

Succinylcholine and Decamethonium:

Comparison of Depolarization and Desensitization

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Both succinylcholine (SCh) and decamethonium (C10) cause depolarization, or phase I block, and desensitization, or phase II block. Using a frog nerve-muscle preparation *in vitro*, the authors evaluated the relative capabilities of SCh and C10 to cause the two types of block. Indirectly stimulated muscle mounted on a myograph can produce similar records of tension output vs. time following bath application of both SCh and C10. Curves obtained with SCh at 3.75 μ M, 12.5 μ M, and 25 μ M, and with C10 at 25 μ M, 75 μ M, and 150 μ M concentrations were comparable. Using this 1:6 concentration ratio of SCh to C10 in studies of transmembrane potential in single cells, it was found that SCh caused greater and more sustained depolarization than C10. Even when SCh concentrations were increased so as to be equimolar with C10, SCh caused greater depolarization and less desensitization. Compared with SCh, C10 has a limited depolarization capability which cannot be overcome by increasing its concentration. Finally, the desensitization caused by C10 is more difficult to reverse than that caused by SCh. (Key words: Depolarizing drugs; Succinylcholine; Decamethonium; Depolarization; Desensitization.)

Two of the neuromuscular blockers in clinical use, succinylcholine (SCh) and decamethonium (C10), are of the "depolarizing" type. These drugs first stimulate muscle, then cause neuromuscular blockade. With time the quality of the neuromuscular blockade produced by these agents changes. The sequence has been described as "phase I" block and "phase II" block, or "depolarization" block and "desensitization" block.

The muscle paralysis produced by depolarization block occurs promptly following ad-

ministration of either agent. The paralysis can be short-lived but profound, and recovery can occur quickly. Desensitization block develops relatively more slowly. Although many of the characteristics of desensitization block, which appear later, seem curare-like, the muscle paralysis produced by desensitization block is not easily antagonized. In fact, there are no reliable methods for accelerating recovery from desensitization block, because the mechanisms of desensitization block are poorly understood. Therefore, it is reasonable to suppose that the relative clinical safety of SCh and that of C10 depend upon the intensity and duration of the depolarization blocks which they produce relative to their capacities to produce desensitization block. In this study we have attempted to make a quantitative comparison of the depolarization and desensitization blocks produced by SCh and C10.

Methods

The frog sartorius muscle-sciatic nerve preparation was used throughout. The myographic techniques,¹ impalement of single cells by microelectrodes to study transmembrane potential changes,² and methods of microperfusion³ have been described.

A Tektronix storage oscilloscope (#564B) with dual differential amplifier (#3A3) and time base (#2B67) was used, with photographic recording. Stock solutions of C10 (2.5 mM) were prepared weekly from purified powder supplied by Davis and Geck, Inc. These were stored at 4 C and portions used for daily experiments. There was no loss of potency after a month of storage. SCh solutions were prepared daily immediately prior to use from powder supplied by Squibb and Co. (Anectine).

Standard frog Ringer's solution, containing NaCl 111.0 mM, KCl 2.5 mM, CaCl₂ 1.8 mM, NaH₂PO₄ 0.45 mM, Na₂HPO₄ 2.55 mM, and dextrose 5.1 mM, was used. In experiments in

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Received from the Columbia University College of Physicians and Surgeons, New York, New York 10032. Accepted for publication August 26, 1970. Supported in part by NICMS Grant 5 PO1-GM 09069 and Grant 5 RO1-NB-04988. Presented in part at ASA meeting in San Francisco, California, October 1969.

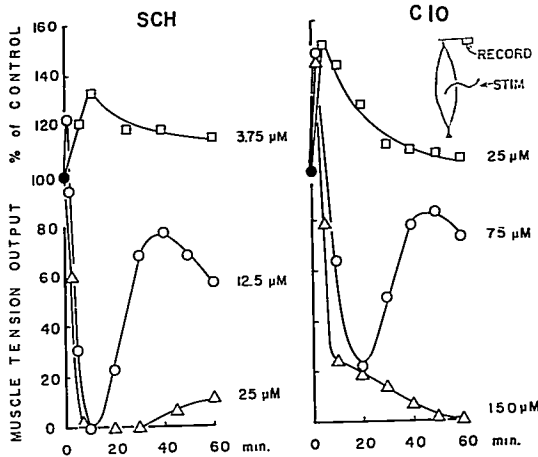


FIG. 1. Plot of tension output (per cent of control) vs. time. Concentrations of SCH and C10 as indicated. The inset is a schematic diagram of the experiment. Plotted points represent averaged results of two to six experiments. Note the similarity of tension outputs resulting from application of SCH and application of C10.

which we wished to stop muscle contraction either sucrose, 300.0 mM, or tetrodotoxin, 0.1 μ M, was added. Bathing solutions were changed every 20 minutes. Temperature was maintained at 18–20 C.

Statistical significance was evaluated by Student's *t* test applied to the means of non-paired data. Results were considered significant when *P* was 0.01 or less as derived from tables for $N_1 + N_2 - 2$ where N_1 is the number of observations in the first group and N_2 is the number of observations in the second group.

Results

Muscles were mounted on a myograph and stimulated via the motor nerve by supramaximal pulses 0.1 msec in duration delivered once per minute. The preparations were bathed in Ringer's solution containing various concentrations of SCH or C10.

Figure 1 shows the mean tension outputs from two to six preparations at intervals following drug application in the indicated concentrations. Although three typical curves for each agent are shown, additional data (not shown) indicate that a continuous spectrum of curves is obtained by varying the concentrations of SCH or C10.

At low concentrations of the drugs (upper curves in figure 1) tension output of muscle

is increased without any evidence of block. Next, with increases in drug concentrations (middle curves), the periods of potentiation are markedly abbreviated, followed by reduced tension output. Thereafter, partial recovery occurs, followed spontaneously by another period of decrease in tension output. In this diphasic response, the early decrease in tension output depends considerably on postjunctional membrane (pjm) depolarization, and the later decrease in tension output depends largely on pjm desensitization.⁴ Finally, with still higher concentrations of either SCH or C10 (lower curves in both panels of figure 1) the periods of potentiation are further shortened and rapidly become a deepening, uninterrupted monophasic muscle block. It is noteworthy that the curves of tension output obtained with C10 and SCH are closely similar in form, but to achieve this parallel behavior, C10 must be applied at a sixfold greater concentration than SCH.

The three patterns of responses to SCH and C10 shown in figure 1 are not unique. They have been demonstrated for many depolarizing drugs, in many species, including man, in many fast-acting twitch muscles both *in vitro* and *in vivo*.⁴ However, even though the experiments on whole-muscle preparations shown in figure 1 produce similar tension output curves for C10 and SCH, the mechanisms un-

derlying the parallel responses are not identical. Quantitative differences between SCh and C10 were found in studies of transmembrane potential changes produced by these agents. We used for these further studies bathing media containing intermediate and high concentrations of SCh and C10, as indicated in the myograph experiments (fig. 1).

Figure 2 shows the results of transmembrane potential recordings following bath application of SCh, 30.0 μ M and 12.5 μ M, and C10, 150 μ M and 75 μ M. Plotted points represent mean values obtained from 2-13 cells during successive 15-minute periods. Each cell was impaled only once at the junctional area. The junctional site was identified visually and impaled verified by the appearance of an endplate potential (EPP) following nerve stimulation. In figure 2, during the initial period, the depolarization produced following SCh application exceeded that produced by C10. In figure 1 we have shown that SCh and C10 produce "similar tension output curves." The values (table 1) recorded for membrane potential during the intervals following application of SCh at 30 μ M are significantly different ($P < 0.005$ or better) from the results of C10 application at 150 μ M concentration. Similarly, there were significant differences between fibers treated with 12.5 μ M SCh and those treated with 75 μ M C10.

Returning to figure 2, we see that the rate of repolarization of SCh-treated fibers was less than that of fibers treated with C10. Average

repolarization rates were: SCh, 30 μ M, 0.21 mV/min; SCh, 12.5 μ M, 0.12 mV/min; C10, 150 μ M, 0.33 mV/min; C10, 75 μ M, 0.25 mV/min. The rate of membrane repolarization following exposure to C10 always exceeded that for SCh in these experiments. The repolarization of the *pjm* during continuous exposure to depolarizing quaternary ammonium compounds has been interpreted as indicating the occurrence of inactivation or desensitization of *pjm* receptors.⁵ On this basis, the relatively more rapid *pjm* repolarization obtained in C10-treated fibers may be taken to indicate that C10 causes more rapid receptor inactivation (desensitization) than SCh.

Immediately following bath application of either SCh or C10, repetitive muscle activity occurs. Early in the experiments, for a few minutes following application of the drug it was not possible to place an electrode inside a cell. To overcome this difficulty, in the next experiments the contractile responses were blocked by placing the muscle in hyperosmotic modified Ringer's solution (sucrose added). Under these conditions, the muscle fiber can be electrically stimulated without subsequent contraction,⁶ and thus we can follow changes in membrane potential from the instant of application of the drug. The junctional area was impaled and a continuous intracellular recording made during microperfusion of the region with hypertonic modified Ringer's solution containing SCh or C10. Figure 3 shows membrane depolarization vs. time during the sec-

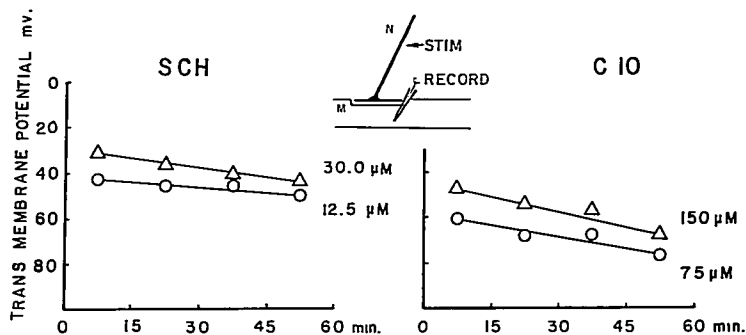


FIG. 2. Plot of transmembrane resting potentials at *pjm* averaged for 15-minute intervals from two to 13 single fiber impalements. The inset is a schematic diagram of the experiment. SCh produces more profound depolarization than C10 but repolarization occurs more slowly.

TABLE 1. Comparison of Postjunctional Membrane Potentials* at Various Times after Application of SCh and C10

	0-14 Min	15-29 Min	30-44 Min	45-59 Min
SCh 30 μ M	(5) 31.0 \pm 1.5	(12) 36.3 \pm 0.6	(10) 40.4 \pm 2.1	(13) 43.8 \pm 1.8
C10 150 μ M	(9) 47.9 \pm 3.5 <i>P</i> < 0.005	(9) 54.9 \pm 1.9 <i>P</i> < 0.001	(6) 57.0 \pm 3.6 <i>P</i> < 0.001	(2) 68.0 \pm 3.0 <i>P</i> < 0.001
SCh 12.5 μ M	(8) 43.0 \pm 1.4	(12) 46.1 \pm 3.5	(10) 46.2 \pm 1.0	(10) 50.6 \pm 1.6
C10 75 μ M	(6) 61.2 \pm 4.6 <i>P</i> < 0.002	(6) 68.0 \pm 2.7 <i>P</i> < 0.001	(5) 67.4 \pm 6.3 <i>P</i> < 0.001	(2) 76.5 \pm 5.5 <i>P</i> < 0.001

* Transmembrane potentials in mV \pm SE. The number in parenthesis is the number of recordings from individual neuromuscular junctions. *P* values were determined by Student's *t* test for the significance of the difference between the means of non-paired data.

onds immediately following perfusion with the drug. It is evident that SCh caused greater depolarization than C10. These results parallel those obtained in the bath application experiments (fig. 2), but the intensity of the depolarization was less. With the microperfusion technique some drug dilution occurred, and probably fewer receptors were activated. This probably accounts for the difference in levels of depolarization reached by the two methods. The attempt to increase depolarization by increasing C10 concentration to 625 μ M was unsuccessful (fig. 3, right panel). Instead, repolarization (or desensitization) rapidly developed. A parallel sixfold increase in SCh concentration (to 150 μ M) caused increased pjm

depolarization without showing desensitization during perfusion. In these experiments SCh depolarization approached a limiting value of -30 mV transmembrane potential; the limit of C10 depolarization approximated -60 mV.

It might be supposed that the differences between SCh and C10 in causing depolarization and desensitization arose because the drugs were applied in different concentrations (previous work of our laboratory⁴ has shown that drug concentration is an important factor in desensitization). To test this point, individual junctions of a single muscle preparation were perfused (using identical perfusion pipettes) with modified Ringer's solution containing either SCh, 625 μ M, or C10, 625 μ M.

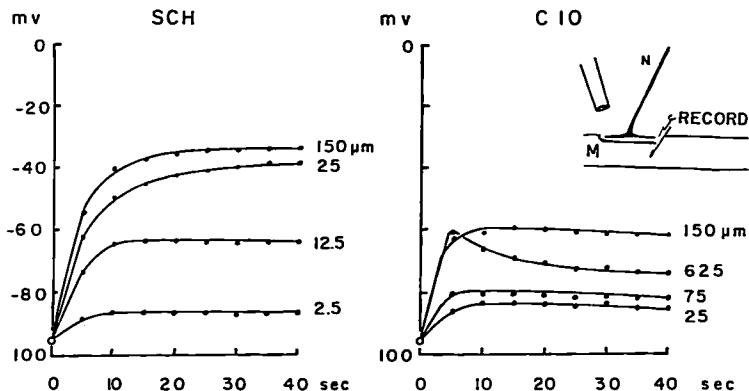


FIG. 3. Plot of transmembrane resting potentials during microperfusion at indicated SCh or C10 concentrations. The inset is a schematic diagram of the experiment. A sixfold increase in C10 concentration did not increase the degree of depolarization but did increase the rapidity of desensitization.

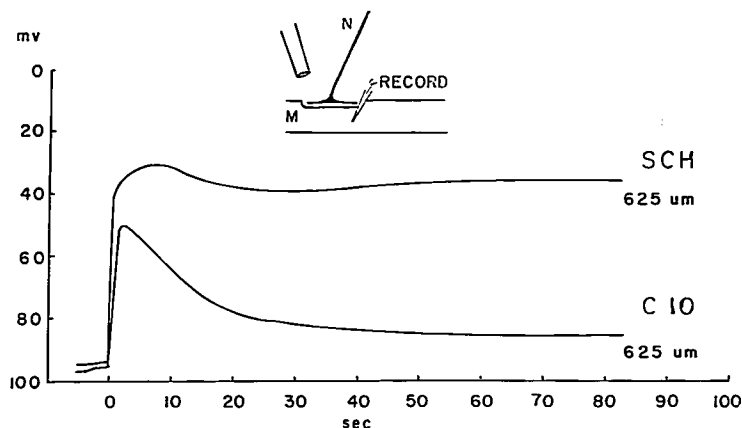


Fig. 4. Plot of transmembrane resting potentials during microperfusion at $625 \mu\text{M}$ SCH and $625 \mu\text{M}$ C10. The inset is a schematic diagram of the experiment. Under identical experimental conditions SCH produces greater and more prolonged depolarization than C10.

A typical result is shown in figure 4. In all trials depolarization caused by SCH was more intense and was better maintained for longer than that caused by equimolar concentrations of C10.

Receptor desensitization (which conceivably begins at the moment of application of the drug and in parallel with depolarization) becomes apparent later in time because it has a slow rate of development. In the next experiments we examined the onset and offset of desensitization caused by SCH and C10. Following prolonged *pjm* exposure to SCH or C10, receptor activity was reduced (desensitized). In the next experiment we tested the residual capability for activation that remained in *pjm* receptors following such prolonged drug application. Acetylcholine (ACh) was used as the receptor activator to test for residual available receptors.

Figure 5 presents the results of such an experiment. Transmembrane potential was recorded during perfusion of the junction with either SCH, $25 \mu\text{M}$, or C10, $150 \mu\text{M}$. After 10, 25, or 90 seconds the perfusion was stopped and immediately switched to a solution containing ACh, $55 \mu\text{M}$, plus the previously used concentration of either SCH or C10 (fig. 5, inset). The upper panel in figure 5 shows that

even after 90 seconds of SCH perfusion, ACh caused an additional rapid intense depolarization. In contrast, perfusion with C10 caused a progressive loss of receptor response to ACh application (lower panel). These results indicate that C10 is more effective than SCH in causing receptor desensitization.

The diphasic block in neuromuscular transmission produced by prolonged application of a depolarizing drug depends critically upon the concentration of drug applied (fig. 1). The neuromuscular block produced under these circumstances can be reversed by washing the preparation in drug-free modified Ringer's solution. One can suppose that complete recovery has occurred if reapplication of the depolarizer in the same concentration produces a similar diphasic response in tension output. We used this approach to test the recovery of the nerve-muscle preparation from SCH and C10. Figure 6 presents the results. The mean tension output is plotted in the upper panel for six muscles during an hour of bath exposure to SCH, $12.5 \mu\text{M}$. Thereafter, all the muscle preparations were washed vigorously in modified Ringer's solution free of SCH. After 45 minutes of washing, two muscles were again immersed in modified Ringer's solution containing SCH, $12.5 \mu\text{M}$. Tension output showed

a diphasic response like that obtained initially. The same result was obtained from two muscles washed 75 minutes, and from two muscles washed 90 minutes. In a similar experiment with C10, 75 μM , 150 minutes of vigorous washing with modified Ringer's solution free of C10 were needed to restore a diphasic response to C10 like that produced initially. It appears that it is more difficult to reverse the effects of C10 than to reverse those of SCH. It should be emphasized that during most of the period of washing with modified Ringer's solution the muscle contractile responses to neural stimulation were normal in amplitude, that is, there was no apparent impairment of tension output. However, a long-lasting re-

sidual effect of C10 which modifies the muscle response became apparent only when the muscle was challenged by reapplication of the depolarizing drug.

Discussion

In clinical situations, muscle relaxation can be produced reasonably well with SCH or C10. Our results, as might be expected, show that both are effective neuromuscular blockers. What is of concern in clinical applications is that it is sometimes difficult to reverse the blocks produced. These prolonged blocks are difficult to treat and potentially dangerous to the patient. One might argue that the most desirable neuromuscular blocking agent is one

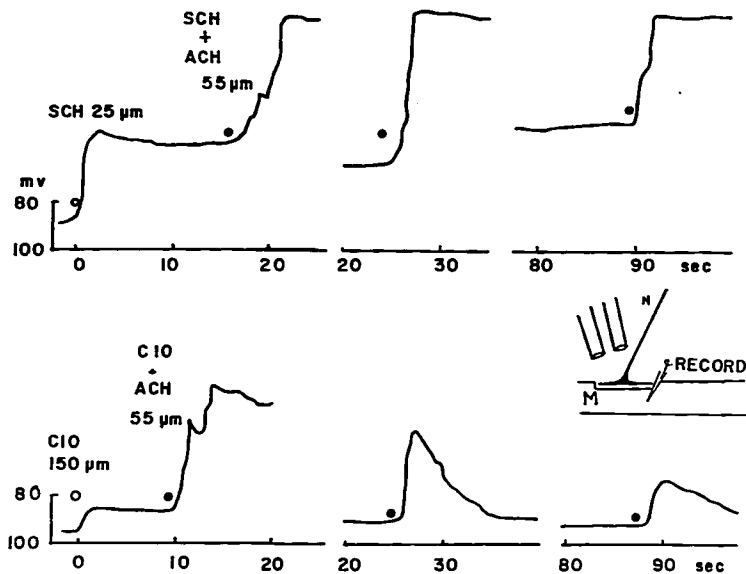


FIG. 5. Plot of transmembrane potentials recorded during micropfusion with two successive solutions. The upper panel represents results of perfusion with SCH, 25 μM , followed in 10, 25 or 90 sec by perfusion with SCH, 25 μM , plus ACh, 55 μM . The lower panel represents results of perfusion with C10, 150 μM , followed in 10, 25 or 90 sec by perfusion with C10, 150 μM , plus ACh, 55 μM . The inset is a schematic diagram of the experiment. After prolonged exposure to SCH excitable membrane still can be activated by ACh to produce large permeability changes. Prolonged exposure to C10 results in a rapid decrease in the response to ACh (desensitization).

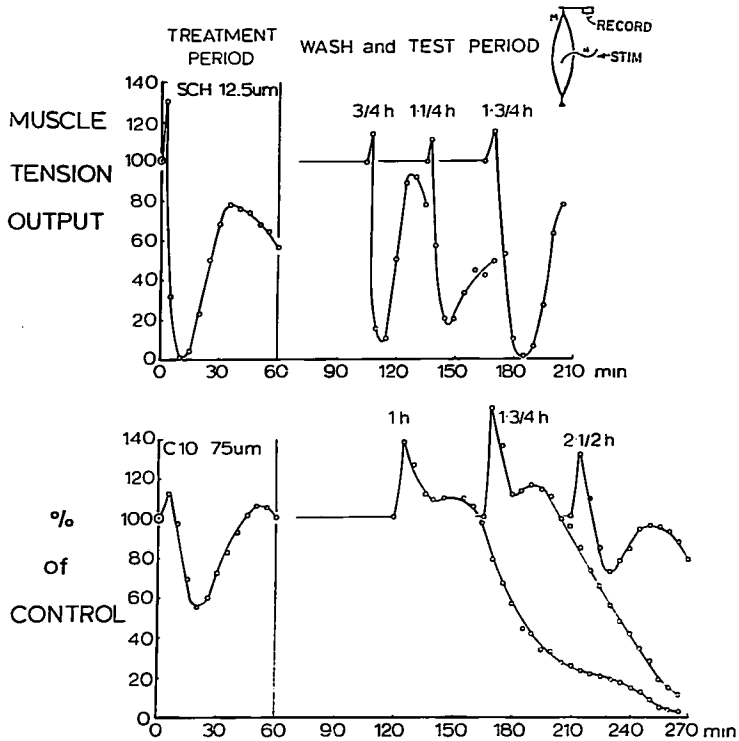


FIG. 6. Recovery of the diphasic response. Plot of tension output (per cent of control) vs. time. The upper panel represents results for six muscles initially bathed in SCh, 12.5 μ M, for an hour. After the indicated period of washing in frog Ringer's solution free of SCh, two muscles each were again treated with SCh, 12.5 μ M. The lower panel shows the results when six muscles were similarly treated with C10, 75 μ M. The inset is a schematic diagram of the experiment. The effects of an hour of exposure to SCh on tension output can be washed away in less than 45 minutes. C10 requires 150 minutes to return to the initial state.

that produces maximal postjunctional membrane depolarization and little receptor desensitization. On this basis, our data indicate that SCh is superior to C10 for clinical applications. Thus, although there is a wide gap between our experiments and clinical situations, we might conclude from our results that SCh rather than C10 is the relaxant drug of choice.

Because SCh is hydrolyzed rapidly *in vivo* by

plasma cholinesterase (pseudocholinesterase), its activity following injection is extremely short, and this also favors the clinical use of SCh rather than C10. However, in interpreting our experimental results, we should point out that SCh is not hydrolyzed appreciably by isolated nerve-muscle preparations.⁷ Thus, SCh breakdown was not a factor in the differences in behavior between SCh and C10 seen in our ex-

periments. Apparently the difference between SCH and C10 depends on other factors.

In many previous studies it has been reported that C10 causes slower activation of receptors and more pronounced desensitization than other depolarizing compounds, including both hydrolyzable and stable nonhydrolyzable quaternary ammonium compounds.^{8,9} del Castillo and Katz raised the possibility that SCH and C10 might have different rates of access to the active sites on the *pjm* because of the presence of a differential diffusion barrier. However, on the basis of their iontophoresis experiments^{8,10} utilizing acetylcholine, carbamylcholine, SCH, and C10, they concluded that all of these compounds had approximately equal rates of access to the receptor sites.

Our experimental data indicate that the important difference between SCH and C10 lies in their capacities to desensitize postjunctional membrane receptors. Our view parallels that of del Castillo and Katz, whose experiments showed that the initial effect of applied C10 is to inhibit the response of *pjm* receptors.^{8,10}

We find it interesting that closely similar diaphasic tension output curves can be produced by adjusting the concentrations of SCH and C10. The usual explanation for the early phase I block (depolarization block) is that it occurs if the potential difference across the postjunctional membrane is reduced below approximately -57 mV (that is, between 0 mV and -57 mV).¹¹ At this potential level the conductile membrane of muscle fiber becomes electrically inexcitable, and although a local response may occur, propagated action potentials do not. Yet, surprisingly, during depolarization block produced by C10, muscle fibers have a mean membrane potential of -61 mV or more. Membrane potential records (not shown) of fibers exposed to C10 and in neuromuscular block reveal that large-amplitude endplate potentials are often generated in response to nerve stimulation but action potentials are not initiated. Although the potential level of -60 mV to -65 mV is a transitional zone of responsiveness,¹¹ we would expect fibers depolarized by such large EPP's to initiate propagated action potentials. Our laboratory is now exploring possible differences between our results, obtained in C10-treated

fibers, and those obtained by Jenerick and Gerard¹¹ on KCl-depolarized fibers. The experiments we report here were not designed to explore these questions.

In conclusion, results of our *in-vitro* experiments indicate that SCH causes more profound depolarization and less desensitization than C10. The desensitization resulting from C10 lasts longer and is more resistant to corrective procedures than that produced by SCH. Although there is a large gap between our experiments and clinical practice, the results indicate that SCH is the drug of choice to produce uncomplicated surgical relaxation.

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