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The Margin of Safety of Neuromuscular Transmission in the Muscle of the Diaphragm

B. E. Waud, M.D., and D. R. Waud, M.D.†*

Recovery from neuromuscular block is commonly assessed by examining the response of a limb muscle to stimulation of its motor nerve. However, it is in the muscles of respiration, particularly the diaphragm, that the degree of recovery is most crucial. Therefore, the diaphragm has been compared with the tibialis anterior with respect to margin of safety of neuromuscular transmission. When the twitch of the tibialis anterior has returned to normal during recovery from *d*-tubocurarine, the diaphragm has almost twice as many receptors free as are barely necessary to produce a maximal twitch response. Thus, recovery in the periphery is a conservative index of recovery in the diaphragm. (Key words: Margin of safety; Neuromuscular transmission; Diaphragm; *d*-Tubocurarine; Succinylcholine.)

IT HAS BEEN DEMONSTRATED that there is a large margin of safety of neuromuscular transmission.[1] Specifically, in a limb muscle such as the tibialis anterior 75–80 per cent of the receptors have to be blocked by *d*-tubocurarine before the most sensitive muscle fibers fail to respond to single nerve stimuli, and 90–95 per cent must be blocked before transmission fails at the more resistant neuromuscular junctions.

Clinically, nerve stimuli can conveniently be applied to peripheral muscles only. On the other hand, the muscles of prime interest in connection with recovery of a patient from the effect of a neuromuscular blocking agent are those concerned with respiration, particularly the diaphragm. There is some suggestion that the diaphragm is more resistant to the action of *d*-tubocurarine than the peripheral musculature,[2-5] but a direct measurement of this difference in terms of the relative margins of safety in the two muscles is not available. We have, therefore, determined the margin of safety of neuromuscular transmission in the diaphragm.

Methods

The preparation (fig. 1) was essentially an extension of that previously described.[1, 6] Fourteen experiments were done in cats of unselected sex and weighing 2.4–3.5 kg. Seven experiments were done in dogs (7.6–11.3 kg) to obtain some indication of the extent of variation among species. Cats were anesthetized with chloralose, 80 mg/kg, iv, after induction with ether, dogs with pentobarbital, 25 mg/kg, iv, supplemented as necessary. Periodic determinations of arterial P_{O_2}, P_{CO_2} and *p*H were made and respiration was adjusted to bring these values into the normal range.[7]

In the early experiments, the chest was opened anteriorly by a midsternal incision, but later by a left paramedian incision through the costal cartilages. The latter approach allowed better hemostasis, since ligatures could readily be placed around both ends of each rib as it was cut. For recording the mechanical response of the diaphragm, an isometric strain-gauge transducer was coupled to the costal cartilages, which serve as origin for the anterior part of the left hemidiaphragm. The muscle attachments adjacent to this part of the diaphragm were freed from the rest of the thoracic cage so as to provide a mobile connection to a slip of the muscle. Such a recording system monitors cardiac activity and inflation of the lungs, as well as the twitch elicited from the diaphragm. To minimize the respiratory artifact, mechanical ventilation was stopped

* Assistant Professor of Anaesthesia.
† Associate Professor of Pharmacology.
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Address reprint requests to: Dr. B. E. Waud, Department of Anaesthesia, Peter Bent Brigham Hospital, 721 Huntington Avenue, Boston, Massachusetts 02115.

periodically for a few seconds to give a tidier record of the twitch. The phrenic nerve on the left side was crushed high in the chest and the distal segment laid over a pair of platinum electrodes. Supramaximal stimuli of 0.3-msec duration were applied to the nerve every 10 seconds throughout the experiment. The phrenic nerve on the other side was crushed. The chest was covered with a plastic sheet to prevent drying and was kept at about 35 C with an infrared lamp.

An indifferent wick electrode was mounted on the membranous part of the diaphragm. A scanning wick electrode was set up so that it could be swept by a synchronous motor drive from the membranous part of the diaphragm along the muscle fibers towards their origin on the costal margin. The length of the muscle was scanned in about 5 seconds. An XY recorder was connected so as to record the voltage difference between the scanning and indifferent electrodes on the y axis against distance along the muscle fibers on the x axis. Normally, such a tracing (*cf.* fig. 2, bottom) is essentially horizontal, except for a slight negativity towards the costal end of the muscle fibers (this negativity presumably reflects an injury potential where the costal cartilage was freed from the rest of the chest wall).

A fine intra-arterial catheter [6] was inserted through the left common carotid artery into the descending part of the thoracic aorta so that the tip lay slightly above the origin of the arterial supply of the diaphragm. Test doses of succinylcholine were then injected through this cannula, and the muscle surface was scanned periodically thereafter. The result was a series of records of the potential profile along the muscle. These show the development of negativity over the endplate region of the muscle (fig. 2), followed by a return to normal. The peak negativity was taken as a measure of the intensity of the depolarizing action of the succinylcholine.

After a series of graded doses of succinylcholine had been administered to obtain a control depolarization dose–response curve, an intravenous dose of *d*-tubocurarine sufficient to abolish the twitch response of the diaphragm was given. The rest of the experiment consisted of periodically giving a test dose of succinylcholine and recording the depolarization for comparison with the magnitude of the twitch response. Doses of succinylcholine were not given more frequently than at hourly intervals. With such spacing, cumulative desensitization is not seen; stable sensitivity is obtained.[1, 6] The details of the analysis of the succinylcholine-induced depolarizations in terms of receptor occlusion by *d*-tubocurarine have been given previously.[1, 6] The result of such an experiment is a series of measure-

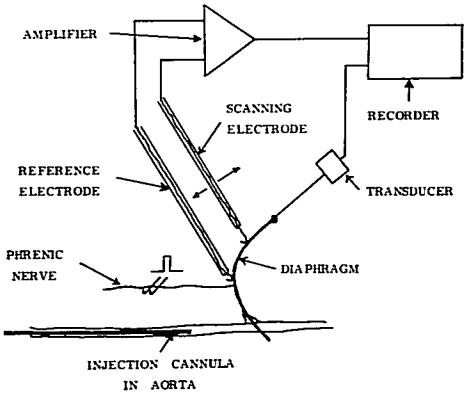

FIG. 1. The experimental arrangement. The phrenic nerve was stimulated every 10 seconds and the resultant twitch response was recorded by connecting an isometric transducer to the segment of costal cartilage serving as origin for the anterior part of the left hemidiaphragm. Succinylcholine was injected intra-arterially into the diaphragm through a cannula placed in the descending aorta. The resultant depolarization was recorded by comparing the potential of a wick electrode scanned along the muscle fibers with that of another on the inert membranous part of the diaphragm.

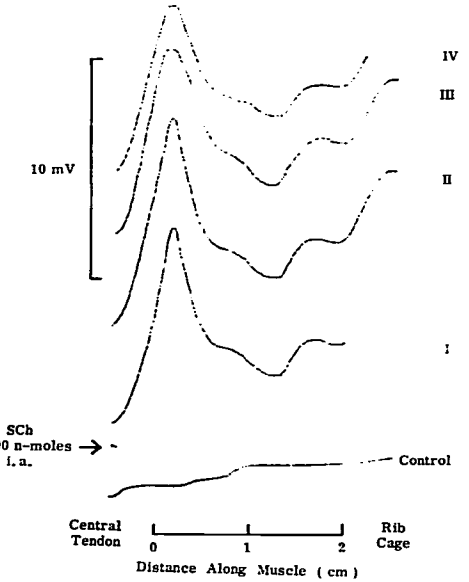

FIG. 2. Example of electrical record from the diaphragm. *Ordinate:* depolarization (mv). *Abscissa:* distance along muscle (cm). The bottom trace is the voltage profile obtained before the administration of 200 nmoles of succinylcholine intra-arterially. The tracings above are those recorded about every 30 seconds after succinylcholine was given. Between successive tracings, the pen was moved upwards slightly so that the tracings would not overlap. The control tracing shows a slight negativity at the right-hand end. This probably reflects a slight injury potential. With administration of succinylcholine an area of negativity is produced in the center of the tracing. This is the result of the depolarizing action of succinylcholine, which is localized in the endplate region of muscle fibers.

ments of interference with the twitch response and associated measurements of receptor occlusion by *d*-tubocurarine.

The drugs used were succinylcholine iodide (K and K) and *d*-tubocurarine chloride (K and K).

Results

The results in the cat are summarized in figure 3. In this figure, fractional twitch height is plotted against receptor occlusion by *d*-tubocurarine. Thus, on the scale of ordinates a value of 1 means a normal twitch was obtained; a value of zero means no twitch was obtained. Similarly, along the scale of abscissae, a value of 1 means *d*-tubocurarine had blocked all the receptors; a value of zero means no receptors were blocked. The values obtained in the diaphragm are given in the right panel. The left panel gives values from the tibialis anterior for comparison. Examination of the figure indicates that the response of the diaphragm is very similar to that of the tibialis anterior. Specifically, the twitch is normal until about four fifths of the receptors are blocked and is not completely abolished until nine tenths or more are blocked. Figure 4 shows analogous results in the dog; a similar picture is obtained.

The receptor occlusion corresponding to 50 per cent reduction of twitch height was used as an index for comparison of the two muscles, because the twitch height–receptor occlusion curve is steepest here. For each animal a line was fitted by eye to the values in the plot of twitch heights against receptors blocked. Thus, sets of values of receptor occlusion that reduced twitch height 50 per cent could be obtained for both tibialis anterior and diaphragm. These two sets were compared by calculating the mean difference and its 95 per cent confidence limits. In the cat, 10 per cent more receptors (limits 2.8 to 17.2) must be blocked in the diaphragm to halve the twitch response.

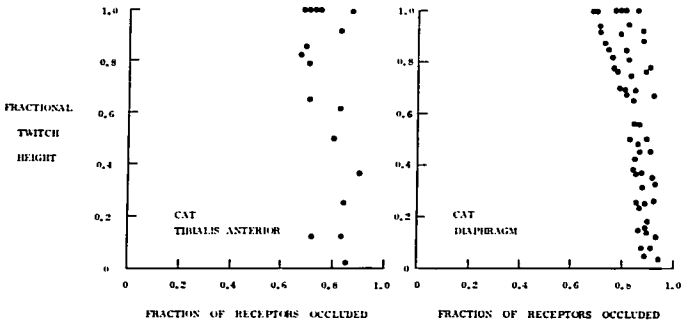

FIG. 3. The margin of safety of neuromuscular transmission in the cat. *Ordinate:* twitch response as fraction of control. *Abscissa:* fraction of receptors blocked by *d*-tubocurarine. *Left:* tibialis anterior, eight cats. *Right:* diaphragm, six cats.

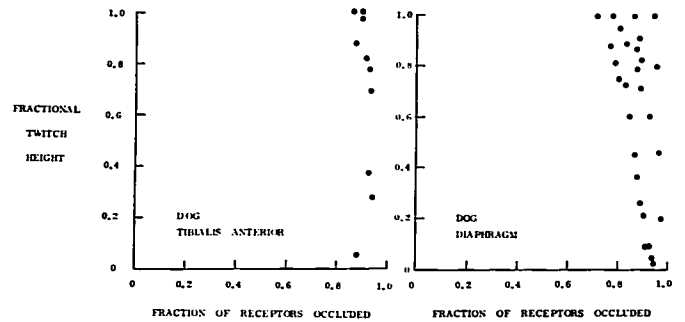

FIG. 4. The margin of safety of neuromuscular transmission in the dog. Plot as in figure 3. Four diaphragm and three tibialis preparations. Behavior is similar to that seen in the cat.

In the dog, the mean difference was only 0.6 per cent, and the 95 per cent limits, −6.6 to +7.8, include zero. Thus, in the cat there seems to be a slight difference between the two muscles, while in the dog any difference is obscured by variation between animals.

In order directly to examine this more closely, we made three preparations in the cat and one in the dog in which the margin of safety was measured not only in the diaphragm but also simultaneously in the tibialis anterior (by the method previously described [6]). The results from two such animals are given in figure 5. In each case, the curve for the diaphragm lies to the right of that for the tibialis anteriors, *i.e.*, the margin of safety was slightly greater in the diaphragm. For 50 per cent reduction of the twitch responses in three such paired experiments in cats, an average of 8.3 per cent more diaphragmatic receptors had to be occupied by *d*-tubocurarine (95 per cent confidence limits were 7.3 to 9.3 per cent). This mean difference corresponds well to the 10 per cent obtained in unpaired experiments,

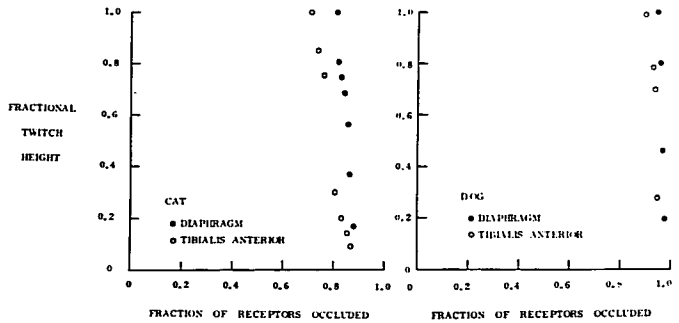

FIG. 5. Margin of safety of neuromuscular transmission measured simultaneously in the tibialis anterior and in the diaphragm. Examples from a cat (*left*) and a dog (*right*). Plot as in figure 3. Closed circles: diaphragm. Open circles: tibialis anterior. In each animal more receptors have to be blocked to interfere with transmission in the diaphragm.

while the smaller error limits obtained with pairing confirm that variation between animals contributed considerably to the spread found when the measurements were not made in the same animal.

The effect of *d*-tubocurarine on the indirectly-elicited twitch response is consistent with the above measurements of margin of safety (fig. 6). More *d*-tubocurarine is needed to block transmission in the diaphragm, and the diaphragm recovers sooner.

Discussion

A small but consistent difference between the margins of safety in the diaphragm and limb muscles was found. The difference between muscles seems less in the dog than in the cat. This probably reflects, to some extent, the tendency to a higher margin of safety in the limb of the dog (a species in which evolution might reasonably have been expected to select individuals with a sounder apparatus for running). But even in the dog,

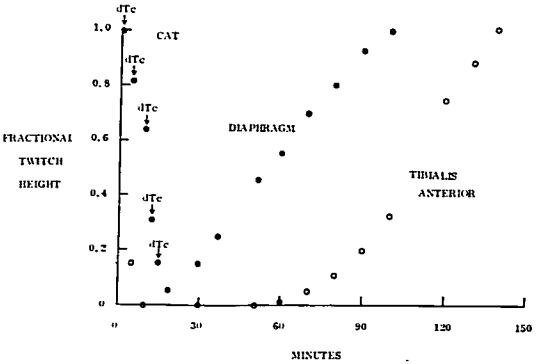

FIG. 6. The effect of *d*-tubocurarine on the indirectly-elicited twitch responses in the diaphragm and the tibialis anterior of a cat. *Ordinate:* twitch height as fraction of control. *Abscissa:* time. Closed circles: diaphragm. Open circles: tibialis anterior. At the arrows, 1 μmole of *d*-tubocurarine was administered intravenously. More *d*-tubocurarine is needed to block the diaphragm, which also recovers sooner.

when precision is increased by simultaneous measurement in both muscles the diaphragm is slightly more resistant.

This difference between the margins of safety in the diaphragm and the tibialis anterior is of the same order of magnitude as variation among individuals. Thus, it might seem that the difference is inconsequential. However, in any given individual, the diaphragm recovers before the tibialis, and this edge is accentuated if one focuses not on the receptors blocked by *d*-tubocurarine but on those *not* blocked. It is, after all, the latter which matter when transmission is considered. To illustrate, consider the two animals whose records are shown in figure 5. For the recovery of the diaphragm, roughly 5 per cent of the receptors must be free in the dog, and 18 per cent in the cat. For recovery in the tibialis, the values are 10 and 29 per cent, respectively. Thus, the diaphragm needs only about half as many receptors as the tibialis anterior for a normal twitch response. Hence it is not surprising that figure 6 shows a clear difference between the two muscles (as has been seen frequently previously[2-5]).

The physiologic drive to the diaphragm is tetanic in nature. Therefore, a few tetanic responses were examined. The diaphragm gave results similar to those seen in limb muscles,[6] that is, the fade of a 5-second tetanus at 30/sec recovered *pari passu* with the twitch, while at 100/sec roughly half the receptors had to be available before a normal response was obtained.

It is not possible to determine the reason for the difference between muscles from the present experiments. Lu[9] found no difference between the affinities of respiratory and limb muscles for *d*-tubocurarine. However, differences in amounts of transmitter released, amounts destroyed by cholinesterase, geometry of the synaptic clefts, depolarizing efficiencies of the transmitters, or electrical thresholds of the muscle fibers still remain as possible explanations. Temperature differences between leg and diaphragm do not seem responsible. In the three experiments in which the tibialis anterior and the diaphragm of the cat were examined simultaneously, the two muscles were at 34 C in two animals, while the diaphragm was 3 C higher in the third. However, all three animals behaved similarly.

Conclusions

The diaphragm has a greater margin of safety than peripheral muscles. Thus, the response of peripheral muscles to nerve stimulation provides a slightly conservative index of diaphragmatic recovery from competitive neuromuscular block.

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