Succinylcholine: Mechanism of Fasciculations and Their Prevention by d-Tubocurarine or Diphenylhydantoin

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Administration of d-tubocurarine (dTC) or diphenylhydantoin (DPH) was evaluated as a pretreatment to prevent succinylcholine (Sch) evoked fasciculations. Experiments were designed to determine the nature of the drug–drug interactions, sites of interaction, and site of fasciculation suppression. Sch is known to evoke repetitive discharge generation by motor nerve terminals (MNTs). Transmission of these prejunctional discharges causes fasciculations. A cat soleus neuromuscular preparation in situ, which enables recording of nerve action potentials initiated by MNTs, their transmitted muscle action potentials, and the resultant contractile responses, was used to explore Sch effects before and after iv pretreatment with dTC or DPH. dTC is known to act prejunctionally to suppress repetitive discharges initiated by facilitatory drugs and tetanic conditioning of MNTs. Accordingly, pretreatment with dTC 50 μg·kg⁻¹ suppressed the Sch-induced MNT repetitive discharging and correspondingly suppressed generalized fasciculations without affecting twitch. This dTC dose, however, also reduced Sch blocking potency by 33%, slowed its rate, and shortened block duration. These latter effects represent competitive postjunctional antagonism. DPH is also known to suppress MNT repetitive discharging. Correspondingly, Sch-induced repetitive firing and ensuing fasciculations were suppressed by DPH (30 mg·kg⁻¹) without affecting twitch. Unlike dTC, this DPH dose increased Sch blocking potency by 50%, increased the initial rate of block, and did not alter block duration. These DPH effects were dose-dependent and within the anticonvulsant range for cats. Therefore, patients with anticonvulsant levels of DPH may not require pretreatment before Sch. It is concluded that the effectiveness of a pretreatment regimen for prevention of Sch fasciculations depends on a prejunctional suppression of repetitive firing generated by MNTs. The cat soleus preparation serves as a clinically relevant in situ method for evaluating prejunctional and postjunctional effects of drugs and should serve as a reliable test for other pretreatment candidates. (Key words: Neuromuscular Junction: diphenylhydantoin (phenytoin); motor nerve terminal; prejunctional activity. Neuromuscular relaxants: succinylcholine; d-tubocurarine.)

SIDE EFFECTS of the depolarizing neuromuscular blocker, succinylcholine (Sch), include muscle pains, elevation of intragastric intracranial and intraocular pressures, hyperkalemia, myoglobinuria, and increased creatinine kinase (CK). The occurrence of fasciculations has been associated with all of these effects and especially postoperative myalgias. While there is no absolute relationship between the degree of gross fasciculations and the subsequent side effects, these effects are minimized by suppressing fasciculations.

To suppress complications associated with Sch fasciculations, small, subparalytic doses of nondepolarizing neuromuscular blockers such as d-tubocurarine (dTC) are often administered prior to a paralyzing dose of Sch. While nondepolarizing neuromuscular blocker pretreatment is a common practice, the basis by which it prevents Sch fasciculations and the quantitation of this effect have not been clarified.

The present experiments were designed to evaluate the neuromuscular effects of Sch with and without dTC present. The presumption was that dTC suppression of Sch fasciculations results from a prejunctional dTC action to suppress Sch-evoked repetitive discharging of motor nerve terminals. Evaluation of this interaction necessitated definition of dose–response and time–action relationships both prejunctionally and postjunctionally. Awareness of such relationships clinically is essential to obtain prejunctional selectivity and to avoid complications of pretreatment with nondepolarizing neuromuscular blockers. Specifically, these latter include inadvertent compromise of spontaneous respiration, as well as antagonism of the desired level of depolarizing neuromuscular blockade with Sch.

Diphenylhydantoin (DPH) pretreatment also suppresses repetitive activity arising from motor nerve terminals. Because DPH, over a wide dose range, does not block twitch responses, it may prove a more selective prejunctional blocker of Sch actions on motor nerve terminals than dTC. Therefore the DPH–Sch dose–response and time–action relationships were also examined prejunctionally and postjunctionally.

For these experiments, a cat soleus neuromuscular preparation was employed. In situ preparation appears to be a clinically relevant model, both qualitatively and quantitatively, for evaluating neuromuscular blockers, their drug–drug interactions, and their antagonists.
Methods

All experiments were performed on anesthetized cats of both sexes weighing between 1.75–3.5 kg. Anesthesia was induced with halothane and maintained with alpha chloralose 90 mg · kg⁻¹ dissolved in 0.9% normal saline administered through the saphenous vein. Cats were hemodynamically stable throughout induction. Experimental recordings were made at least 1.5 h after halothane was discontinued. Cats were ventilated via a tracheostomy with a Harvard ventilator. Ventilation was adjusted to maintain an end-tidal P<sub>CO₂</sub> at 28–32 mmHg, which is normal for cats. For this measurement an Instrumentation Laboratory End-TidIL 200® CO₂ monitor was used. Arterial blood pressure was monitored continuously via a carotid artery cannula attached to a pressure transducer (Statham Instruments, Inc., # 9797 P23AA). Pressures were displayed on a dual-channel Gould recorder (Gould Brush 220® with a Gould preamplifier model 13 421800). The Statham pressure transducer was calibrated with a Tycos sphygmomanometer (#E71157). Blood and dehydration losses were replaced with 0.9% NaCl (40–60 ml iv) through a percutaneous cephalic vein cannula. An electric heating pad was placed under the cat for maintenance of body temperature. The experimental methods were similar to those described in previous publications. Briefly, the procedures are described in the following sections.

Contractile Response of Muscle

A sciatic nerve was sectioned at its exit from the sciatic notch. The ipsilateral popliteal fossa was dissected to expose the innervation of the soleus muscle. The nerve to the soleus muscle was freed from the lateral head of the gastrocnemius muscle; all other branches of the sciatic nerve were sectioned. The soleus muscle was exposed by excision of both heads of the gastrocnemius. The calcaneus was divided and with its attached soleus tendon connected by a light steel rod to a Grass strain gauge (Grass force displacement transducer FT® 10C). The strain gauge was calibrated with standard brass 500-g weights. With the cat in the prone position, the leg was fixed horizontally in a modified Brown-Schuster myograph with bone pins through the tibial head and both malleoli. Contractile tensions were recorded with a Gould direct writing recorder (Gould Brush 220®) (see fig. 1).

A basin was formed by suspension of the popliteal fossa skin flaps and filled with mineral oil. The oil pool was maintained at 37° C with a regulated heat lamp. The nerve to the soleus was placed on platinum electrodes for stimulation. Muscle length was adjusted to achieve recording of maximal isometric twitch tension in response to supramaximal (8–10 V) single stimuli delivered to the nerve at 0.4 Hz. The stimuli were square wave pulses of 0.25 ms duration. The nerve was intermittently stimulated at 400 Hz for 10 s (tetanic stimulation).
MOTOR AXON RECORDING

In order to monitor motor nerve activity in situ, the leg was prepared as discussed earlier, except the sciatic nerve was not sectioned. A dorsal laminectomy exposed the lumbar spinal cord and ventral roots L-7 and S-1. These roots were sectioned close to the cord and further microdissected until filaments containing 1–3 active soleus motor axons were obtained. Filaments were placed on bipolar platinum electrodes for recording and stimulating. Nerve and muscle action potentials were viewed on a dual beam oscilloscope (Tektronix Type RM 565 Dual Beam Oscilloscope®) and recorded on magnetic tape (Vetter® Model B 4 Channel recorder). A basin was formed by retracting and fixing skin edges; the cord and ventral roots were then covered with mineral oil maintained at 37° C.

POSTTETANIC REPETITION (PTR) AND POSTTETANIC POTENTIATION (PTP)

Detailed description of these phenomena can be found in previous publications and are only briefly described here: cats were prepared for motor axon recording as described earlier. The experimental arrangement and principal data recorded are schematized in figure 1. In the control circumstance (see fig. 1), a one-to-one relationship exists between stimulus, nerve action potential, and muscle twitch tension. When stimulated at high frequency (400 Hz for 10 s), motor nerve terminal excitability is altered such that the subsequent single impulses reaching the conditioned terminals generate repetitive discharging. This phenomenon is called posttetanic repetition (PTR). These stimulus-triggered neural bursts are transmitted, and their postjunctional summation leads to increased twitch tension (i.e., posttetanic potentiation, PTP).

PTR and PTP were evoked in these experiments to assess motor nerve terminal changes caused by Sch, dTC, and DPH. The prejunctional actions of these drugs were detected, depending on the drug, either by contractile potentiation or by a reduction or loss of PTR–PTP. Because the PTP of the cat soleus muscle reflects the PTR generated by its motor nerves, PTP was determined at intervals throughout all experiments in order to test and measure the capacity of motor nerve terminals to generate repetitive discharges.

FASCICULATIONS AND DRUG-INDUCED ACTIVITY (DIA)

Certain drugs (e.g., neostigmine and Sch) evoke asynchronous repetitive firing of motor nerve terminals in the absence of nerve stimulation (DIA) (see fig. 2, upper). Transmission of these asynchronous discharges results in generalized muscle fasciculations.

POSTDRUG REPETITION (PDR) AND POSTDRUG POTENTIATION (PDP)

So-called facilitatory drugs (e.g., neostigmine and phystigmine), like high-frequency stimulation (PTR–PTP), can condition motor nerve terminals such that each subsequent single impulse results in repetitive activity (see fig. 2, center). The neural repetition initiated in this way is referred to as postdrug repetition (PDR). The resulting summated twitch tension is called postdrug potentiation (PDP). (PDR and PDP correspond to PTR and PTP, respectively.) PDP, like PTP, is representative of repetitive firing in the motor nerve population. Therefore, PDP and PTP afford an integral measure of repetitive discharging either evoked or suppressed by a drug.

DRUGS

Drugs were administered intravenously at varying doses in the following concentrations: Sch (Quelicin®, Abbott Laboratories) 100 µg · ml⁻¹ by rapid bolus injection, dTC (Tubocurarine Chloride®, E. R. Squibb and Sons, Inc.) 100 µg · ml⁻¹ by rapid bolus injection, and DPH (Dilantin®, Parke-Davis) 30 mg · kg⁻¹ diluted in 10 ml infused over 10–15 min (never exceeding 3 mg · kg⁻¹ · min⁻¹). All dilutions were made with 0.9% NaCl.
**STATISTICAL ANALYSIS**

The resultant paired data (pretreatment and post-pretreatment) were analyzed using paired \( t \) tests. Fasciculation data were evaluated by the sign test, except when a more "efficient test" was indicated.\(^{16}\) In these instances the signed-ranks test was employed.\(^{16}\) Linear regression, parallelism, and common slope analyses were performed as described by Tallarida, Murray, and Jacob.\(^{17,18}\) Statistical significance was accepted at \( P = 0.05 \).

**Terminology of Data and Calibration**

Per cent blockade was calculated as follows: \( \frac{1 - \left( \text{twitch tension at peak block/control twitch tension} \right)}{1} \times 100\% \). Time to peak block was defined as the time from injection until peak twitch depression. Recovery from blockade was defined as the time from peak twitch depression to 50% recovery of predrug tension. Fasciculations were recorded as present or absent.

**Results**

\( d'TC \) PRETREATMENT

*Sch Block.* Sch administered intravenously to cats caused neuromuscular blockade as a function of dose (50–250 \( \mu g \cdot kg^{-1} \)). This relationship is shown in figure 3. The linear portion of the log dose–response is defined by the range 50–150 \( \mu g \cdot kg^{-1} \).

Pretreatment with \( d'TC \) 50 \( \mu g \cdot kg^{-1} \) iv shifted the Sch dose–response regression to the right over the dose range examined (fig. 9). The linear portions of the dose–response relationships for both control and \( d'TC \) pretreatment groups have slope values that are not significantly different \( (P > 0.5) \). Therefore, in figure 3, the regressions are plotted with a common slope value. From this, it can be seen that the \( d'TC \) pretreatment decreased the blocking potency of Sch; the Sch ED\(_{50} \) increased by approximately 50%. Over the 200–250 \( \mu g \cdot kg^{-1} \) range of Sch doses, neuromuscular blockade converged toward 95% block for both groups.

*Time Course of Sch Effects.* For cats pretreated with \( d'TC \) (50 \( \mu g \cdot kg^{-1} \) iv), the first evidence of Sch action was usually block without fasciculations (37/56 trials). The usual sequence can be seen in figure 4. Although \( d'TC \) pretreatment did not alter the time to onset of Sch effects or the time to peak block, it did significantly slow the initial rate of block. Rates of initial block were determined from negative slope values, i.e., 50% change in twitch tension \( \cdot \) time\(^{-1} \). These values are shown in table 1. With \( d'TC \) present, at Sch doses of 100 and 150 \( \mu g \cdot kg^{-1} \), block rates were slowed to 7.0% and 10% of control, respectively. While this \( d'TC \) effect was lessened by higher Sch doses (200–250 \( \mu g \cdot kg^{-1} \)), the differences remained significant \( (P < 0.01) \); at these Sch doses, Sch block rates after \( d'TC \) pretreatment were slowed to 33% and 50% of control, respectively.

\( d'TC \) pretreatment also significantly shortened the duration of Sch block caused by doses of 50–150 \( \mu g \cdot kg^{-1} \) \( (P < 0.01) \). At these doses, average times to 50% recovery ranged from 20 to 70% of control (table 1).

*Fasciculations/PDR.* Sch administered iv over the dose range explored caused generalized fasciculations in 53 of 56 trials. Because these fasciculations arise from a pre-junctional action of Sch,\(^{15}\) the repetitive nerve firing evoked was monitored and recorded from ventral root motor axons. In three cat preparations, following Sch ad-
Succinylcholine: Fasciculations, dTC, Phenytoin

Table 1. Rate and Duration of Sch Block without and with dTC 50 µg·kg⁻¹ 1 IV Pretreatment

<table>
<thead>
<tr>
<th>Sch µg·kg⁻¹</th>
<th>Rate (g·0.04 s⁻¹)</th>
<th>Duration (min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Without dTC</td>
<td>With dTC</td>
</tr>
<tr>
<td>50</td>
<td>—</td>
<td>0.30 ± 0.08*</td>
</tr>
<tr>
<td>100</td>
<td>4.29 ± 1.14</td>
<td>—</td>
</tr>
<tr>
<td>150</td>
<td>3.38 ± 0.83</td>
<td>0.33 ± 0.05*</td>
</tr>
<tr>
<td>200</td>
<td>4.17 ± 0.57</td>
<td>1.56 ± 0.43*</td>
</tr>
<tr>
<td>250</td>
<td>4.80 ± 0.54</td>
<td>2.43 ± 0.43*</td>
</tr>
</tbody>
</table>

All data presented ± SEM.

* P < 0.01.

ministration, repetitive firing lasting for 10–30 s (as illustrated in fig. 2, upper) was observed in sampled motor axons. The internal frequency of this discharge peaked between 200–400 Hz.

In contrast to the fasciculatory discharges, nerve stimulation at 0.4 Hz after Sch evokes an impulse-triggered repetitive discharge (fig. 2, center). In turn, postjunctional summation of this repetitive activity underlies the twitch potentiation seen after Sch (fig. 4, upper). Motor axon recordings have been redrawn above the corresponding twitch tracings. Note that comparable repetitive firing follows either high-frequency stimulation (tetanus) or Sch administration.

Pretreatment with dTC significantly reduced or prevented Sch fasciculations (P < 0.05) (fig. 4). After dTC pretreatment, fasciculations occurred in only 19 of 56 trials. In those preparations in which ventral motor axons were sampled, dTC pretreatment suppressed the repetitive activity following Sch (fig. 2, lower). In those trials in which repetitive activity and, therefore, generalized fasciculations did not occur (fig. 4, lower), Sch twitch potentiation was also abolished.

PPT of soleus twitch tension was assessed at intervals throughout all experiments in order to test the capacity of motor nerve terminals to generate repetitive firing. Repetitive firing, a neural function, is suppressed by dTC; therefore, it is important to emphasize for the present experiments that the extent of PTP suppression by dTC pretreatment corresponded to the degree to which Sch twitch potentiation and fasciculations were suppressed. The redrawn motor axon recordings above the twitch tracings in figure 4 show that suppression of Sch-evoked repetitive activity is suppressed by prior dTC administration.

Notably, the subblocking dose of dTC suppressed these prejunctional phenomena for approximately 2 h. This is more than twice as long as the observed t½ of a dTC dose reference.

DPH Pretreatment

Sch block. An example of the sequence and time course of Sch effects on indirect twitch with and without DPH pretreatment is illustrated in figure 5. Unlike dTC, DPH (30 mg·kg⁻¹ iv) pretreatment, approximately 15 min prior to Sch, significantly enhanced the Sch blocking potency (P < 0.01). Sch dose–response regressions with and without DPH pretreatment are shown in figure 6. Within the Sch dose range of 50–100 µg·kg⁻¹, these dose-re-

![Fig. 4. Twitch tracing recordings (with motor axon recordings above) of Sch without (upper) and with (lower) dTC pretreatment. The tracing on the left illustrates a control PTP. The right tracing shows the initial fast onset of fasciculations and twitch potentiation that follow Sch administration. These brief events are promptly followed by rapid block development. The time to onset of Sch action, time to peak block, and rate of development of block remained constant over the dose range studied.](image-url)
response relationships are linear, with slope values that do not differ significantly \((P > 0.5)\). Therefore, the regressions were plotted with a common slope. From this, it can be seen that after DPH, the \(ED_{50}\) for Sch block is reduced to two-thirds of the control \(ED_{50}\). This shift in Sch potency is opposite to that occurring after \(dT\)C pretreatment. At the highest Sch dose tested \((150 \mu g \cdot kg^{-1})\), the neuromuscular blockade converges toward 95% for both control and DPH pretreatment groups as found for the Sch–\(dT\)C interaction.

**Time Course of Sch Effects.** In the DPH pretreated group, the first evidence of Sch action was block without fasciculations in 25 of 29 experimental points. However, DPH pretreatment did not change the time to peak Sch block \((P > 0.05)\) or the time for recovery from peak block \((P > 0.05)\). Consequently, the duration of Sch block after DPH pretreatment was not significantly different from controls. DPH pretreatment significantly increased the initial rate of Sch block \((P < 0.01)\) (see table 2 and fig. 5, upper). This DPH effect was opposite that seen with \(dT\)C.

**Fasciculations/ PDR.** In the control groups, Sch administration was followed by generalized fasciculations in 31 of 31 trials across the entire dose range studied. In cats, in which activity in ventral root motor axons was sampled, corresponding asynchronous, repetitive discharges were observed as described earlier. These are redrawn in figure 5, upper. There also occurred a stimulus synchronized repetition with obligatory post-Sch twitch potentiation.

**Fig. 6.** Dose–response of Sch blockade without and with DPH pretreatment. Average values \pm SEM shown for each dose pair. Numbers in parentheses: paired trials at each dose. Per cent fasciculations with and without DPH pretreatment tabulated below each dose pair.
Pretreatment with DPH suppressed the generalized fasciculations following Sch administration ($P < 0.01$) (fig. 5). Following DPH pretreatment, generalized fasciculations were observed in only 6 of 29 trials, as compared with 29 of 29 trials prior to DPH administration. Accordingly, post-Sch repetition was not seen in sampled ventral root axons (fig. 5, lower). The corresponding post-Sch twitch potentiation (PDP) was likewise suppressed. DPH pretreatment was also seen to suppress PTP, as Raines and Standaert have previously described.6

Although the pretreatment dose of DPH (50 mg·kg$^{-1}$) suppressed these Sch-induced excitatory activities, it did not impair indirect twitch transmission (see fig. 5, lower). On occasion, slight potentiation of indirect twitch tension was observed following the administration of DPH.

**Discussion**

The effect of low-dose dTC (50 μg·kg$^{-1}$ iv) to decrease the neuromuscular blocking potency of Sch, to slow the rate of Sch block, and to shorten its duration are all compatible with a postjunctional, competitive antagonism between these drugs. Accordingly, regression slopes for Sch block with and without dTC present are parallel. Interestingly, the small, fixed subblocking dose of dTC exerted a competitive anti-Sch effect over a three-fold range of Sch dose (50–150 μg·kg$^{-1}$). However, the extent of this dTC antagonism was overcome by further increasing Sch dose (200–250 μg·kg$^{-1}$; fig. 3), as expected from their competitive interaction. Clinically it is well known that dTC diminishes the blocking potency of Sch.2,5,19 This antagonism of Sch block often results in inadequate relaxation for intubation.5 Therefore in this circumstance, it is common practice to increase the Sch dose to achieve the desired level of blockade.2

Average levels of transmission block and their time courses, without and with low-dose dTC pretreatment, are shown in figure 3 and table 1. From figure 3 it can be seen that after dTC pretreatment, Sch doses of 150 and 200 μg·kg$^{-1}$ are needed to match, respectively, the blocks caused by 100 and 150 μg·kg$^{-1}$ Sch without dTC present. Further, from table 1, these dose comparisons show that times to recovery from *equal levels of block* are not changed by dTC pretreatment. This suggests that recovery time from Sch block is determined primarily by Sch dissociation from acetylcholine (Ach) receptors and that dTC presence does not alter the recovery time.

Apart from the competitive blocking interaction between dTC and Sch, low-dose dTC also prevents Sch-induced fasciculations. In control preparations, Sch causes motor nerve terminals to fire repetitively, hence PDR and DIA are observed. Riker has noted that these repetitive neural phenomena are responsible for Sch fasciculations.16 Therefore, in each experiment, the capacity of mammalian motor nerve terminals to generate fasciculations was assessed periodically, as described in “Methods,” by testing for PTR/PTP.

The fixed subblocking dose of dTC (50 μg·kg$^{-1}$), tested in the present work, suppressed PTR/PTP, confirming the findings of Standaert.11 Correspondingly, this low-dose dTC pretreatment suppressed fasciculations in response to the full range of Sch doses explored. It is concluded, therefore, that the capacity of low-dose dTC to suppress Sch fasciculations represents a dTC action that prevents repetitive firing of motor nerve terminals.

There is a dose-dependent selectivity of dTC for suppression of these prejunctional repetitive phenomena. As Standaert reported, and as confirmed during present work, the dose of dTC suppressing repetitive firing is 50 μg·kg$^{-1}$, whereas the dTC dose causing complete transmission block is 400 μg·kg$^{-1}$.11 It is important also to add that the time course of this dTC suppression of repetitive firing is four to five times longer than the time course of transmission blockade with 400 μg·kg$^{-1}$. These large time course and dose differences reinforce other evidence disclosing that prejunctional dTC action is distinct from that of postjunctional blockade.

The dose-dependent prejunctional selectivity of dTC was used to reveal further details of Sch actions. The sequence of Sch effects are seen in figure 4, showing twitch potentiation followed by block; block and potentiation occur concurrently. Nevertheless, it should be noted that after Sch administration, contractile tension reflects a resultant of two Sch actions, namely potentiation and block. The measured contractile tension at any given moment will depend on the algebraic sum of these opposing actions on fractions of the motor unit population. Sch PDR with resultant increased tension (PDP) may occur in a sufficient number of motor units such that their increased tension obscures the level of a simultaneous blockade in the remaining units. Conversely, a reduced contractile tension can obscure a simultaneously occurring twitch potentiation in a smaller fraction of motor units. These events were resolved with the low-dose dTC pretreatment. This eliminated the Sch fasciculations and twitch potentiation, while the blocking action of Sch remained. In this circum-

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**Table 2. Rate of Sch Block Development without and with DPH (50 mg·kg$^{-1}$ iv) Pretreatment**

<table>
<thead>
<tr>
<th>Sch μg·kg$^{-1}$</th>
<th>Without DPH</th>
<th>With DPH</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1.01 ± 0.33</td>
<td>2.57 ± 0.22*</td>
<td>7</td>
</tr>
<tr>
<td>75</td>
<td>1.6 ± 0.27</td>
<td>3.13 ± 0.23*</td>
<td>9</td>
</tr>
<tr>
<td>100</td>
<td>2.53 ± 0.49</td>
<td>3.60 ± 0.30*</td>
<td>9</td>
</tr>
<tr>
<td>150</td>
<td>3.62 ± 0.24</td>
<td>5.00 ± 0.84</td>
<td>4</td>
</tr>
</tbody>
</table>

All data presented ± SEM. * $P < 0.01$ compared to without DPH.
stance, the time-to-onset of Sch block was the same as that for twitch potentiation in controls. Thus, the two actions of Sch when viewed separately are seen to be concurrent events.

Additional support for a prejunctional site of dTC action in suppressing Sch fasciculations was gained through study of the DPH–Sch interaction. The fixed pretreatment dose of DPH (30 mg·kg⁻¹) suppressed PTR/PTP; this confirms the previous work of Raines and Standaert. Following DPH pretreatment, Sch administration, in the dose ranges explored, failed to cause either generalized fasciculations or repetitive firing in motor axons. In this regard, DPH was more effective than was dTC. DPH appears to protect the motor nerve terminals from depolarization by Sch. It is concluded that DPH and dTC act prejunctionally to prevent a Sch action on motor nerve terminals that would otherwise lead to the asynchronous repetitive firing responsible for generalized fasciculations.

The prejunctional mechanisms by which dTC and DPH act to prevent Sch-induced fasciculations obviously differ. Prejunctional Ach receptors have been identified morphologically and functionally. Therefore, dTC prevention of motor nerve terminal depolarization by Ach is thought to result from dTC interaction with prejunctional Ach receptors. Likewise, dTC prevention of Sch depolarization of motor nerve terminals, as shown herein, is concluded to result from prejunctional cholinergic receptor antagonism. DPH antagonism, however, in accordance with its known actions on nerve, is concluded to stabilize motor nerve terminal membranes by a nonreceptor action. DPH action on motor nerve terminals may result from a reduction of Ca²⁺ fluxes responsible for transmitter mobilization and release. In this regard, dTC and DPH pretreatments may have the same ultimate effect on motor nerve terminals, considering that dTC action on mammalian motor nerve terminals is thought to impair transmitter mobilization.

DPH pretreatment, in contrast to that of dTC, did not antagonize Sch blocking potency. Notably, Sch blocking potency was enhanced. The increased blocking potency of Sch in the presence of DPH is compatible with the view that DPH decreases motor nerve terminal membrane responsiveness similar to its known action on cardiac Purkinje fibers. A resulting decrease in rate of rise and amplitude of nerve terminal action potential could translate directly to a diminished transmitter output. Such DPH action would augment the effective Sch blocking potency. Although DPH in these doses does not affect twitch response as measured in these studies, a sizeable safety factor may prevail in motor nerve terminal responsiveness, as is the case with Purkinje fibers.

For these reasons, DPH may be a better choice for pretreatment than dTC. The chosen dose was 30 mg·kg⁻¹ because previous studies in the cat had shown that this dose, when given acutely by slow iv administration, was maximally effective at suppressing repetitive discharging of motor nerve terminals without effect on twitch. This dose also corresponds to that which suppresses maximal electroshock seizures in the cat. Dose requirements in humans for suppression of motor nerve terminal repetitive activity need to be determined; the cat dose of 30 mg·kg⁻¹ would be twice the recommended loading dose for humans. Numerous patients, especially those presenting for neurosurgery, often have anticonvulsant levels of DPH (10–20 μg·mL⁻¹). This DPH level following chronic administration in patients with seizure disorders may be as effective in suppressing Sch fasciculations as is the large dose given acutely to cats in these experiments, i.e., an interspecies therapeutic equivalent. It is reasonable to speculate, then, that these patients may not require additional pretreatment before Sch. For other patients, however, the cardiac depressant action of acute dosing with DPH may preclude its elective use.

Both the dTC–Sch and the DPH–Sch interactions reveal motor nerve terminals as a specific site of action for drugs effective in the pretreatment of Sch fasciculations. Other drugs have been suggested as effective "pretreatments", including gallamine, pancuronium, lidocaine, diazepam, and dantrolene. Of these, pancuronium, lidocaine, and the new nondepolarizing neuromuscular blocker, atracurium, have been shown experimentally to suppress PTR/PTP in the cat soleus. It is reasonable to expect, therefore, that the reported suppression of fasciculations following pretreatment with these drugs also results from their capacity to prevent Sch depolarization of motor nerve terminals. The value of effective pretreatments clearly lies in the prevention or limitation of the adverse post-Sch effects, which include: increased intragastric and intraocular pressures; hyperkalemia; myoglobinuria; increased CK; postsuccinylcholine myalgias; and bradycardia.

In the studies described herein, the cat soleus neuromuscular preparation provided a reliable model system for evaluating the effectiveness of pretreatment regimens. This system allows the examination in situ of drug activity on mammalian motor nerve terminals. The dTC dose is the same as that used in humans for pretreatment. The Sch doses used are also comparable if the markedly greater serum esterase activity in humans is considered. The anticonvulsant effect of acute DPH dosing (30 mg·kg⁻¹) used in these experiments compares with the therapeutic anticonvulsant level achieved with chronic human dosing. Therefore, these dose equivalencies suggest that the prejunctional and postjunctional actions of these drugs are similar in cats and humans. Thus, the cat soleus preparation permits a clinically relevant examination of elemental components involved in neuromuscular drug actions, i.e., axons and motor units, as well as the integrated
response of these units, namely twitch tension. It should prove predictive in the evaluation of other pretreatment drug candidates.

In summary, pretreatment with dTC results in a competitive antagonism of the Sch neuromuscular blockade consistent with a postjunctional site of this action. On the other hand, prejunctional suppression of Sch fasciculations by dTC results from dTC suppression of a motor-nerve-terminal-generated repetitive activity. DPH also suppresses Sch fasciculations at a prejunctional site, although its mechanism differs from that of dTC.

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