Relative contribution of neurotransmission failure to diaphragm fatigue

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KUEI, JOSEPH H., REZA SHADMEHR, AND GARY C. SIECK. *Relative contribution of neurotransmission failure to diaphragm fatigue.* J. Appl. Physiol. 68(1): 174–180, 1990.—Two procedures were used to estimate the relative contribution of neurotransmission failure (NF) to fatigue of the rat diaphragm at different rates of phrenic nerve stimulation. In one, direct muscle stimulation was intermittently superimposed on neural stimulation of the diaphragm, and the relative contribution of NF was estimated by the difference in generated tension. In a second procedure, diaphragm fatigue was induced by using either direct muscle stimulation (with complete blockade of the neuromuscular junction by *d*-tubocurare) or phrenic nerve stimulation. The relative contribution of NF to diaphragm fatigue was then estimated by comparing the force loss during these two modes of stimulation. With both procedures, it was observed that *1*) the relative contribution of NF to diaphragm fatigue was <45% at each frequency of phrenic nerve stimulation; *2*) the relative contribution of NF to diaphragm fatigue increased at higher rates of phrenic stimulation, reaching a maximum at 75 pulses/s; and *3*) the relative contribution of NF to diaphragm fatigue reached a plateau after 2 min of repetitive stimulation.

muscle; phrenic nerve; tension

PERIPHERAL FATIGUE of the diaphragm muscle may result from either a failure in transmission of the neural signal (i.e., neurotransmission failure, NF) or a failure in the muscle itself after the neuromuscular junction (i.e., muscle failure, MF). NF depends on the rate of stimulation (6, 20, 25) and can occur at several locations along the axon (e.g., a failure of action potential propagation at axonal branch points) or at the neuromuscular junction (e.g., diminished transmitter release or reduced endplate excitability). MF also has several potential underlying mechanisms (e.g., decreased sarcolemmal excitability, failed excitation-contraction coupling, altered contractile mechanisms, or an insufficient energy supply).

The relative contributions of NF and MF to diaphragm fatigue have been estimated by comparing the force loss during direct muscle stimulation (bypassing neural sites of failure) to that loss occurring while the phrenic nerve was stimulated (1, 2, 15). In a study by Kelsen and Nochomovitz (15), direct stimulation of the rat diaphragm was intermittently superimposed on phrenic nerve stimulation, and the differences in force generated by these two modes of stimulation were used to estimate the relative contributions of NF and MF to diaphragm fatigue. These authors estimated that after 6 min of repetitive stimulation at 15 pulses/s, NF accounted for only 18% of diaphragm fatigue. These results are in contrast to a later study by Aldrich et al. (2) in which fatigue of the rat diaphragm was also induced by repetitive stimulation of the phrenic nerve at 15 pulses/s for 6 min. Diaphragm forces generated by direct muscle (with *d*-tubocurare) or phrenic nerve stimulation were then compared at different rates of stimulation (i.e., force-frequency responses). With this procedure, Aldrich et al. (2) estimated that the relative contribution of NF to diaphragm fatigue was 82%. These authors also reported that when diaphragm fatigue was induced by stimulating the phrenic nerve at 50 pulses/s, NF actually contributed less to diaphragm fatigue than it did at 15 pulses/s (71 vs. 82%). These results do not agree with other studies that have reported that the extent of NF is greater with increasing rates of neural stimulation (6, 20, 25).

Procedural differences between the studies of Kelsen and Nochomovitz (15) and Aldrich et al. (2) included *1*) Ca^{2+}/Mg^{2+} (2.5 vs. 1.6, respectively), *2*) the duration of the stimulus pulse (0.2 vs. 2.0 ms, respectively), and *3*) the temperature of the tissue bath (26 vs. 37°C, respectively). Lowered Ca^{2+}/Mg^{2+} in the tissue bath can depress transmitter release at the neuromuscular junction (12) and thus increase the amount of NF contributing to diaphragm fatigue. A shorter stimulus pulse duration may produce less efficient direct muscle stimulation especially during repetitive stimulation when the threshold for generating muscle fiber action potentials can increase (14, 16). It is possible that with the shorter pulse duration, Kelsen and Nochomovitz (15) may have been subthreshold for activation of some muscle fibers. Finally, a higher temperature tissue bath may have caused rapid deterioration of the preparation (16) and/or decreased transmitter release (13), both of which could have resulted in a greater extent of NF. The purpose of the present study was to determine the relative contribution of NF and MF to diaphragm fatigue at different rates of neural stimulation. In addition, the influence of Ca^{2+}/Mg^{2+}, stimulus pulse duration, and the temperature of the tissue bath were examined.

METHODS

General procedures. Adult male Sprague-Dawley rats (body wt range 250–300 g) were anesthetized with pen-

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tobarbital sodium (60 mg/kg ip). The diaphragm was excised together with the phrenic nerves, and a strip of innervated muscle (~10 mm wide) was prepared from the costal region of the right hemidiaphragm with the insertion of fibers at the ribs and central tendon intact. This nerve/muscle strip preparation was mounted vertically in a glass chamber containing Krebs solution (Ca^{2+} 2.5 mM; Mg^{2+} 1.0 mM) and aerated constantly with 95% O_2-5% CO_2. The pH (7.30–7.40), P_{CO_2} (30–40 Torr), and P_{O_2} (380–450 Torr) of the Krebs solution were periodically monitored during different phases of the experiment and found to remain constant. The temperature of the Krebs solution was also kept constant by circulating water through the outer jacket of the tissue chamber. In most studies, the temperature was maintained at 26°C.

The costal margin was fixed by a clamp mounted on a micropositioner. The central tendon was secured superiorly by another clamp connected in series with a force transducer (Grass model FT10). Muscle fiber length was adjusted by using the micropositioner until maximal isometric twitch force responses were obtained (i.e., optimal fiber length). This usually corresponded to a preload tension of ~10 g.

The muscle was stimulated directly (Grass model S-88 stimulator and SIU-5 isolation unit) by using monophasic rectangular pulses of anodal current (in most experiments at 2.0 ms duration, see below) delivered through a stranded stainless steel wire electrode (Cooner wire; 32 gauge), insulated except for 1 cm in contact with the muscle. This electrode was sutured into the muscle near the costal margin. The anodal current pulses were referenced to a large Ag-AgCl plate electrode (2.0 x 1.5 cm) placed ~5.0 mm from the muscle. In preliminary studies, several other electrode configurations were compared, including plate electrodes placed on either side of the muscle, bipolar stainless steel electrodes inserted into the muscle at different points along the length of muscle fibers, and surface electrodes in contact with the muscle. By means of the Grass stimulator, maximal forces could be consistently achieved only when electrodes were sutured directly into the muscle.

Stimulation of the phrenic nerve was achieved by using 0.2-ms duration pulses delivered through a suction electrode. In both direct muscle and phrenic nerve stimulation, stimulus intensity was increased until maximal twitch force responses were obtained. Thereafter, stimulus intensity was set at 1.2–1.5 times this maximal level (i.e., supramaximal). In some experiments, to verify the maximization of stimulus intensity during repetitive direct muscle stimulation, the stimulus intensity was increased to three to four times the original maximal levels. Although no further increments in force were observed, stimulus intensity was set at two times maximum during the fatigue tests.

In five animals, the duration of the stimulus pulse was systematically varied from 0.1 to 5.0 ms during nerve and direct muscle stimulation. The stimulus intensities required to generate maximal twitch force responses at each pulse duration were then compared.

The evoked isometric force responses were displayed on a storage oscilloscope and recorded on magnetic tape and a chart recorder. The stimulation paradigm was precisely controlled by a computer (IBM AT) program, and two procedures were used to estimate the relative contributions of NF and MF to diaphragm fatigue.

Procedure 1. In 15 animals, the relative contributions of NF and MF to diaphragm fatigue were estimated by superimposing direct muscle stimulation on repetitive stimulation of the phrenic nerve at 20 (n = 5), 40 (n = 5), or 75 pulses/s (n = 5). Both nerve and muscle stimulation were presented in 330-ms duration trains with one train repeated each second (i.e., a duty cycle of 33%). Every 15 s, instead of nerve stimulation, the muscle was directly stimulated at the appropriate rate for two stimulus trains; thereafter, nerve stimulation was resumed. This alternation between nerve and direct muscle stimulation was continued for 5 min. To determine the effects of a lower Ca^{2+}/Mg^{2+} and a higher temperature (37°C) tissue bath on the estimations of NF and MF, the nerve/muscle strips (n = 5) were fatigued by using the same procedure described above (20 pulses/s stimulation), except that the bath temperature was set at 37°C and the Ca^{2+} and Mg^{2+} concentrations were 1.4 and 0.9 mM, respectively (Ca^{2+}/Mg^{2+} of 1.6).

Procedure 2. In this procedure, the relative contributions of NF and MF to diaphragm fatigue at activation rates of 20, 40, and 75 pulses/s were estimated by comparing force loss during repetitive nerve stimulation with that during repetitive direct muscle stimulation in two separate groups of animals. Because direct muscle stimulation also activates intramuscle nerve branches, neural stimulation was prevented by adding 12 μM d-tubocurare to the Krebs solution. Blockade of neuromuscular transmission usually took ~5 min and was verified by the absence of evoked responses to nerve stimulation.

Thirty-seven rats were used in this experiment. In 20 animals, diaphragm fatigue was induced by phrenic nerve stimulation at 20 (n = 5), 40 (n = 10), or 75 (n = 5) pulses/s. In another group of 17 animals, diaphragm fatigue was induced by direct muscle stimulation at 20 (n = 5), 40 (n = 7), or 75 (n = 5) pulses/s. In both groups, stimuli were presented in 330-ms duration trains with one train repeated each second for 5 min.

Statistical analysis. To normalize the evoked tension responses across animals, the results were expressed as percent of the initial maximum tension produced at each rate of stimulation. Two-way analysis of variance for repeated measures (7) was used to determine the significance of the relationship between the time-dependent decline in tension and the independent grouping effects.

RESULTS

Stimulation efficacy. Maximum twitch force responses were observed during nerve stimulation at 4- to 8-V intensity with pulse durations ranging from 0.1 to 1.0 ms. Longer pulse durations triggered multiple action potentials; therefore pulse duration was limited to 0.2 ms and stimulus intensity was set at 1.2–1.5 times maximum. Increasing the intensity of stimulation during the fatigue tests had no effect on the forces generated by the diaphragm.

The stimulus intensities required to generate maximum twitch tensions during direct muscle stimulation varied considerably with pulse duration. With shorter pulse durations (0.1–0.4 ms), maximum twitch tensions could not be consistently achieved even with stimulus intensities up to 150 V (maximum intensity of the Grass stimulator). With pulse durations of 0.5–1.0 ms, the stimulus intensity required to produce maximum twitch tensions decreased progressively, until a plateau was reached at 40–100 V. Increasing pulse durations from 1.0 to 4.0 ms had no effect on this maximal stimulus intensity. Pulse durations >4.0 ms generated additional force, indicating that multiple muscle action potentials were triggered. This is consistent with the duration of muscle fiber action potentials (~5.0–10.0 ms) reported in other studies (11, 14, 16, 18). Based on these results, a pulse duration of 2.0 ms was selected to ensure consistent maximal stimulation of the muscle. Stimulus intensities were set to a level that was at least 1.5 times that at which maximum twitch tension responses were obtained.

In preliminary studies, it was noted that with shorter pulse durations (0.5–1.0 ms) the stimulus intensity required to produce maximal force responses increased during continued repetitive stimulation of the muscle. This suggested that the threshold for generating muscle action potentials increased with time during repetitive stimulation. During the fatigue tests which employed direct muscle stimulation (at 2.0-ms pulse duration), increasing the stimulus intensity to 150 V (1.6–4.0 times maximum) had no effect on the force generated. Thus, although an increase in muscle fiber threshold to direct muscle stimulation undoubtedly occurs, stimulus intensities during the fatigue tests remained at or above maximum. The possibility that changes in muscle fiber threshold (i.e., reduced end-plate excitability) contributed to the fatigue observed with phrenic nerve stimulation could not be excluded by the procedures employed in the present study.

Procedure 1. Figure 1 shows an example of an experiment in which direct muscle stimulation was intermittently superimposed on trains of phrenic nerve stimulation. For each frequency of stimulation, the tension developed with phrenic nerve stimulation was significantly less than that observed with direct muscle stimulation ($P < 0.001$). This difference was evident throughout the period of phrenic nerve stimulation but remained relatively constant after 2 min (Fig. 2). The relative contribution of NF to diaphragm fatigue was estimated by assuming that *1*) force loss during the superimposed direct muscle stimulation reflects only MF and *2*) the difference in tension generated by direct muscle vs. phrenic nerve stimulation reflects NF. The relative contributions of NF and MF to diaphragm fatigue were calculated every 15 s (Fig. 3). It should be noted that, for each frequency of stimulation, the relative contribution of NF to diaphragm fatigue varied over the course of phrenic nerve stimulation, reaching a maximum at ~2 min. Subsequently, little additional NF was observed. The maximal relative contributions of NF to diaphragm fatigue at each rate of phrenic nerve stimulation are summarized in Table 1. Fatigue attributed to NF was greater at higher frequencies of phrenic nerve stimulation. At each rate of stimulation, the loss of tension attributed to MF was consistently greater than that caused by NF. A lower Ca^{2+}/Mg^{2+} and a higher temperature of the tissue bath were associated with a more rapid decline in tension and a higher magnitude of NF [25.9 ± (SD) 8.4%; Fig. 4].

The relative contribution of NF to diaphragm fatigue was plotted as a function of the number of pulses delivered to the phrenic nerve (Fig. 5). For each frequency of stimulation, the contribution of NF to diaphragm fatigue appeared to depend on the number of pulses during the initial period of stimulation (up to ~1,000 pulses at 20-pulses/s stimulation and 2,000 pulses at 40- and 75-pulses/s stimulation). Thereafter the contribution of NF to diaphragm fatigue was independent of the number of pulses delivered.

Procedure 2. Figure 6 compares the decline in diaphragm force during phrenic nerve and direct muscle stimulation at different rates. For each frequency of stimulation, the initial rate of force decline with nerve

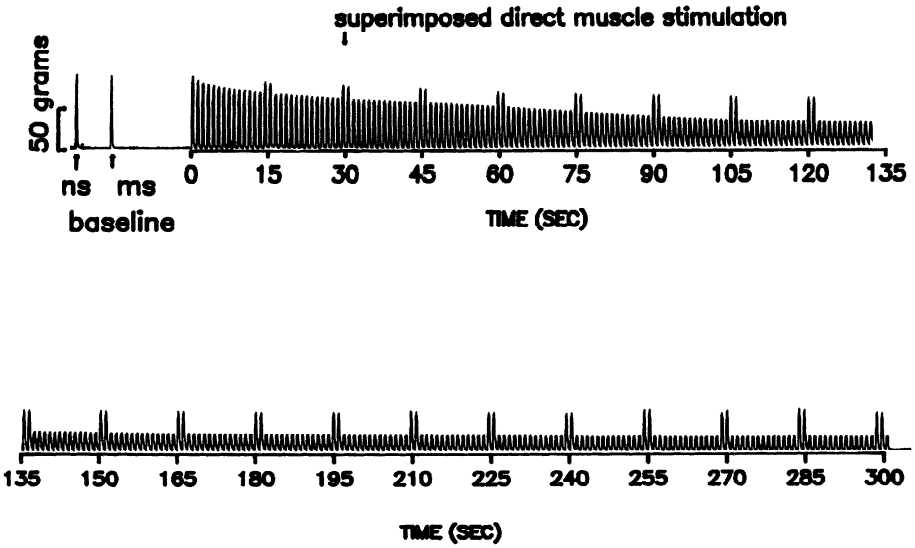

FIG. 1. Experimental recording in which direct muscle stimulation was intermittently superimposed on trains of phrenic nerve stimulation (*procedure 1*). Arrow, tension developed with superimposed direct muscle stimulation. ns, phrenic nerve stimulation; ms, direct muscle stimulation.

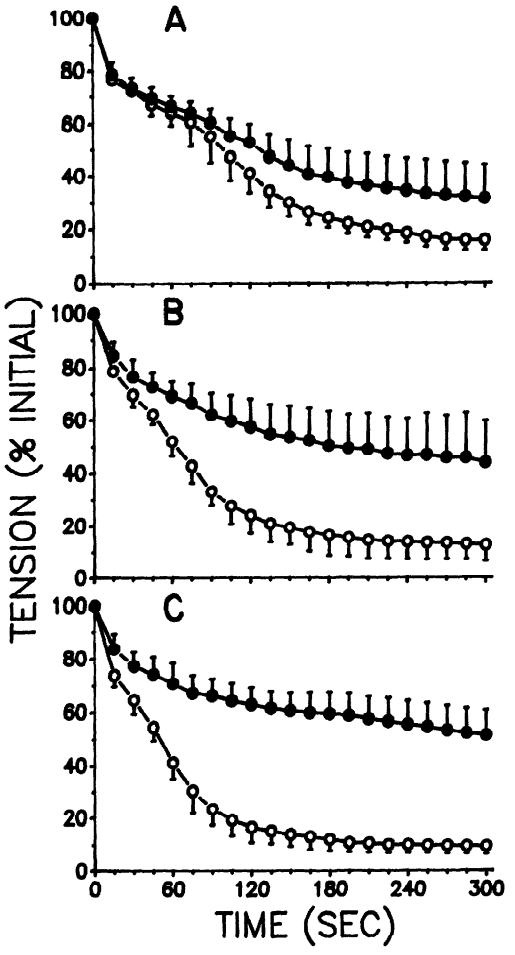

FIG. 2. Force loss during superimposed direct muscle stimulation (●) compared with that during phrenic nerve stimulation (○) at 20 (*A*), 40 (*B*), and 75 pulses/s (*C*). Values are means ± SD. Note that tension developed with nerve stimulation was less than that observed with direct muscle stimulation for each frequency of stimulation. Difference in tension generated by direct muscle stimulation and phrenic nerve stimulation increased at higher frequencies of stimulation.

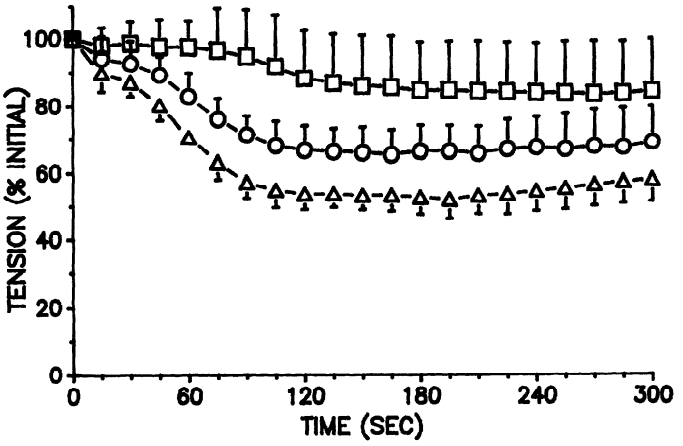

FIG. 3. Relative contribution of neurotransmission failure to diaphragm fatigue at various times during repetitive phrenic nerve stimulation at 20 (□), 40 (○), and 75 pulses/s (△). Values are means ± SD. Fatigue attributed to neurotransmission failure was greater at higher frequencies of phrenic nerve stimulation.

TABLE 1. *Estimated maximal relative contribution of neurotransmission failure to diaphragm fatigue*

Procedure	Frequency of Stimulation, pulses/s	Maximal Contribution of NF to Diaphragm Fatigue, % of failure
1	20	15.7
	40	31.2
	75	42.0
2	20	16.2
	40	40.0
	75	44.6

NF, neurotransmission failure.

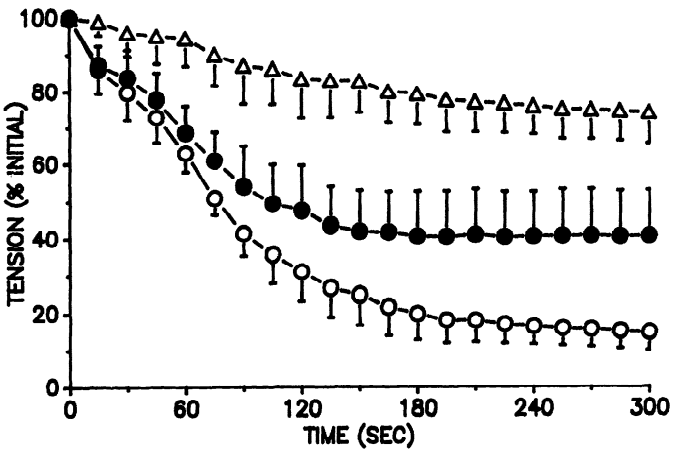

FIG. 4. Diaphragm force generated by 20-pulse/s direct muscle stimulation (●) compared with that generated by phrenic nerve stimulation (○) in a Krebs solution containing lower Ca^{2+} concentration (1.4 mM) and maintained at a higher temperature (37°C). Force loss attributed to neurotransmission failure (△) was calculated from difference in force developed by using these 2 modes of stimulation. Values are means ± SD.

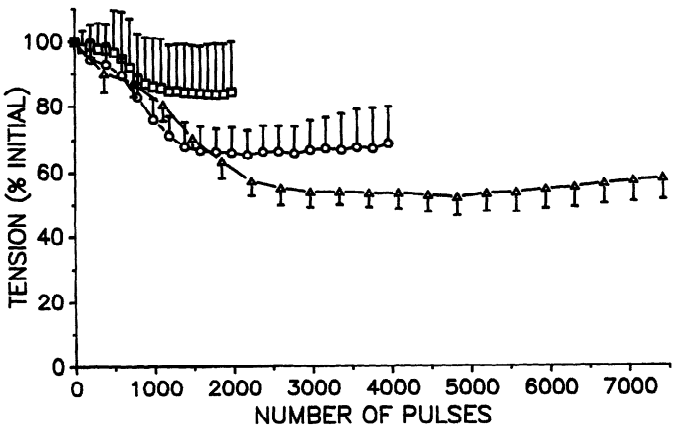

FIG. 5. Relative contribution of neurotransmission failure to diaphragm fatigue at 20 (□), 40 (○), and 75 pulses/s (△) compared with number of pulses delivered. Values are means ± SD.

stimulation was significantly faster than that with direct muscle stimulation ($P < 0.05$). Subsequently, force decline during both nerve and direct muscle stimulation reached an asymptote at ~15% of the initial tension. The relative contributions of MF and NF to diaphragm fatigue were estimated by assuming that the force decline during direct muscle stimulation reflected only the contribution of MF, whereas the force decline during nerve

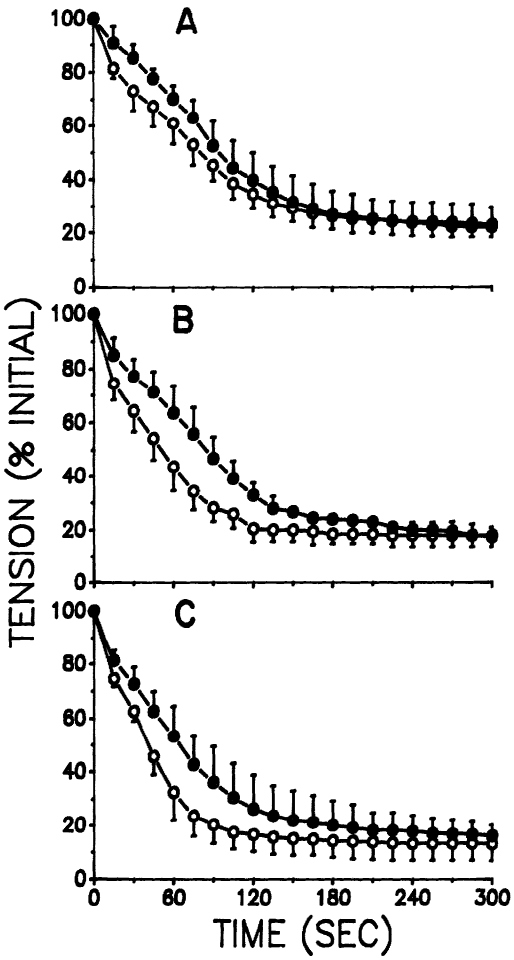

FIG. 6. Force loss during repetitive direct muscle stimulation in presence of curare (●) in 1 group of animals compared with force loss during repetitive phrenic nerve stimulation (○) in a second group at 20 (*A*), 40 (*B*), and 75 pulses/s (*C*). Values are means ± SD.

stimulation reflected the combined contribution of both MF and NF. The following formula, proposed by Aldrich et al. (2), was used to estimate MF and NF

$$NF = (F - MF)/(1 - MF)$$

where NF is neurotransmission failure, F is force loss during nerve stimulation, and MF is contractile failure.

The relative contributions of NF and MF to diaphragm fatigue at each rate of activation varied with time. The maximum contribution of NF occurred within the initial 120 s of activation. Table 1 compares the maximum contribution of NF to diaphragm fatigue at each stimulation rate as estimated by the two different procedures.

DISCUSSION

The results of the present study demonstrated that, for each frequency of stimulation, the contribution of NF to diaphragm fatigue was less than that attributed to MF. As might be expected, the contribution of NF to diaphragm fatigue increased at higher rates of phrenic nerve stimulation. When the phrenic nerve was stimulated supramaximally at 20 pulses/s, ~16% of diaphragm fatigue was attributed to NF, whereas at 75 pulses/s 43% of diaphragm fatigue resulted from NF. These results are in basic agreement with the previous report of Kelsen and Nochomovitz (15), who estimated that with phrenic nerve stimulation at 15 pulses/s, 18% of diaphragm fatigue was attributable to NF. Our results differ from the estimate of Aldrich et al. (2) that 82% of the diaphragm fatigue induced by phrenic nerve stimulation at 15 pulses/s was attributable to NF. Moreover, when these authors induced diaphragm fatigue using a higher rate of phrenic nerve stimulation (i.e., 50 pulses/s for 6 min), they found less NF than at 15 pulses/s (71 vs. 82%). These results are inconsistent with previous studies that have demonstrated that NF is dependent on stimulus rate (6, 20, 25).

NF may occur as the result of a block in action potential propagation along the axon [e.g., at axonal branch points (16, 20) or a failure at the neuromuscular junction (e.g., decreased transmitter release or diminished endplate excitability)]. Aldrich et al. (2) suggested that the increased NF they observed relative to Kelsen and Nochomovitz (15) was the result of lower Ca^{2+}/Mg^{2+} (1.6 vs. 2.5) and a higher temperature (37 vs. 26°C) tissue bath. By lowering the Ca^{2+} concentration in the tissue bath, it is possible to reduce the amount of transmitter released at the neuromuscular junction (12) and thus decrease synaptic efficacy. The temperature of the tissue bath also affects transmitter release at the neuromuscular junction (13). In the present study, lowering the Ca^{2+}/Mg^{2+} and increasing the temperature of the tissue bath significantly increased the contribution of NF to diaphragm fatigue from 16 to 26%. However, even when using the same Ca^{2+} concentration and temperature of the tissue bath as used by Aldrich et al. (2), the relative contribution of NF to diaphragm fatigue was much lower than that reported by these authors. Based on the results of our study, it is impossible to discern whether NF was the result of an axonal block of action potential propagation or to a failure at the neuromuscular junction.

NF has also been assessed by changes in evoked electromyographic (EMG) responses. In the rat diaphragm, Krnjevic and Miledi (16) noted that single-fiber action potentials were not always evoked during repetitive phrenic nerve stimulation, indicating NF. Similarly, in motor units of the cat medial gastrocnemius, Sandercock et al. (20) observed that repetitive stimulation of the motor axon failed to evoke action potentials in some unit fibers. In cat diaphragm motor units, we have observed abrupt changes in motor unit action potentials during the fatigue test, suggesting NF in some unit fibers (21). Other changes in evoked EMG responses in cat diaphragm motor units during the fatigue test included a prolongation of the compound motor unit action potential and a decrease in the integrated (root mean square calculation) area. The changes in diaphragm motor unit action potentials occurred only in more fatigable motor units (i.e., fast-twitch fatigable and fast-twitch fatigue intermediate) and not in fatigue-resistant units (i.e., slow- and fast-twitch fatigue resistant). Although these diaphragm motor unit EMG changes occurred only in fatigable units, there was no correlation with the extent of unit fatigue (21). This is important in light of the use of changes in amplitude of the evoked compound muscle action potential to index the extent of neuromuscular

transmission failure (1, 4, 5, 10, 19). In the rat diaphragm, Pagala et al. (19) reported that the compound muscle action potential decreased in amplitude during fatigue induced by repetitive stimulation of the phrenic nerve at 30 pulses/s. The change in the evoked muscle action potential varied with time, showing a slight potentiation for up to 90 s before declining. Changes in the evoked muscle action potential did not parallel the changes in diaphragm force. We also found that changes in the evoked compound action potential of the cat diaphragm did not parallel the decline in force during the fatigue test (21). It is important to note that changes in evoked muscle action potentials could be affected by either a failure in neural transmission or by alterations in the generation or propagation of muscle fiber action potentials. Thus changes in the evoked muscle action potential would not discriminate between NF and MF.

In the present study, MF was assessed by stimulating the muscle directly, thus bypassing the neuromuscular junction. It should be recognized, however, that the mechanisms for generating action potentials in muscle fibers are not bypassed by direct muscle stimulation. Thus the diaphragm fatigue observed with direct muscle stimulation could have resulted from a failure in the generation or propagation of muscle action potentials, in addition to any one of several other potential sites, e.g., impaired excitation-contraction coupling, inadequate Ca^{2+} release from the sarcoplasmic reticulum, altered contractile mechanisms, or insufficient energy production within muscle fibers (4). It has been demonstrated that the threshold for generating muscle fiber action potentials can increase during repetitive stimulation (14, 16). Thus, during repetitive direct muscle stimulation, maintenance of maximal stimulus intensities is essential. In the present study, we found that maximal intensities for direct muscle stimulation could not always be achieved by using pulse durations <0.5 ms, even with stimulus intensities of 150 V. Only with pulse durations >0.5 ms could maximal stimulus intensities be consistently reached by using the Grass stimulator and our electrode configuration. With pulse durations of 1.0–4.0 ms, the stimulus intensity for maximal twitch responses remained constant. The increase in force generated by pulse durations >4.0 ms suggests that multiple muscle action potentials were triggered in some fibers. This pulse duration is comparable with the duration of the muscle fiber action potential reported in the rat diaphragm (11, 14, 16, 18). We also observed that if pulse durations during repetitive direct muscle stimulation were <1.0 ms, the stimulus intensity eliciting maximal force response increased, and eventually supramaximal intensity could not be achieved by using the Grass stimulator. In contrast, with a pulse duration of 2.0 ms, increasing stimulus intensity by as much as four times maximal during the fatigue test caused no additional increment in force. This indicated that supramaximal stimulus intensity was maintained throughout the fatigue test. In this regard, it should be noted that the studies of Aldrich et al. (2) and Kelsen and Nochomovitz (15) differed in the duration of the stimulus pulse when stimulating the muscle directly. Aldrich et al. (2) used a pulse duration of 2.0 ms instead of 0.2 ms (15), arguing that the longer duration pulse more reasonably approximated the duration of the end-plate potential during generation of muscle fiber action potentials (3, 11, 16, 18). Furthermore, Aldrich et al. (2) indicated that the longer pulse duration for direct muscle stimulation was necessary to achieve supramaximal stimulation. The results of the present study would support this contention. However, these results do not exclude the possibility that a failure in the generation or propagation of muscle fiber action potentials contributed to the MF observed during the fatigue test.

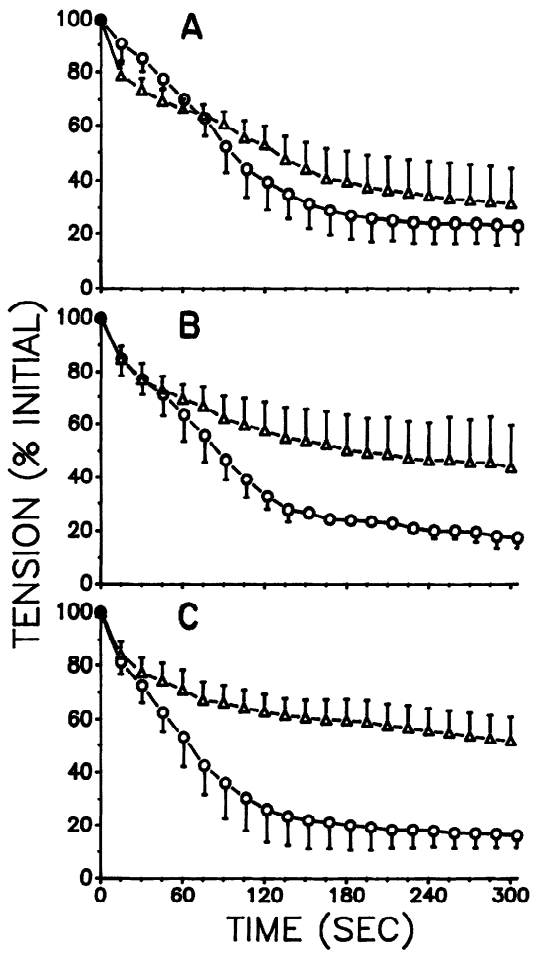

FIG. 7. Estimated contribution of muscle failure to diaphragm fatigue during nerve stimulation (△) compared with that measured during direct muscle stimulation in presence of curare (○) at 20 (*A*), 40 (*B*), and 75 pulses/s (*C*). Values are means ± SD.

Previous studies (6, 20, 25) have suggested that NF is greater with increasing rates of nerve stimulation and is more prevalent among specific types of motor units [i.e., fast-twitch fatigable and fatigue-intermediate units (6, 20, 21)]. It is likely that NF might affect only the activation of a subset of muscle fibers. In such a case, those diaphragm muscle fibers affected by NF might be spared from developing MF during phrenic nerve stimulation. In contrast, with direct muscle stimulation all muscle fibers would be activated. Thus a greater extent of MF might be expected with direct muscle stimulation than with nerve stimulation. Figure 7 compares the estimated relative contribution of MF to diaphragm fatigue during nerve stimulation with that observed during direct mus-

cle stimulation in the presence of curare. As expected, at each frequency of stimulation the amount of MF estimated during nerve stimulation was less than that observed during direct muscle stimulation. This difference was greater at higher frequencies of stimulation.

In previous studies (17, 23), we observed that 38% of rat diaphragm muscle fibers were type I [i.e., belonging to slow-twitch motor units (9, 22)] and 62% were type II (i.e., belonging to fast-twitch motor units). The cross-sectional areas of type I fibers in the rat diaphragm were found to be smaller than those of type II fibers (1,100 vs. 2,100 μm^2, respectively). Based on these observations, we estimated that the relative contributions of type I and II fibers to diaphragm area were 24 and 76%, respectively (23). Eddinger and Moss (8) reported that the specific tension (force per fiber area) of type I fibers in the rat diaphragm was 68% of type II fibers. Based on the relative contribution of each fiber type to total diaphragm area and these differences in specific tension, we estimated the relative contribution of type I and II fibers to the maximum tetanic tension generated by the rat diaphragm to be 18 and 82%, respectively. These estimates are very similar to the amount of residual diaphragm force remaining after 5 min of repetitive nerve or muscle stimulation (Figs. 2 and 6). Thus the plateau in diaphragm force observed in each case probably reflects the continued contribution of fatigue-resistant muscle fibers.

In summary, the present study demonstrated that *1*) NF is present during repetitive phrenic nerve stimulation but contributes less to diaphragm fatigue than MF, *2*) the relative contribution of NF to diaphragm fatigue varies with time, reaching a maximum after 2 min of phrenic nerve stimulation, and *3*) diaphragm fatigue attributed to NF is greater at higher frequencies of phrenic nerve stimulation.

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