the human muscle sodium channel by the V1589 M mutation. *J Physiol* 1994; 478:395-402
- Wagner S, Lerche H, Mitrovic N, Heine R, George AL, Lehmann-Horn F: A novel sodium channel mutation causing a hyperkalemic paralytic and paramyotonic syndrome with variable clinical expressivity. *Neurology* 1997; 49:1018-25
- Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 1981; 391:85-100
- Maurer W: Multiple comparisons in drug clinical trials and pre-clinical assays: A priori ordered hypotheses, Testing Principles in Clinical and Preclinical Trials. Edited by Vollmar J. Stuttgart, Fischer Verlag 1995, pp 3-8
- Kahn A, Sandow A: The potentiation of muscular contraction by the nitrate-ion. *Science* 1950; 112:647-9
- Horowicz P: The effects of anions on excitable cells. *Pharmacol Rev* 1964; 16:193-221
- Hodgkin A, Horowicz P: The effect of nitrate and other anions on the mechanism response of single muscle fibres. *J Physiol* 1960; 153:404-12
- Kao C, Stanfield P: Actions of some anions on electrical properties and mechanical threshold of frog twitch muscle. *J Physiol* 1968; 198:291-309
- Dani J, Sanchez J, Hille B: Lyotropic anions. *J Gen Physiol* 1983; 81:255-81
- Bennett E, Urcan M, Tinkle S, Koszowski A, Levinson SR: Contribution of sialic acid to the voltage dependence of sodium channel gating. *J Gen Physiol* 1997; 109:327-43
- Hille B: Modifiers of gating, Ionic Channels of Excitable Membranes, 2nd Edition. Edited by Hille B. Sunderland, MA, Sinauer Associates, 1992, pp 445-72
- Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J: Voltage-gated cation channels are evolutionarily and structurally related, Molecular Biology of the Cell, 3rd Edition. Edited by Robertson M. New York, Garland Publishing, 1994, pp 534-5
- Catterall WA: Cellular and molecular biology of voltage-gated sodium channels. *Physiol Rev* 1992; 72(Suppl):15-48
- Chahine M, Bennett P, George A, Horn R: Functional expression and properties of the human skeletal muscle sodium channel. *Pflügers Arch* 1994; 427:136-42
- Matzner O, Devor M: Na<sup>+</sup> conductance and the threshold for repetitive neuronal firing. *Brain Res* 1992; 597:92-8
- Nordgren K, Forney RB, Carroll FT, Holmstedt BR, Jaderholm-Eriksson I, Pettersson BM: Analysis of succinylcholine in tissues and body fluids by ion-pair extraction and GC-MS. *Arch Toxicol Suppl* 1983; 6:339-50
- Meyer E, Lambert WE, De Leenheer A: Succinic acid is not a suitable indicator of suxamethonium exposure in forensic blood samples. *J Anal Toxicol* 1997; 21:170-1
- Folbergrova J, Ljunggren B, Norberg K, Siesjö BK: Influence of complete ischemia on glycolytic metabolites, citric acid cycle intermediates, and associated amino acids in the rat cerebral cortex. *Brain Res* 1974; 80:265-79
- Iles RA, Barnett D, Strunin L, Strunin JM, Simpson BR, Cohen RD: The effect of hypoxia on succinate metabolism in man and the isolated perfused dog liver. *Clin Sci* 1972; 42:35-45
- Antognini JF, Gronert GA: Succinylcholine causes profound hyperkalemia in hemorrhagic, acidotic rabbits. *Anesth Analg* 1993; 77:585-8