Anesthesiology
V 35, No 5, Nov 1971

The Relation between Tetanic Fade and Receptor Occlusion in the Presence of Competitive Neuromuscular Block

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The relation between receptor occlusion by a competitive neuromuscular blocking agent and fade of an indirectly elicited tetanus at frequencies of 30, 100 and 200/sec has been determined. Tetanic fade at 30/sec returned to normal when one-fifth to one-quarter of the receptors became available, and thus recovery approximated closely that of the single twitch response. The tetanic fade at 100/sec was not normal unless half the receptor pool was available, and a normal response at 200/sec required two-thirds of the pool. It is suggested that recovery of the response to tetanic stimulation at 100/sec would provide a practical index of adequate recovery from curarization. (Key words: Competitive neuromuscular block; Tetanus; Fade; Receptor occlusion; Margin of safety; *d*-Tubocurarine; Succinylcholine.)

NEUROMUSCULAR TRANSMISSION involves the release of acetylcholine, which diffuses to the surface of the muscle cell to react with specific sites called receptors. Competitive neuromuscular blocking agents act by combining with these receptors to occlude them and thereby hinder access of the transmitter. Teleological considerations indicate that neuromuscular transmission would not be borderline, *i.e.*, more receptors would be available than those barely necessary for transmission of the signal. The term "margin of safety of neuromuscular transmission" is used to refer to this excess. The magnitude of the margin of safety has been measured. Paton and Waud[1] have shown that only 20–25 per cent of the receptor pool is necessary for transmission to all of the fibers in a muscle. In other words, the twitch response to a nerve–muscle monitoring system can be completely normal when 75–80 per cent of the receptors are blocked by *d*-tubocurarine.

Tetanic fade, especially at high frequencies of stimulation, is more sensitive to interference by a competitive blocking agent than is the simple twitch.[2, 3] This suggests that more receptors are needed for a sustained tetanus than are needed for a single muscle twitch. Therefore, the extent of tetanic fade might prove to be a more sensitive index of the fraction of receptors occluded than the single twitch. To see if this were the case, we used *d*-tubocurarine to occlude receptors to various extents and determined the associated degrees of tetanic fade. Thus, tetanic fade in the presence of *d*-tubocurarine was calibrated in terms of the fraction of receptors blocked.

Methods

The preparation was essentially that used by Paton and Waud.[1] Experiments were done on cat tibialis anterior, sartorius, and soleus muscles, and on rabbit tibialis anterior and dog tibialis anterior. In cats, anesthesia was usually induced with ether for about 5 minutes and then chloralose, 80 mg/kg, was given intravenously. (About 2 hours of surgical preparation separated the ether induction from the beginning of recording.) Some spinal cats were used to show that effects obtained were not those of the chloralose. Rabbits were anesthetized with urethane, 1.25 g/kg, iv, and dogs with chloralose, 140 mg/kg, or pentobarbital, 25 mg/kg, iv, supplemented as needed.

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Received from the Department of Anesthesia, Peter Bent Brigham Hospital, and Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115. Accepted for publication May 20, 1971. Supported by USPHS Research Grant NS 04618 from NINDS.
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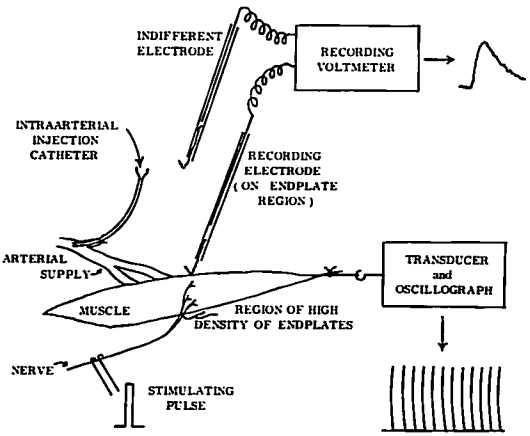

FIG. 1. Experimental preparation. Bottom part of figure: the response to indirect stimulation is obtained by stimulating the motor nerve to the test muscle and recording the isometric twitch. Top part of figure: the response to a test dose of succinylcholine is obtained by injecting the drug intra-arterially into the muscle and recording the subsequent depolarization of the endplate region of the muscle as a function of time. The voltage was measured relative to an indifferent electrode on a tendon of the contralateral limb.

Respiration was controlled on the basis of periodic determinations of Pa_{O_2}, Pa_{CO_2} and *p*H, which were kept in the range reported for normal cats.[4] Ringer's lactate solution with 5 per cent dextrose was infused intravenously to help maintain arterial pressure and urinary flow.

The experimental arrangement is indicated diagrammatically in figure 1. The preparation was a combination of two techniques. The first was a standard nerve–muscle preparation in which the muscle was stimulated indirectly through its motor nerve with 0.3-msec shocks of approximately twice maximal intensity and the resulting mechanical response obtained on an oscillographic recorder by means of an isometric force transducer. (Even when the tibia is fixed with a metal pin, there is still enough elasticity in the system that the response is best considered auxotonic, not isometric.) Tetanic stimuli were given in trains lasting 5 seconds and were spaced not less than 5 minutes apart.

The second part of the preparation was used to measure receptor occlusion. The surface of the muscle was exposed and the skin edges fixed to a brass oval so as to form the walls of a liquid paraffin bath over the muscle. The paraffin was kept at about 35 C with an infrared lamp. The potential difference between a wick electrode on the endplate region of the muscle and an indifferent electrode on a tendon of the contralateral limb was recorded as a measure of the depolarizing effects of test doses of succinylcholine. These were injected intra-arterially in a constant volume of 0.2 ml through a fine catheter inserted in a small muscular side branch of the femoral artery, as described by Waud and Waud.[12] The animals were heparinized; 20 mg were given initially and 10 mg every hour thereafter.

The strategy of the experiments can best be illustrated by describing the procedure. After the muscle had been prepared, the recording electrode was moved along the surface of the muscle to confirm that it was isopotential. Then a test dose of succinylcholine (about 20 nmoles) was injected intra-arterially and the muscle surface scanned again. Areas of negativity were found over regions of endplate density, and the electrode was left over one such area. (This placement could be checked periodically by seeing if the response decreased when the electrode was moved along the fiber during an elicited depolarization.)

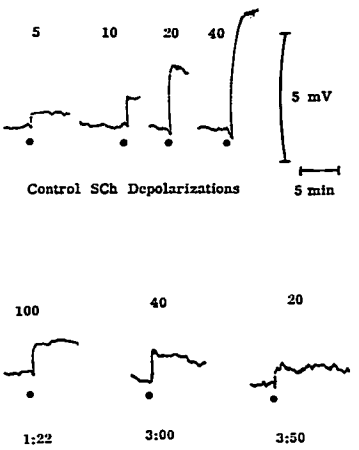

FIG. 2. Record from a typical experiment. Top row: control responses to graded doses of 5, 10, 20 and 40 nmoles succinylcholine, ia. Next, at 1:20 PM, *d*-tubocurarine was given in two doses (of 500 μmoles iv) sufficient to produce about 50 per cent depression of the twitch response. The rest of the experiment consists of following recovery of the depolarizaton response to succinylcholine. At 1:22 PM 100 nmoles succinylcholine produced a depolarization matching the control response to 10 nmoles, thus the dose-ratio was 100/10 = 10. Similarly, dose-ratios of 4 and 2.6 were obtained at 3:00 and 3:50 PM.

The subsequent course of the experiment consisted of three stages. Control responses were obtained. *d*-Tubocurarine was administered. Recovery was followed. Focus first on depolarization responses to succinylcholine (fig. 2). Initially, a control series of injections was given and resulting responses to give a control dose–response curve were recorded (figure 3 gives a more conventional plot). After *d*-tubocurarine had been given, sensitivity of the endplate area to succinylcholine was tested periodically. In the experiment of figure 2, the first test came soon after the *d*-tubocurarine and, in anticipation of considerable receptor occlusion, a dose of 100 nmoles of succinylcholine was given. The response obtained matched closely that obtained with 10 nmoles of succinylcholine originally. This dose-ratio of 100/10 = 10 can be interpreted as follows. Since 10 times as much succinylcholine was needed after tubocurarine, only 1/10 of the receptors were free, *i.e.*, 90 per cent were blocked. The analytical expression for fractional receptor occlusion can be confirmed [7, 11] to be y = (dose-ratio − 1)/dose-ratio), or in the above case, 9/10.

Periodically during further recovery, test doses of succinylcholine were given. For example, the response to a later test dose of 40 nmoles again matched the 10-nmole control response, so the dose-ratio was 4. That is, receptor occlusion had fallen to 75 per cent. Still later, 20 nmoles matched 7.5 to give a dose-ratio of 2.6 or a receptor occlusion of about 60 per cent.

Since frequently repeated doses of succinylcholine might lead to desensitization, we spaced test doses about an hour apart. This seems to have kept desensitization negligible, since the sensitivity returned to control levels when we waited long enough for *d*-tubocurarine concentrations to fall to low levels. The price paid for such wide spacing of test doses of succinylcholine was that a test dose could not be paired with every tetanic stimulation. Consequently, we had to obtain dose-ratios corresponding to any given tetanus by interpolation.

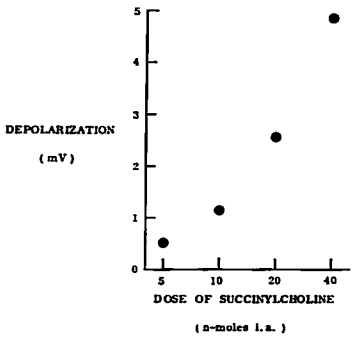

FIG. 3. Example of control dose–response curve to succinylcholine (from experiment of figure 2). Ordinates: depolarization (mV). Abscissae: doses of succinylcholine (nmoles ia). Graded responses result from graded doses to form a suitable basis for the bioassay.

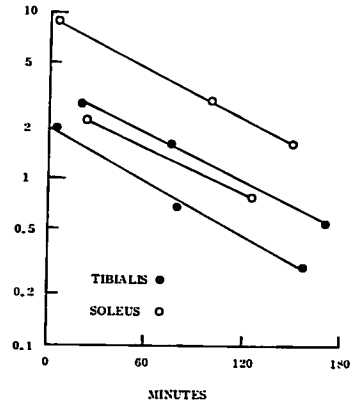

FIG. 4. Decrease in concentration of *d*-tubocurarine with time. Examples from four cats (top curve from preparation of fig. 2). Ordinates: logarithms of concentration of succinylcholine as dose-ratio minus 1 (see text). Abscissae: time in minutes after iv administration of *d*-tubocurarine. ● tibialis anterior. ○ soleus. The concentration falls exponentially, with a half-life of about 70 minutes.

We have based the interpolation on the following model. *d*-Tubocurarine is a charged compound restricted essentially to the extracellular space and eliminated by a first-order process with a half-life of about 70 minutes (*cf.*, Cohen,[1] table 1, line 1). Since (dose-ratio − 1) varies as concentration (*cf.*, Waud,[11] equation 31), a plot of (dose-ratio − 1) on a logarithmic scale against time should be linear and yield a half-life of about 70 minutes. Fig. 4 gives examples of four such plots. The system behaved as expected; therefore, we interpolated linearly in plots like those in figure 4. For example, consider the top curve in figure 4 and suppose a tetanus had been obtained at 60 minutes. We would interpolate between measured (dose-ratio − 1) at 2 minutes and at 100 minutes to get an estimate of 4.5 for the (dose-ratio − 1) at 60 minutes. (Hence, we would estimate fractional receptor occlusion as $4.5/5.5 = 0.82$.)

Now turn to the tetanic responses (fig. 5) obtained in the experiment of figure 2. The control tetani at 30 and 100/sec were well-maintained (panels A and B). After *d*-tubocurarine (which reduced the twitch to about half normal), the response to a 100/sec tetanic stimulus was markedly attenuated and faded rapidly and completely (panel C). Subsequently, the response to 100/sec tetanic stimuli became progressively better sustained, until finally recovery was achieved. The 30/sec tetani were not followed as extensively in this preparation, but it can still be seen that the 100/sec tetanus faded markedly when the 30/sec tetanus had recovered completely (panels D, E, F).

Receptor occlusion can be measured directly in terms of the fraction of receptors occupied by the competitive blocking agent. However, because the tetanic response is complicated, it is impossible to say *a priori* what the most natural index of interference with the process would be. We arbitrarily defined a tetanic fade-ratio as the ratio of the final to the maximal response during the tetanus. For example, in the control tetanic responses of figure 5 the final tension equals the maximal tension so the fade-ratio would be unity. On the other hand, the tetanic response in panel C faded completely; the final tension was zero so the fade-ratio was zero. In some animals the control tetanus faded somewhat. In such instances we normalized all fade-ratios by dividing by the control value. Thus, the fade-ratio goes from a value of unity, meaning the control value, to zero, meaning complete fade. Like Paton and Waud,[7] we have expressed the twitch response as a fraction of its control value.

d-Tubocurarine was used as the competitive blocking agent in most experiments. In a few

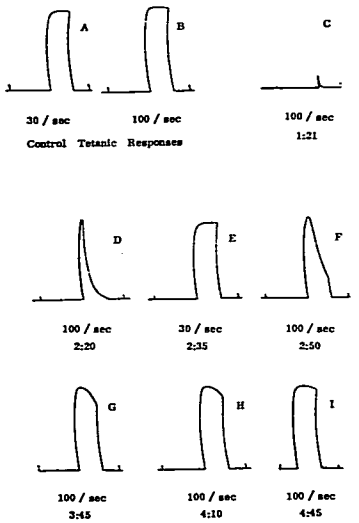

FIG. 5. Tetanic responses from the preparation in figure 2. Panels *A* and *B* show control responses to tetanic stimulation at 30 and 100/sec. *d*-Tubocurarine given at 1:20 PM. *C–I* are responses during recovery. At *C* the twitch was depressed about 50 per cent. At *D–I* the twitch response had returned to normal.

animals gallamine was used to confirm that the results did not depend on choice of antagonist. The *d*-tubocurarine, succinylcholine, and gallamine were the chloride, iodide, and triethiodide, respectively. All were obtained from K & K Laboratories.

Results

Cat Tibialis Anterior

Most experiments were done on the tibialis anterior of the cat. Like Paton and Waud,[7] we found that the twitch recovered as the fractional receptor occlusion fell from about 0.9 to 0.75 (fig. 6, top left). When the fade response to a 30/sec tetanus was examined, a similar picture was obtained (fig. 6, lower left). However, when a frequency of 100/sec was used for the tetanic stimulus, the curve shifted to the left (fig. 6, top right); that is, the tetanic response faded markedly when receptor occlusion was about 0.8 and did not recover until about half the receptors became free. When a 200/sec tetanic stimulus was examined, still more receptors had to be free before any given degree of recovery was obtained (fig. 6, lower right). Specifically, at 200/sec the tetanic responses still faded markedly when receptors were 60 per cent occluded and did not return to normal until only 20–30 per cent of the receptors were still blocked.

Cat Soleus

The tibialis anterior is principally a white, or fast, muscle. For comparison we also examined a representative red, or slow, muscle, the soleus. The response to a 200/sec tetanus faded almost completely even in the absence of *d*-tubocurarine and was not examined further. Otherwise, the results (fig. 7) were very similar to those found in the tibialis. Specifically, the twitch response and the fade of a 30/sec tetanus behaved similarly, and both recovered by the time fractional receptor occlusion had fallen to about 0.75–0.8. The response to a 100/sec tetanus was again more sensitive to receptor block and did not return to normal until about half the receptors became free.

Rabbit Tibialis Anterior

To obtain some indication of species variation, we examined responses in the rabbit tibialis anterior. The results (fig. 8, left) resembled those found in the cat. The twitch response and the fade of a 30/sec tetanus were essentially indistinguishable, while the responses to 100 and 200/sec tetanic stimuli were more sensitive to receptor occlusion.

Dog Tibialis Anterior

The response of the tibialis anterior of the dog was also examined (fig. 8, right). The margin of safety appeared larger in the dog; all the curves lay slightly to the right of the corresponding curves in the cat and rabbit. However, the same general picture was obtained. The twitch response and the fade of a 30/sec tetanus went hand in hand, while the 100/sec tetanus did not recover until more re-

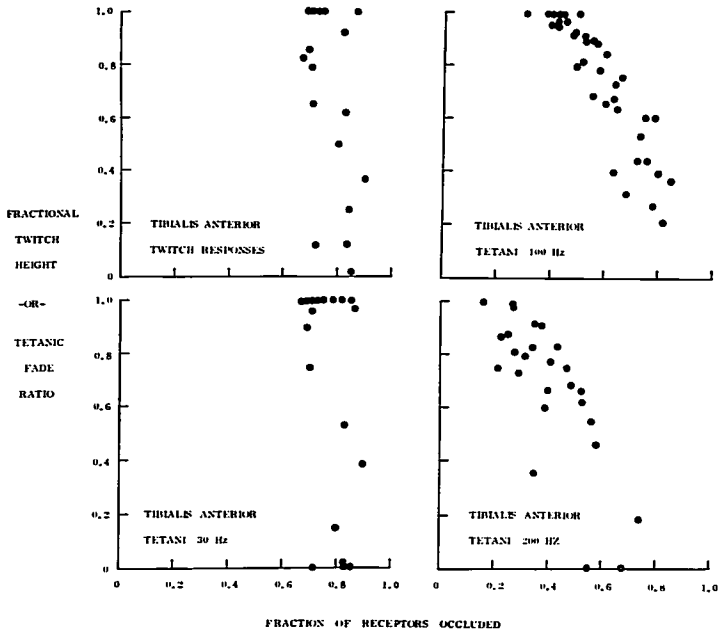

FIG. 6. Relation between twitch response or tetanic fade and receptor occlusion. Top left panel: twitch response plotted against receptor occlusion. When 20–25 per cent of the receptors become free the twitch response returns to normal. Remaining panels, tetanic responses. Bottom left: tetanic fade at 30/sec recovered at the same range of receptor occupancy at which the twitch recovered. Right-hand panels: tetani at 100/sec are not well-maintained until half the receptor pool becomes available, while tetani at 200/sec are not normal until two-thirds of the receptors are free.

ceptors became available. A few values for 200/sec tetani indicate that they recovered still later, as in the other two species.

Discussion

Consideration of the margin of safety of neuromuscular transmission is essential in both clinical and experimental examination of the effects of competitive neuromuscular blocking agents such as *d*-tubocurarine. For example, a patient may show a normal twitch response to the ulnar nerve stimulation and still have 75–80 per cent of his receptors blocked by *d*-tubocurarine. While the remaining 25 per cent of the receptors would be adequate for transmission to occur, there would be no receptor reserve, *i.e.*, no margin of safety. Thus, the patient would be in a very precarious position. If any change (temperature, electrolytes, or fall in transmitter output) were to occur, muscle response could fail.

Consideration of the margin of safety also indicates why tetanic responses are normally well-sustained but fade in the presence of *d*-tubocurarine. Acetylcholine output can be expected to decrease progressively during a tetanic stimulation. Normally, the margin of safety is so large that the acetylcholine out-

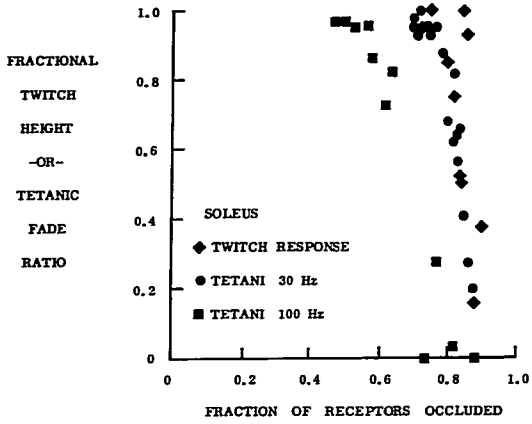

FIG. 7. Relation between mechanical response (twitch or tetanus) and receptor occlusion in cat soleus muscle. ◆ twitch responses; ●, ■ tetanic responses at 30/sec and 100/sec, respectively. Ordinates: fractional twitch height or tetanic fade-ratio. Abscissae: fractional receptor occlusion. The results parallel those in figure 6.

put does not fall low enough to cause transmission failure, *i.e.*, tetanus does not fade. Now suppose 75 per cent of the receptors were blocked by *d*-tubocurarine. A single indirectly elicited twitch response would be normal, as would the initial part of a tetanus. In other words, the amount of acetylcholine released would be just sufficient to activate the muscle fiber. However, as the tetanus continued, acetylcholine output would fall to the point where in more and more fibers it would no longer be sufficient to initiate a muscle response. As fewer and fewer fibers responded, the mechanical response would fade.

Such considerations suggested the use of tetanic response as an indirect measure of receptor occlusion below the levels reflected in the twitch response.

The frequency of tetanus which would be optimal for this purpose can only be determined empirically. When tetanic stimulations were applied for 5 seconds every 5 minutes, we found that a frequency of 30/sec offered little advantage over the twitch responses, *i.e.*, there was no tetanic fade when 75 per cent of the receptors were blocked. On the other hand, stimulation at 100/sec or at 200/sec allowed examination of lower levels of receptor occlusion.

It has been suggested that neuromuscular blocking agents might exert some of their action by affecting the presynaptic nerve terminals.[9] However, this does not affect the conclusions reached in the present paper. Whether or not blocking agents act presynaptically as well as postsynaptically, the fact remains that half the receptors have not become available until the 100/sec tetanus is well-sustained.

A considerable scatter was observed in the degree of receptor occlusion corresponding to any given degree of depression of a tetanic response. Some of this scatter simply reflects the fact that the experiments are very complex and hence carry with them a certain amount of random variation of estimation. However, much of the variation seems to represent variance among animals. It is conceivable that the slightly larger margin of safety seen in our three dogs reflects just such individual differences rather than species variation. To illustrate the variation between animals, results from two cats are given in figure 9. The cats were not different on any *a priori* grounds, so it seems that the margin of safety varies significantly among cats and, presumably, among people. The cat represented by the closed symbols consistently gave responses to the left of those for the other cat (*i.e.*, showed a lower margin of safety).

This consistency is of interest in connection

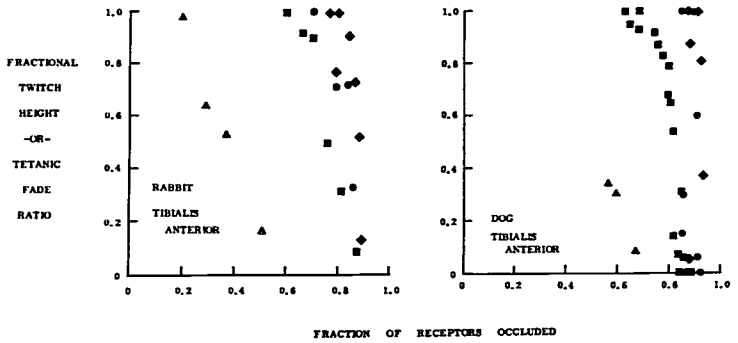

FIG. 8. Relation of mechanical response to receptor occlusion in the rabbit (left) and dog (right). Tibialis anterior. ◆ twitch response; ● tetani at 30/sec, ■ tetani at 100/sec, ▲ tetani at 200/sec. Ordinates: fractional twitch height or tetanic fade-ratio. Abscissae: fractional receptor occlusion. The results are similar to those in the cat.

with use of a 100/sec tetanic response to monitor recovery from *d*-tubocurarine. An index of recovery should reflect not just the fraction of receptors free but rather free receptors above and beyond those barely necessary for transmission, *i.e.*, the receptor reserve. Figure 9 shows that the tetanic response to a 100/sec tetanus behaves appropriately. The cat that needed more free receptors for recovery of the twitch response also needed more before the 100/sec tetanic response was well-sustained.

The type of experiment described here cannot easily be done in man. However, the similarity of responses seen in the three species (cat, dog, rabbit) makes it seem unlikely that man would behave differently. On the other hand, since respiratory muscles are peri-

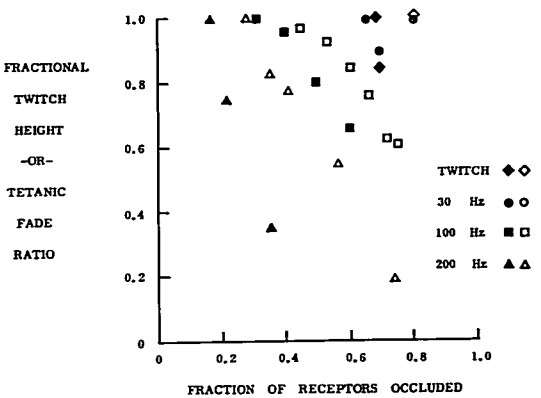

FIG. 9. Variation in margin of safety between animals. Values from two cats (open *vs.* closed symbols). Diamonds represent twitch responses; circles, squares, and triangles responses to tetanic stimuli at 30, 100, and 200/sec, respectivley. The values for one cat (closed symbols) lie consistently to the left of those for the other.

odically reported to be less sensitive to neuromuscular block than are muscles of the extremity,[5, 8, 10] extrapolation from limb muscles to those of respiration seems less likely to be successful than extrapolation across species. Recently, Lu[6] reported no difference in affinity of *d*-tubocurarine for respiratory as opposed to other muscles. This suggests that the measurement of receptor occlusion in the periphery reflects closely the occlusion in respiratory muscles (but does not mean the margins of safety are the same).

Ideally, recovery from *d*-tubocurarine would be followed by determining the number of receptors still blocked, but direct measurement in patients is not practical. The results presented above show that an indirect measure of receptor occlusion can be obtained by following the tetanic response. Both tetanic and twitch responses vary consistently with the numbers of receptors occluded. The twitch response, however, has recovered completely by the time a fourth of the receptors are free, and at this point ceases to be a useful index of receptor block. The tetanic response at 30/sec shares this disadvantage. On the other hand, the tetanic responses at 100/sec and 200/sec provide useful measures of receptor occlusion until half to two-thirds of the receptors become free. Since the response at 200/sec can fade considerably in some muscles (for example, the cat soleus), the 100/sec response may prove to be more convenient in practice.

In summary, we suggest that the clinical stimulators in use enable us to measure receptor occlusion above 75 per cent only. Thus, normal responses could be obtained, yet there might be a negligible margin of safety for neuromuscular transmission. On the other hand, use of tetanic stimulation at 100/sec allows the practicing anesthesiologist to measure receptor occlusion down to 50 per cent and thus ensure the existence of an appreciable receptor reserve.

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