

# Recovery of Neuromuscular Function in the Perfused Rat Diaphragm after Succinylcholine and Pancuronium Blockade

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The recovery of the rat diaphragm from neuromuscular blockade was studied in order to separate the contributions of drug binding and tissue washout. The *in vitro* rat diaphragm preparation was perfused with a cholinesterase-free solution via the phrenic vein and stimulated electrically via the phrenic nerve. Muscle paralysis was induced by infusion of a depolarizing blocker succinylcholine or by the nondepolarizing blocker pancuronium. The time for recovery from 25–75% recovery averaged  $1.0 \pm 0.1$  min for pancuronium and  $0.8 \pm 0.1$  min for succinylcholine at the fast (1.9 ml/min) perfusion rate. This was prolonged to  $2.0 \pm 0.3$  min for pancuronium and  $1.4 \pm 0.1$  min for succinylcholine at the slower (0.76 ml/min) rate. More sensitive paired tests of recovery utilizing recovery time and rate demonstrated no drug difference in three comparisons and a 44% longer recovery time for pancuronium only at the lower perfusion rate. In general, recovery times were similar for both drugs. The rapid recovery from pancuronium blockade in this mammalian system as perfusion rate was increased suggests that recovery from this drug is not impaired by drug binding but is strongly dependent on organ perfusion. (Key words: Neuromuscular relaxants; pancuronium; succinylcholine.)

DEGRADATION OF SUCCINYLCHOLINE by plasma esterases leads to a rapid return of normal neuromuscular function. The nondepolarizers in use today have durations of action of 30–60 min depending on dose, route of administration, and condition of recovery.<sup>1</sup> If they were removed from the circulation as rapidly as succinylcholine, would their action terminate as rapidly? Feldman and Tyrrell<sup>2</sup> addressed this question in an arm isolated from the circulation by a tourniquet, and concluded that there was an intrinsically slower recovery from a nondepolarizing block<sup>2,3</sup> as a result of slow drug receptor dissociation. If tissue factors limited the recovery from nondepolarizing block, these factors would present a major obstacle to the development of a very short-acting nondepolarizing drug.

In order to eliminate the effect of degradation of succinylcholine in the plasma, we used an *in vitro* rat diaphragm preparation perfused by a solution free of circulating cholinesterase. Recovery of neuromuscular block from succinylcholine and pancuronium was studied at two rates of perfusion. In this way, the contribution of organ perfusion could be separated from effects of tissue binding since the latter would be expected to be independent of perfusion rate.

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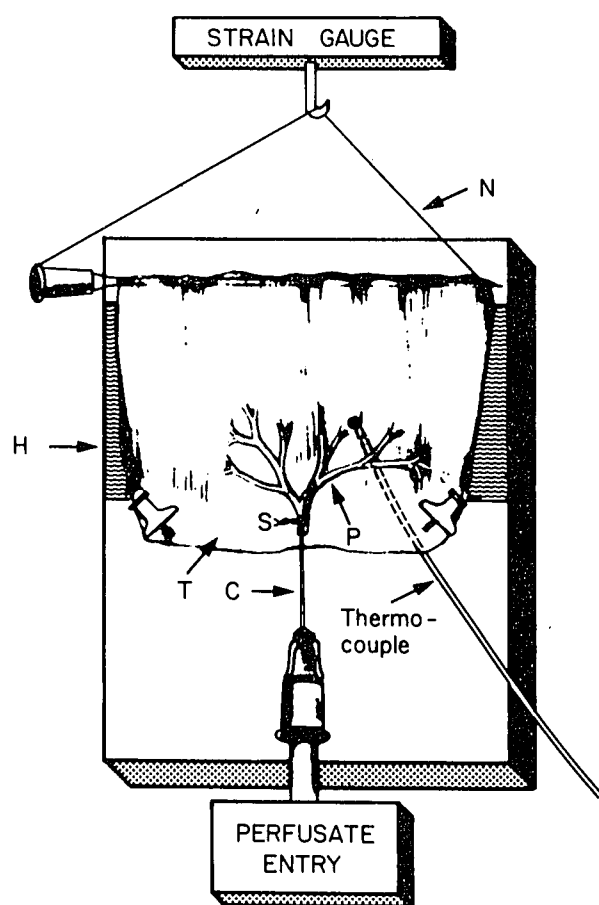
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## Materials and Methods

### ANIMALS AND APPARATUS

The left hemidiaphragm and phrenic nerves of 12 male Wistar rats (300–500 g) were mounted in the apparatus of figure 1 immediately following decapitation. A tie through the costal tendons coupled the diaphragm to a calibrated Grass® FTO3C strain gauge force transducer. The transducer position was adjusted until it showed an initial resting tension of 6–8 g. Position was then fixed for the duration of the experiment. A polyethylene cannula, connected to a calibrated syringe infusion pump, supplied retrograde perfusion<sup>4</sup> via the left phrenic vein. The perfusate was a Tyrodes solution modified to provide levels of ionized calcium and magnesium in the normal range for rats and humans. It contained constituents at the following levels in mM/l: sodium 143, potassium 4.4, calcium 1.25, magnesium 0.6, chloride 124, bicarbonate 25, sulfate 1.25, phosphate 1.1, glucose 11, and choline 0.01. In addition, 200 mU/l insulin and albumin at 50 mg/l as an insulin carrier were added prior to infusion. The solutions were bubbled with an O<sub>2</sub>–CO<sub>2</sub> mixture to provide a pH of 7.4 and a P<sub>CO<sub>2</sub></sub> of 35–40 mmHg at 37° C. The solution was heated to the working temperature 37° C by a feedback controlled in-line heater. Another heater kept the plate on which the muscle was mounted at the same temperature. A thermocouple in contact with the lower surface of the muscle measured diaphragm temperature. The upper surface of the diaphragm was covered with polyethylene (0.002-cm thickness) to prevent evaporative heat and moisture loss. The entire apparatus was housed in a clear enclosure warmed by a heat lamp to minimize thermal gradients.

The phrenic nerve was mounted on two silver electrodes. These were connected to a stimulator to deliver pulses at 1.5–2.0 times the voltage necessary to provide maximal tension. The pulses were 150- $\mu$ s duration and typical voltages were 1.5–2.0 volts. A Commodore® PET 4016 digital computer controlled the timing of the stimulating pulses. This device also received the tension data through an analog-digital converter. The computer displays the time and the maximal tension for each stimulus and produces a printed record. The shape of the tension time curve, *i.e.*, the twitch, is observed continuously on an oscilloscope screen to ensure reliable data.



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|------------------|--------------------|
| C Cannula        | H Baseplate heater |
| T Central tendon | S Suture           |
| P Phrenic vein   | N Nylon tie        |

FIG. 1. Schematic of the perfused diaphragm apparatus. The muscle lies horizontally on the platform. The cannula, C, connected to an infusion pump perfuses the diaphragm via the phrenic vein, P. The nylon tie, N, couples the strain gauge transducer to the costal tendons. The phrenic nerve passing over stimulating electrodes originates from the lower surface of the diaphragm and is not seen in this view.

#### PLAN

Drug-free solution was infused at 37°C until the twitch tension value was stable for 10 min. In six diaphragms, 14–15  $\mu\text{M}$  succinylcholine was perfused until the twitch tension reached 5% of the initial drug-free tension. The perfusion rate for the drug was 1.9 ml/min, the maximum rate used in these experiments. The perfusion rate was reset immediately to either 1.9 (N = 3) or 0.76 ml/min (N = 3) and the solution changed to a drug-free medium. The time of beginning washout was noted at the time drug-free medium first entered the diaphragm. The computer triggered stimuli to the muscle at 20-s intervals and recorded peak de-

veloped tension along with the time. This continued until tension recovered to a stable value.

Drug-free perfusion continued for 30 min to allow washout of succinylcholine. The identical procedure was repeated using 2.4–2.6  $\mu\text{M}$  pancuronium. After 30 min of drug-free perfusion, the entire sequence for succinylcholine and pancuronium was repeated at the other perfusion rate.

In six additional animals the first drug perfused was 2.4–2.6  $\mu\text{M}$  pancuronium at the higher (1.9 ml/min) rate. Perfusion for the initial recovery was either 1.9 (n = 3) or 0.76 ml/min (n = 3). The entire sequence listed for succinylcholine was repeated with the order of drug administration reversed. In this way, complete data were collected for 12 animals, one-half with pancuronium first and one-half with succinylcholine first.

#### ANALYSIS

To assess the rate of recovery of twitch, two indices were used. Recovery index (RI) is the time from 25% to 75% recovery of twitch. Recovery index is a measure of the average recovery rate over the mid-portion of the recovery process. As a measure of the initial recovery rate the slope of the twitch tension *vs.* time curve at 20% of maximal tension was chosen. A single observer drew a tangent to the recovery curve at 20% recovery and measured the slope from the graph in units of per cent maximal twitch per minute. An index for comparison of the two drugs was formed by calculating the ratio of the pancuronium value and the succinylcholine value for a single diaphragm at the same perfusion rate. This allows each animal to serve as its own control and can eliminate some of the animal-to-animal differences. Student's *t* test, corrected for multiple comparisons by the Bonferroni method,<sup>5</sup> was used for comparison with *P* < 0.05 considered significant.

#### Results

At the 15  $\mu\text{M}$  concentration of succinylcholine, 7–10 minutes were required to reach a stable depressed twitch tension. No evidence of tachyphylaxis or increasing tension, as described for a changing or type II succinylcholine block,<sup>6</sup> was seen in any preparation. Pancuronium, 2.4–2.6  $\mu\text{M}$ , took a comparable time to depress developed tension to 5% of maximum value. The recovery data for the 12 rat diaphragms are presented in table 1. The data showed no significant differences between pancuronium and succinylcholine recovery. There were no differences between numbers 1–6 that had succinylcholine first and numbers 7–12 that had pancuronium first. There were also no differences based on the order of high and low perfusion rates. Figure 2

TABLE 1. Two Measures of Recovery from 95% Block Induced in a Rat Diaphragm by Approximately Equipotent Levels of Pancuronium and Succinylcholine

Experiment* Number	Pancuronium (1.9 ml/min)†		Succinylcholine (1.9)		Pancuronium (0.76)		Succinylcholine (0.76)	
	Slope‡	RI§	Slope	RI	Slope	RI	Slope	RI
1	86	0.8	63	0.8	61	1.6	43	1.2
2	108	0.8	78	0.5	58	1.5	49	1.0
3	54	1.6	85	0.9	22	3.5	48	1.4
4	128	0.5	67	0.5	55	1.1	43	1.3
5	75	0.9	68	0.7	33	1.9	43	1.2
6	60	1.3	71	1.0	28	2.6	44	1.4
7	65	0.8	52	0.9	27	3.0	31	2.0
8	146	0.6	129	0.5	80	0.8	74	0.7
9	47	1.6	60	1.1	26	2.8	22	1.8
10	56	1.1	54	1.0	25	1.8	40	1.7
11	53	1.5	46	1.3	26	2.5	30	2.2
12	150	0.5	113	0.5	64	0.9	78	0.7
MEAN	86	1.0	76	0.8	42	2.0	45	1.4
± SEM	11	0.1	7	0.1	6	0.3	5	0.1

\* Diaphragm numbers 1-6 had succinylcholine first; numbers 7-12 had pancuronium first.

† Perfusion rate.

‡ Per cent/min of recovery curve measured at 20% maximal tension.

§ Min for recovery from 25-75% maximal tension.

displays the data for one of the preparations along with a graphic representation of the recovery parameters used to quantitate the results.

Using the ratio of the pancuronium to succinylcholine value of each rat and for each of the two recovery parameters of table 1 gives a more sensitive paired test for drug effects. The averages  $\pm$  SEM of the pancuronium/succinylcholine value for initial slope were  $1.12 \pm 0.08$  (1.9 ml/min) and  $0.93 \pm 0.09$  (0.76 ml/min). The averages of this ratio for recovery index were  $1.23 \pm 0.08$  (1.9 ml/min) and  $1.44 \pm 0.12$  (0.76 ml/min). Only the last value was significantly ( $P < 0.05$ ) different from unity by *t* test when corrected for four comparisons. This implies that pancuronium took 44% longer than succinylcholine to span 25-75% recovery at the slower perfusion rate.

A ratio of each recovery parameter at one perfusion rate to that at the other rate was calculated for each experimental animal to provide a paired test for perfusion effects. The average  $\pm$  SEM for the initial slope (1.9/0.76 ml/min) for pancuronium was  $2.10 \pm 0.09$  while that for succinylcholine was  $1.72 \pm 0.10$ . For recovery index which decreased with faster recovery the ratio was calculated by taking the values at 0.76 ml/min divided by the value at 1.9 ml/min to give a result comparable to those above. The average of the 12 values for pancuronium was  $2.03 \pm 0.17$  and for succinylcholine was  $1.74 \pm 0.11$ . Since faster recovery leads to a larger value of slope and a smaller value of recovery index, the values for both indices represent fractional increases in the rate of recovery. There were no sig-

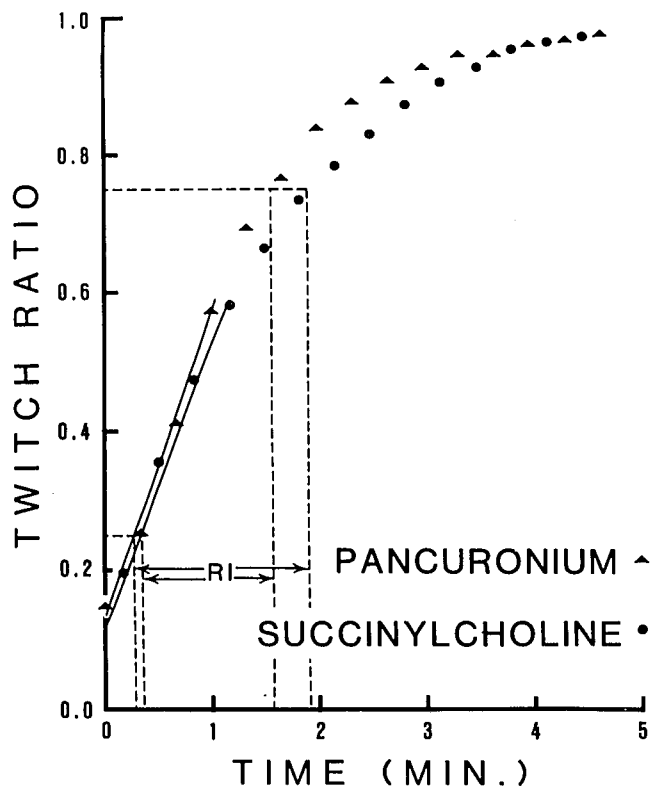


FIG. 2. Recovery of the twitch tension in a single preparation during washout with drug-free medium. Twitch ratio is the tension divided by the maximal or drug-free tension. Illustrated are the two recovery parameters used in the analysis. The solid lines show the slope drawn to fit the recovery curve at 20% twitch recovery. The dashed lines show the Recovery Index (RI) representing the time from 25-75% recovery.

nificant differences among these results for either index or drug. Not seen in the averages, but easily appreciated in table 1, was that every rat diaphragm for both drugs and both recovery indices recovered faster at the higher perfusion rate. The ratios of recovery parameters formed from the two rates of perfusion have a range from 1.33–3.76.

### Discussion

These experiments sought to determine the relative contribution of tissue factors such as drug binding *vs.* tissue washout on the rate of recovery of neuromuscular block induced by approximately equipotent doses of pancuronium or succinylcholine. The experimental model is the rat diaphragm. This preparation allows control over the muscle perfusion rate which can be varied as an independent factor to distinguish perfusion-related changes. The effect sought was a difference in the rate of recovery. For this two measures were used, recovery index and initial slope of recovery. The recovery index was used by Feldman<sup>2,3</sup> because he found it independent of the level of depression from which recovery began provided that there is no recirculation of drug to the perfusing vessels. The latter assumption may not be entirely accurate<sup>1</sup> in the isolated human arm preparation that he employed. The slope of the recovery curve at 20% of control tension appears useful because it is a measure of initial recovery rate while the RI measures the mid-portion of the recovery phase. The 20% point was chosen because the recovery curves (fig. 2) appear sigmoidal with an inflection near 20% of control tension. Because an inflection is a region of maximal and relatively constant slope, determination of the recovery rate is more reliable in this region.

The recovery curves were drawn through values spaced 15–20 s apart. This spacing was chosen to avoid problems with fade or depressed response that can occur with stimuli closer than 10 s apart in a blocked preparation. The recovery from succinylcholine also can be affected by the character of the block, whether phase I or II. Although tachyphylaxis or increasing tension at constant drug level seen by Galindo and Kennedy<sup>6</sup> in a rat diaphragm at the onset of Phase II was not observed here, it is possible that some change of block occurred. To minimize this, the lowest concentration of succinylcholine consistent with 95% block was used and recovery was begun immediately after reaching this level. It is difficult to maintain pure phase I succinylcholine block in practice, even in humans,<sup>7</sup> but lower doses and shorter exposure times are associated with phase I. The concern about phase II comes from the possibility of slower recovery when the ratio of the block

changes.<sup>8</sup> The evidence against a prolonged recovery is in table 1, which shows a very short recovery time of 0.8 min for succinylcholine at the higher perfusion rate (1.9 ml/min). Because the perfused area of the diaphragm had a weight of 1.2–2.5 g, the observed washout time is consistent with a simple picture of tissue washout. The short time and consistency with predicted washout argue against any significant prolongation of recovery.

A study of the maximal rate of recovery from nondepolarizing block may seem to be redundant since a curare block has been found to have a rapid recovery and short dissociation time (less than 0.1 s) in frog neuromuscular junction treated with collagenase<sup>9</sup> to expose the neuromuscular receptors. Other mechanisms for tissue effects, however, such as restricted or buffered diffusion in the synaptic cleft were proposed to explain the slower rate of recovery observed for this block in untreated preparations.<sup>9</sup> These experiments showed that Feldman's proposed mechanism, *i.e.*, slow drug reception dissociation prolonging recovery from nondepolarizing could not be correct. They did not explain his findings of slower recovery of nondepolarizing block which could occur by another mechanism such as restricted diffusion by receptor or basement membrane binding. Slow recovery of nondepolarizing block also has been seen in a dog hind limb perfused by a roller pump.<sup>10</sup> In spite of a perfusion rate increase from 50 to 400 ml/min the recovery index remained unchanged in the 5- to 30-min range. This was taken as further evidence of tight drug binding and intrinsically slow recovery for nondepolarizing blockers. This topic recently has been reviewed in detail.<sup>11</sup> The experiments described here examine the rate of recovery when drugs are delivered and removed via the circulation in a mammalian system.

The comparisons of recovery using the pancuronium-succinylcholine ratios found only small differences. Overall, the recovery of neuromuscular function in the perfused rat diaphragm is similar for pancuronium and succinylcholine. In this preparation, both drugs encounter identical conditions. Perfusion rate is the same for both. The concentrations of drug in the perfusing medium are reduced to zero in a few seconds. Both drugs then wash out of the tissue with a gradient determined by the tissue level. This compares with the *in vivo* situation where the drug is washed out at a rate determined by the difference between tissue and plasma level. Succinylcholine, however, differs from other drugs *in vivo* by its metabolism in plasma by the circulating esterases. This accounts for its rapid elimination and termination of action. In the setting of our experiments, both drugs are eliminated by perfusion and thus

are on an equal footing. Under these circumstances both terminate their blocking action at a similar rate.

The reason for the difference between the recovery times measured here and those observed clinically is addressed in the experiments which vary the perfusion rate. It has been suggested<sup>12</sup> that blood flow is the rate-limiting factor preventing recovery of nondepolarizing relaxants in the isolated arm experiments of Feldman. Washout of the drug from the muscle to bloodstream raises capillary blood levels, lowers the gradient for drug outflow, and limits recovery. Using estimates of blood flow, the clinically observed recovery was reported to be consistent with this hypothesis. The effect of slowing perfusion rate in the rat diaphragm is to slow recovery. In this system, the prediction that recovery is slowed at lower flow rates is verified. Looking at the converse, higher flow rates lead to more rapid recovery. As table 1 demonstrates, the recovery time for several of the preparations was less than one minute at the high rate of perfusion. If there is an intrinsic barrier to the recovery of function after nondepolarizing block as suggested by Feldman,<sup>2,3</sup> the rat data imply that it is very brief. The recovery times are so short for these animals as to be insignificant in practice because drugs are not eliminated from the plasma this rapidly. By using an *in vitro* preparation where vascular perfusion is controlled and freely variable, the independent effect of perfusion on recovery from neuromuscular block is demonstrated.

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