Equipment

Refractoriness of neuromuscular transmission: determination by computer subtraction of neurally evoked compound electromyograms

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Shortly after elicitation of a muscle response by supramaximal stimulation of its motor nerve, there exists a period of refractoriness of neuromuscular transmission when a second stimulation elicits only a submaximal response or no response at all. Many anaesthetics and neuromuscular blocking and facilitatory drugs change the refractoriness of neuromuscular transmission. Measurement of this refractoriness may improve our understanding of the neuromuscular actions of these drugs and be useful in differential diagnosis. However, the neuromuscular refractory periods (RP) are difficult to measure, and the unavailability of normal values renders accurate determination of drug effects impossible. Based on an existing technique of digitization and time expansion of the neurally evoked compound electromyogram (ncEMG), we developed a computer programme of waveform subtraction, and determined in nine

normal awake volunteers the various interstimulus intervals when neuromuscular transmission was refractory (RP_0), 3/4 refractory (RP_{25}), half refractory (RP_5), 1/4 refractory (RP_{75}) or non-refractory (RP_1). We confirmed our hypotheses that computer-based waveform subtraction of the digitized ncEMG is a feasible and necessary technique for the accurate determination of the RPs of neuromuscular transmission, and report that the normal values in humans are: $RP_0 = 1.0 \pm 0.1$, $RP_{25} = 1.3 \pm 0.3$, $RP_5 = 1.9 \pm 0.3$, $RP_{75} = 2.9 \pm 0.5$, and $RP_1 = 6.6 \pm 1.9$ ms (mean $\pm$ SD), respectively, in the ulnar nerve-first dorsal interosseous muscle model.

Key words

MEASUREMENT TECHNIQUES: electromyography;
NEUROMUSCULAR TRANSMISSION: monitoring,
refractoriness.

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Peu de temps après la contraction musculaire provoquée par une stimulation supramaximale, il se produit une période réfractaire au cours de laquelle la stimulation ne provoque qu'une réponse sous-maximale ou ne provoque aucune réponse. Plusieurs anesthésiques, relaxants neuromusculaires et drogues facilitatrices diminuent la réactivité de la transmission neuromusculaire. La mesure de cette hyporéactivité peut nous permettre de mieux comprendre l'activité neuromusculaire de ces drogues et nous aider au diagnostic différentiel. Cependant, la mesure des périodes réfractaires neuromusculaires (RP) est difficile et l'absence de valeurs normales empêche d'évaluer précisément l'action de ces drogues. Grâce à une technique existante de numérisation et l'expansion temporelle de l'électromyogramme à potentiel nerveux évoqué (ncEMG), nous avons pu créer un logiciel pour soustraire des formes d'ondes. Ensuite nous avons déterminé chez neuf volontaires éveillés les différents intervalles entre les stimuli lorsque la jonction neuromusculaire est réfractaire (RP_0), 3/4 réfractaire (RP_{25}), demi-réfractaire

(RP_{50}), 1/4 réfractaire (RP_{75}) ou non réfractaire (RP_1). Nous pouvons confirmer notre hypothèse selon laquelle la soustraction de la forme d'onde par ordinateur du ncEMG numérisé est une technique réalisable et essentielle pour déterminer de façon précise les RP de la transmission neuromusculaire et nous rapportons que chez l'humain, les valeurs normales mesurées sur un modèle utilisant le nerf cubital et le premier muscle interosseux dorsal sont respectivement (moyenne $\pm$ SD): $RP_0 = 1,0 \pm 0,1$, $RP_{25} = 1,3 \pm 0,3$, $RP_5 = 1,9 \pm 0,3$, $RP_{75} = 2,9 \pm 0,5$ et $RP_1 = 6,6 \pm 1,9$ ms.

Neuromuscular transmission has a refractory period during which a second supramaximal stimulus to the motor nerve results in a submaximal muscle response or no response at all. This refractoriness is sensitive to inhalational anaesthetics,¹ anticholinesterases,² depolarizing^{3,4} and nondepolarizing² neuromuscular relaxants, hexafluorenum,⁵ ketamine,⁶ and magnesium (personal information). Analysis of the neuromuscular refractoriness may shed new light into how these various types of neuromuscular effects occur. It might also lead to new monitoring techniques of differential diagnosis.

In studying the refractoriness of neuromuscular transmission, however, we realized that existing techniques were inadequate and reliable normal values were not available. Mechanomyographically, the response of the muscle to double nerve stimuli is complicated by the excitation-contraction coupling processes which are irrelevant to the electrophysiological phenomenon of refractoriness, per se. Electromyographically, the two neurally evoked compound electromyograms (ncEMG) are difficult to quantify separately, because the second response (R_2) is superimposed on to the first (R_1) to make a combined response (R_{1+2}). As illustrated in the Results section, the rapidly changing "baseline" of R_2 makes it difficult to ascertain its amplitude or waveform. Multiplexing of the ncEMG⁷ and repetitive firing⁸ further complicate the picture. Therefore, to determine accurately the refractoriness electromyographically, it is essential that the time course and the amplitude of both the R_1 and R_2 waveforms be determined. To do this, we hypothesized, one must subtract the R_1 waveform from the combined R_{1+2} response digitally, point by point, to obtain the R_2 .

In a clinical setting, all muscle responses should, preferably, be available on-line and displayed immediately on paper, rather than on the computer terminal or the magnetic tape recorder alone. This requires digitization and time expansion of the waveforms.⁹ Furthermore, not only the emergence of the R_2 ("absolute refractory period") but also the endpoints when R_2 becomes 25%, 50%, 75%, and 100% of maximum should be determined

FIGURE 1 Diagram to illustrate the functions of the ncEMG machine (upper panel) and the EMG waveforms (middle panel). The calibrations (lower panel) illustrate a $200\times$ amplification ($10\text{ mV} \times 200 = 2\text{ V}$), and a $160\times$ time-expansion ($16\text{ ms} \times 160 = 2560\text{ ms}$) of the EMG. A stimulus (not shown) has stimulated the motor nerve and triggered the ncEMG machine. Dots represent digital data of the analogue waveform. An adjustable delay (not shown) has adjusted the location of the 16-ms detection period, according to the nerve conduction and neuromuscular transmission times, so that the detection period coincides with the EMG activities. Whereas pen recorders are normally too slow to record the ncEMG signals at the original speed, they are capable of recording the time-expanded signals with high fidelity.

so that drug effects can be investigated as a complete "dose-response" curve. We report here one such technique and the normal values obtained from the human ulnar nerve-first dorsal interosseous muscle model commonly used in anaesthesia practice for the electromyographic (EMG) monitoring of neuromuscular transmission. Since recent anaesthesia papers rarely mention the refractoriness of neuromuscular transmission, we also offer a brief update on the subject.

Methods

The ncEMG technique and the computer-based determination of refractoriness

A method of digital analysis of the ncEMG suitable for clinical use has been described previously.⁹ The published diagram allows for construction of an ncEMG machine with custom specifications (Figure 1). Essentially, a supramaximal stimulus stimulates the motor nerve and triggers the ncEMG analyzer. The trigger initiates a 16-ms time window during which the electrical response of the muscle is amplified and sampled digitally 512 times. The window can be delayed at will to open just before the EMG begins at the end of the latency. The digitized data are then time-expanded and converted back to analogue waveform for display. For this study, we selected a time expansion factor of 160 to allow a pen recorder 2560 ms ($16\text{ ms} \times 160$) to write out the 16-ms information on a strip of paper 12.8 mm long when the paper was

FIGURE 2 Computer-aided determination of the refractoriness of neuromuscular transmission. The upper panel depicts the functions of the ncEMG machine. A software programme commands the computer to obtain the amplified, digitized and time-expanded R_1 and R_{1+2} ncEMG signals, align them point by point, and subtract them to determine the R_2 . The R_1 , R_{1+2} and R_2 data can be stored in the computer or returned to the ncEMG machine for digital-to-analogue reversion and display. Dots represent digital data of the analogue waveforms. Dashed lines indicate the functions of the computer.

run at $5 \text{ mm} \cdot \text{sec}^{-1}$. The slow-motion display allows a pen recorder sufficient response time to write out the very rapid EMG signal with high fidelity. The expanded display also enabled us to examine the waveforms and to read them with accuracy.

A programme was written to command a personal computer to (1) receive and store the R_1 and the R_{1+2} sets of 512 datum pairs from the ncEMG machine, (2) align and subtract the R_1 from the R_{1+2} to produce the new set of 512 datum points of R_2 , (3) store the R_2 , and (4) return the R_1 , R_{1+2} , and R_2 to the ncEMG machine for analogue display (Figure 2).

Human neuromuscular refractoriness

With approval of our institutional Human Subjects Committee and written informed consent, we recruited nine male anaesthesiology colleagues, aged 36–60 yr and weighing 52–76 kg, all in good general health and without neuromuscular disorders. All had experienced neurally evoked twitch of the hand before and were not unduly apprehensive about the experiment. All experiments were done on the non-dominant hands. No premedication was taken.

With the subjects in a recumbent or sitting position, after 10–30 min of rest, the skin sites for the application of the disc electrodes were cleaned with medical alcohol

and dried. The electrodes were disposable discs of silver-silver chloride gel with a diameter of approximately 1.5 cm. Two stimulating electrodes were placed over the ulnar nerve at the elbow, 4 cm apart from each other (centre to centre) with the negative electrode in the distal position. Placement of these electrodes away from the sensing electrodes reduced the stimulus artifacts to minimum. The unipolar EMG sensing electrode was placed on the dorsum of the hand over the belly of the first interosseous muscle. The reference electrode was placed on the distal half of the index finger. The grounding electrode was placed on the ventral aspect of the forearm between the stimulating and the sensing pairs of electrodes. A Grass S88 stimulator generated single or double stimuli of 0.2 ms duration which were passed through a Grass SIU5 stimulus isolation unit. The stimulus voltage required for maximal response (70–110V) was determined, and a supramaximal voltage 50% greater or 150V was used. Clean single-peaked ncEMG waveform of unipolar configuration^{7,9} was verified in every case first. The volunteers were instructed to relax and to let the fingers return to the original resting position after each stimulation. After 5–10 min of acclimatization to trial stimulations, the experiment began. Without exception, the response stabilized immediately. Although the main deflection of the ncEMG is negative, it is traditionally displaced upward. For clarity of presentation, therefore, we refer to any ncEMG deflection in the direction of the main deflection of the R_1 as “upward,” and the amplitude of this main deflection (also normally the first deflection) of R_1 was assigned a relative value of 1, i.e., $R_1 = 1$ throughout the study.

First, the R_1 response was evoked and stored in memory. The R_{1+2} responses were then evoked with double stimuli of the following interstimulus intervals: 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 6.0, 7.0, 8.0, 9.0, 10 ms, in that order. Stimulations were spaced 30 sec apart. Preliminary observations had confirmed that this spacing assured complete return of resting state between stimulations. After completion of data collection, R_1 and selected R_{1+2} responses were evoked again to verify that the responses were stable and reproducible. Corresponding R_{1+2} and R_2 waveforms were then displayed simultaneously for direct comparisons. The following “refractory periods” were determined: the RP_0 (the “refractory period” when $R_2 = 0$, i.e., the interstimulus interval when R_2 was just emerging), $RP_{.25}$, $RP_{.5}$, $RP_{.75}$ (when $R_2 = 0.25, 0.5, 0.75$ of R_1), and the RP_1 (the interstimulus interval when R_2 first approached maximum, i.e., $R_2 = R_1$). As the R_2 related to the interstimulus interval in a sigmoid curve, the RP values of each subject were determined by a log-logit linear regression. However, the RP_0 and RP_1 values must be read directly from the R_2 tracings without the

FIGURE 3 Refractoriness of neuromuscular transmission determined by a computer-based waveform subtraction technique. Upper tracings: combined EMG responses (R_{1+2}) to double supramaximal stimuli to the motor nerve. Lower tracing: response to the second stimulus, R_2 , obtained by subtraction of R_1 from the R_{1+2} . Dotted vertical lines join the corresponding R_2 s. Pairs of arrows locate the RP_0 , RP_{25} , RP_5 , RP_{75} and RP_1 as determined differently, without computer subtraction (upper tracing) and with computer subtraction (lower tracing). Note also that the R_{1+2} tracing over-reads the RP_0 but under-reads the RP_1 . At 1.2 ms and 1.4 ms, the after-potential of the R_{1+2} tracing contains all or part of the hidden R_2 . The correct RPs are: $RP_0 = 1.1$, $RP_{25} = 1.4$, $RP_5 = 1.7$, $RP_{75} = 2.3$, $RP_1 = 5.0$ ms, respectively.

FIGURE 4 Sources of error and difficulty in reading the RPs from the R_{1+2} tracing without computer subtraction, illustrated by a subject: (1) R_2 buried in the elevated or rounded after-potential of R_1 (1.0, 1.2 ms), (2) R_2 buried in the widened R_1 (1.2, 1.4 ms), (3) R_2 under-read (1.0 through 5.0 ms) and shows poor progression (1.4 through 2.6 ms), and (4) R_2 illogically exceeding R_1 in tallness but never equaling R_1 in height (6, 7, 8, 9 ms). Computer-processed results displayed on-line provide sharp contrast. In this subject, accurate determination of the RPs directly from the R_{1+2} tracing is literally impossible.

benefit of linear regression, because their reading requires human judgment and mathematically the log-logit regression does not apply to 0% and 100%. All group averages are expressed as mean $\pm$ SD (range).

Results

Figure 3 illustrates the computer-based waveform subtraction as a feasible technique. The top panel depicts the original R_{1+2} responses, the lower panel the subtracted R_2 . The corresponding R_2 peaks in both panels are joined by dashed lines. From the R_{1+2} tracings, a traditional method (reading the tallness of the rising limb of the R_2) would have read the RP_0 , RP_{25} , RP_{50} , RP_{75} , and RP_1 of this subject as 1.3, 1.6, 2.4, 2.8, and 4.0 ms, respectively (Figure 3, up arrows). From the R_2 waveforms obtained by computer subtraction, these same RP values were read as 1.1, 1.4, 1.7, 2.3, and 5.0 ms (Figure 3, down arrows). The errors of the traditional method thus amounted to 20–40%. Figure 4 illustrates several situations where determination of the RPs with the traditional method would be not only inaccurate but often impossible.

Qualitatively, the following sources of error or difficulty in reading the R_2 from the original R_{1+2} waveforms without computer subtraction were discovered:

- 1 The R_2 superimposes the R_1 in the R_{1+2} tracing with no consistent time relationship. When R_2 peaks, the underlying R_1 may be going upward, turning, or going downward, depending on the interstimulus interval. As a result, the R_2 shows erratic waveform and progression (Figure 4). It may have the same tallness (valley of waveform to peak) or height (line of neutrality to peak) for several interstimulus intervals, only to increase

TABLE 1 The refractory periods of neuromuscular transmission of healthy awake adults (in ms)

Subj.	RP_0	$RP_{.25}$	$RP_{.5}$	$RP_{.75}$	$RP_{.90}$	$RP_{.95}$	RP_1
1	1.1	1.8	2.6	3.9	5.6	6.9	10
2	0.9	1.1	1.9	3.0	4.7	6.2	7
3	1.1	1.6	2.3	3.2	4.4	5.3	7
4	1.1	1.3	1.8	2.5	3.4	4.0	5
5	1.1	1.4	2.1	3.2	4.7	6.0	9
6	0.9	1.0	1.8	2.9	4.8	6.4	8
7	1.1	1.2	1.8	2.5	3.9	4.3	4.3
8	0.9	1.0	1.4	2.0	2.8	3.8	4.6
9	1.1	1.3	1.8	2.5	3.3	3.9	5.9
Mean	1.0	1.3	1.9	2.9	4.2	5.2	6.6
S.D.	0.1	0.3	0.3	0.5	0.8	1.1	1.9
SEM	0.0	0.1	0.1	0.2	0.3	0.4	0.6

FIGURE 5 Average refractoriness of neuromuscular transmission of healthy male adults described by a curve.

markedly for a small further increase in the interstimulus interval.

- 2 A widening of the main deflection of the R_1 may indicate the emergence of R_2 , as the emerging R_2 adds to the R_1 during its initial downward sweep without making a recognizable dent in the waveform (Figure 4). A blunting or elevation of the nadir of the down deflection ("after potential") of the R_1 (Figures 3, 4) may also indicate the emergence of R_2 . In both instances, the "burying" of the R_2 would delay its recognition and result in over-reading of the RP_0 value, to suggest falsely an increased refractoriness or decreased re-excitability.
- 3 When the interstimulus interval is large, the R_2 may appear to begin from below the isoelectric baseline. Furthermore, the R_2 may paradoxically appear taller than the R_1 (Figure 4). If the endpoint when R_2 first equals

R_1 in tallness is taken as the RP_1 , the RP_1 value will be recognized too early (under-read), to suggest falsely a decreased refractoriness or increased re-excitability. In general, the tallness and the height of the second peak of the R_{1+2} waveform differed and did not correlate. Neither represented true R_2 .

By contrast, the computer-processed R_2 invariably showed a smooth, logical progression as the interstimulus interval was increased. It did not get buried. It eventually equaled the R_1 in amplitude and shape, without exceeding it in tallness or height. Furthermore, the R_2 always started from the baseline of electric neutrality, and resembled the R_1 in configuration irrespective of the amplitude (Figures 3, 4).

Table I gives all the RP_0 , 0.25, 0.5, 0.75, 0.9, 0.95 and RP_1 values and the averages. Figure 5 describes a composite curve of the refractoriness of neuromuscular transmission as related to the interstimulus interval.

Discussion

Neuromuscular transmission involved depolarization of the motor nerve terminal by the motor impulse, release of the neurotransmitter acetylcholine, activation of the receptors, generation of the endplate potential, and finally generation of the action potential of the muscle cell ("muscle fibre"). The ncEMG measures the sum of the *extracellularly* detectable electrophysiological responses of all the muscle cells lying beneath the sensing electrode.

In neuromuscular pharmacology involving the whole muscle, the ncEMG is considered a measure of the number of muscle cells firing, and the refractoriness of neuromuscular transmission is a statistic of a subpopulation of muscle cells not transmitting the second motor impulse after the first. This *statistical* concept of refractoriness differs from the traditional electrophysiological concept of "absolute refractory period" and "relative refractory period," which pertains to the excitability of a

TABLE II Normal refractoriness of human neuromuscular transmission (ms)

Authors	MMG or EMG	RP ₀	RP ₂₅	RP ₅₀	RP ₇₅	RP ₁	Remarks
Epstein <i>et al.</i> ²	MMG	1.15 (0.9–1.8)		1.64 (0.95–2.3)		2.40 (1.63–3.0)	(1)
Lee <i>et al.</i> ¹²	EMG (non-computerized)	1.7 ± 0.4		3.3 ± 0.7		4.5 ± 0.9	(2)
Kopec <i>et al.</i> ¹⁰	EMG (computerized)	0.9	1.5–1.8	1.9–2.0	2.3–2.9	7–30	(3)
Present study	EMG (computerized)	1.0 ± 0.1	1.3 ± 0.3	1.9 ± 0.3	2.9 ± 0.5	6.6 ± 1.9	(4)

Remarks: (1) Ulnar nerve-adductor pollicis, N₂O–O₂-vapour anaesthesia ("light plane" of diethyl ether, fluoxene, halothane, or methoxyflurane), mean values and ranges. (2) Ulnar nerve-thenar musculature, awake volunteers, non-computerized, mean values ± SDs. (3) Median nerve-thenar muscle, computer subtraction. Approximate data read by us from their Figure 3. (4) Ulnar nerve-1st dorsal interosseous muscle, awake volunteers, computer subtraction, mean values ± SDs.

single cell membrane. While the action potential of a cell membrane may be "all or none," the compounded EMG response of the muscle is "more or less" and infinitely variable. While a stronger second electrical stimulus can excite the excitable cell membrane during the relative RP, this is not likely in neuromuscular transmission. Accordingly, the terms "absolute" and "relative" should be avoided and specific terminology should be defined for neuromuscular refractoriness.

In the past, the term "average refractory period" has been coined to denote the RP when half the neuromuscular units are refractory.^{1,2,10} We feel that, rather than "average refractory period," statistically this should be called the "median refractory period." It can be abbreviated as "RP_{med}" or "RP₅". Likewise, "RP_{min}" or "RP₀" ("total refractory period") would be the smallest interstimulus interval for the R₂ to be detectable, or the largest interstimulus interval for the R₂ to be undetectable. It is determined by the RP of the *least refractory* neuromuscular unit in the muscle, which is the first to recover from refractoriness. During the RP₀, the entire population of neuromuscular units is "totally" refractory. On the outer limit, the "RP_{max}" or "RP₁" ("non-refractory period") would be the smallest interstimulus interval for the R₂ to have regained the full amplitude of the ncEMG. It is determined by the RP of the *most refractory* neuromuscular unit in the muscle. The large difference between RP₀ and RP₁ indicates the wide scatter of refractoriness of the population of neuromuscular units, not a wide "relative" refractory period. Reciprocally, the "refractory fraction" of the muscle cell population⁴ corresponding to RP₂₅ would be 0.75. A neuromuscular unit with an RP identical to RP₅ can be considered a "typical" unit of the population in the muscle.

Changing neuromuscular refractoriness may manifest as changing RP₀, changing RP₁ or a parallel shift in the entire RP curve. However, it is possible for the RP₅ to remain unchanged while the RP₀ increases and the RP₁ decreases (Table II). A complete study, therefore, calls for determination of the complete RP curve. Where construction of the RP curve is impractical, either the

interstimulus intervals corresponding to certain RPs or the refractory fraction corresponding to several interstimulus intervals can be determined.^{1–6}

Kopec *et al.*¹⁰ pioneered the use of computer-based subtraction technique in the determination of the refractoriness of neuromuscular transmission in man. Instead of the median nerve, we stimulated the ulnar nerve because the ulnar nerve is usually used in neuromuscular monitoring in anaesthesia practice. Instead of displaying the ncEMG on the oscilloscope and storing the results on magnetic tape, we displayed and recorded the response on a paper strip, *on-line*. As pointed out before,⁹ immediate recording on a strip chart facilitates the monitoring of the muscle responses moment-by-moment and enables neuromuscular studies in the clinical anaesthesia setting. Comparison of their results with ours reveals similar refractoriness of neuromuscular transmission from the median and the ulnar nerves to their respective muscles (Table II).

In light of the widely available computer technology, we propose that in the future the refractoriness of neuromuscular transmission not be studied without computer assistance. The R₁₊₂ tracing in Figure 4 is reminiscent of the drawback of our previous work and those of others. The pitfalls we illustrated in the *Results* would invalidate the RP values read from the R₁₊₂ tracing by the traditional visual assessment method. Repetitive firing⁸ and the presence of "cross-talking" from muscle bundles in the vicinity will cause multi-peaking^{7,8,11} of the ncEMG, which will further hinder the determination of the RPs by the visual method. In the past, we have attempted in vain to read the true amplitude of R₂ from the R₁₊₂ waveform by reading its upward stroke, its downward stroke, or the average of these two, from the isoelectric baseline, the turning point, or the projected moving baseline. Data from our previous studies using the same ncEMG method and similar human subjects,¹² agree that without the subtraction technique the RP₀ will be over-read by as much as 70% and the RP₁ will be under-read by 32% (Table II).

Physiologically, the most refractory step involved in

neuromuscular transmission will determine the refractoriness of the neuromuscular transmission. Since the motor nerve has a much shorter RP,^{10,13} it probably can be ruled out as the rate-limiting factor. The acetylcholine-receptor interactions are molecular actions and thus non-refractory. Instead, the time required for events at the tortuous unmyelinated motor nerve terminal,¹⁰ the mobilization and release of the transmitter, and especially the refractoriness of the muscle endplate^{11,14} and muscle cell membrane are probably the determinants of the neuromuscular refractoriness under normal conditions.

Recent anaesthesia literature contains no references to the refractoriness of neuromuscular transmission. In contrast, tetanic stimulation, post-tetanic facilitation and train-of-four received thorough investigation in the past two decades. Train-of-four count,¹⁵ post tetanic count¹⁶ and double-burst stimulation¹⁷ have become widely accepted as clinical monitoring techniques. Phenomenologically, these techniques examine the effect of more than one stimulus on the subsequent state of neuromuscular transmission 20 ms to 5s later (20 ms interstimulus in 50 Hz tetanus, 0.5s interstimulus in train-of-four, 0.75s inter-burst in the double-burst, 1s or 5s post-tetanus, and 1s interstimulus between post-tetanic twitches in the post-tetanic count). The refractoriness, by contrast, examines the effect of a single stimulus on the state of neuromuscular transmission within the next 10 ms. We speculate that capturing the immediate post-stimulus events might not only increase our understanding of the neuromuscular pharmacology but possibly also produce new useful monitoring techniques in the future. Since refractoriness represents a stress test of the physiologic reserve with reduced "margin of safety," it might have enhanced sensitivity to certain drug actions relative to the singly evoked twitch. Since the technique involves only two stimuli at a time, the stimulation itself does little to distort the state of neuromuscular transmission. Since the result is quantified by contrasting R_2 against R_1 , it might be useful (as in the case of train-of-four) even without a pre-recorded control response.

Monitoring of the neuromuscular refractoriness may have practical utility. For example, we have observed (unpublished data) that magnesium-induced neuromuscular block drastically reduces the RP_0 to the vicinity of 0.4–0.5 ms. Physiologically, the 0.4–0.5 value approximates the RP of the myelinated motor nerves. The ability of the neuromuscular refractoriness to be reduced to approximate the refractoriness of the myelinated motor nerve suggests that the fine motor nerve terminal can respond as fast as the nerve trunk does. Pharmacologically, the reduction of the refractoriness of the neuromuscular transmission may be an *in vivo* indicator of prejunctional block of the magnesium type. Clinically, markedly re-

duced neuromuscular refractoriness may differentially diagnose magnesium-induced neuromuscular block in patients with toxemia of pregnancy who have received magnesium and another relaxant. Other possible uses of the RP studies include early diagnosis of neuromuscular ischaemia.¹⁴

Concerning the normal RP values of neuromuscular transmission what are determined mechanomyographically and electromyographically differ in value^{1,3,5} and in meaning. Mechanomyographically, the R_{1+2} readily exceed $2\times$ the R_1 in amplitude. In other words, the conditioned R_2 readily exceeds the already "maximal" R_1 ,^{1,2} even when the interstimulus interval is well within the RP_1 . By contrast, the electromyographic R_2 does not exceed the R_1 , or does so slightly^{10,12} only when the interstimulus interval exceeds 10 ms. Table II compares our data with those of previous reports.

In conclusion, computer-based waveform subtraction of the ncEMG response to a single stimulus (R_1) from the response to double stimuli (R_{1+2}) yields an R_2 which is discrete, logically progressive in amplitude, similar to R_1 in shape, and can be read with precision. In normal humans, the physiologic refractoriness of neuromuscular transmission in the ulnar nerve-first dorsal interosseous muscle model is: minimum RP 1.0 ± 0.1 ms, median RP 1.9 ± 0.3 ms, and maximum RP 6.6 ± 1.9 ms. Application of the technique to clinical research may result in new monitoring methods for diagnosis of prejunctional neuromuscular block and differential diagnosis of mixed blocks.

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