Contractile and fatigue properties of the rat diaphragm musculature during the perinatal period

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Martin-Caraballo, Miguel, Paul A. Campagnaro, Yuan Gao, and John J. Greer. Contractile and fatigue properties of the rat diaphragm musculature during the perinatal period: J. Appl. Physiol. 88: 573–580, 2000.—The following two hypotheses regarding diaphragm contractile properties in the perinatal rat were tested. First, there is a major transformation of contractile and fatigue properties during the period between the inception of inspiratory drive transmission in utero and birth. Second, the diaphragm muscle properties develop to functionally match changes occurring in phrenic motoneuron electrophysiological properties. Muscle force recordings and intracellular recordings of end-plate potentials were measured by using phrenic nerve-diaphragm muscle in vitro preparations isolated from rats on embryonic day 18 and postnatal days 0–1. The following age-dependent changes occurred: 1) twitch contraction and half relaxation times decreased approximately two- and threefold, respectively; 2) the tetanic force levels increased approximately fivefold; 3) the ratio of peak twitch force to maximum tetanic force decreased 2.3-fold; 4) the range of forces generated by the diaphragm in response to graded nerve stimulus increased approximately twofold; 5) the force-frequency curve was shifted to the right; and 6) the propensity for neuromuscular transmission failure decreased. In conclusion, the diaphragm contractile and phrenic motoneuron repetitive firing properties develop in concert so that the full range of potential diaphragm force recruitment can be utilized and problems associated with diaphragm fatigue are minimized.

fetal breathing; respiration; muscle

PERIODIC CONTRACTIONS of the diaphragm in utero are an essential component of normal mammalian fetal development (20). Failure to generate adequate fetal breathing movements results in a reduction of stretch-activated processes necessary for normal lung growth and maturation (18, 24). We propose that fetal inspiratory drive transmission also promotes respiratory neuromuscular development to ensure that the rigorous demands of maintaining a 40–45% duty cycle at birth are met. A recent study (29) found that there are pronounced changes in the passive membrane, action potential, and repetitive firing properties of rat phrenic motoneurons (PMNs) during the period spanning from embryonic day (E) 16, 1 day before the commencement of fetal breathing movements (17), through to the birth (gestational period is ~21 days). The focus of the present study was to examine the concomitant changes in diaphragm muscle properties during the perinatal period.

There have been several studies examining the developmental changes in diaphragm contractile and fatigue properties postnatally (5, 12, 21, 33, 39). Those studies found that, with age, 1) the ratio of peak twitch force to maximum tetanic force decreases, 2) twitch contraction and half relaxation times decrease, 3) the maximum unloaded shortening velocity increases, 4) the force-frequency curve is shifted to the right, and 5) neuromuscular fatigue decreases. In the present study, we complement past work by extending the examination of diaphragm properties to earlier perinatal stages, demonstrating that there is a major transformation of contractile and fatigue properties after the inception of inspiratory drive transmission in utero. Furthermore, we demonstrate that these properties develop during the perinatal period to functionally match changes occurring in PMN electrophysiological properties (29).

METHODS

Perinatal rat models. Embryos (E18) were delivered from timed-pregnant Sprague-Dawley rats anesthetized with halothane (1.2–1.5% delivered in 95% O2 and 5% CO2) and maintained at 37°C by radiant heat, following procedures approved by the Animal Welfare Committee at the University of Alberta. The timing of pregnancy was determined from the appearance of sperm plugs in the breeding cages, designated E0. Fetal age was confirmed by comparing the crown-rump length of the embryos with previously published values by Angulo y González (4). Newborn rats (postnatal days (P) 0–1) were anesthetized by inhalation of metofane (2–3%) before surgical procedures.

Phrenic nerve-diaphragm preparation. Embryos and newborns were decapitated, and the thoracic sections, with the rib cage and diaphragm intact, were isolated and transferred to a Sylgard-coated chamber (volume = ~35 ml). The tissue was bathed in a modified Krebs-Ringer solution at 27 ± 1°C during both the dissections and recordings. The solution contained (in mM) 117 NaCl, 6 KCl, 1.3 Na2HPO4, 2.6 CaCl2, 1.3 MgSO4, 24 NaHCO3, and 10 glucose, pH 7.4, after bubbling with 95% O2 and 5% CO2. The diaphragm with the phrenic nerve, ribs, and central tendon attached was dissected free and hemisected along the midline of the central tendon. The connective tissue and excess muscle were trimmed away, leaving a section of the hemidiaphragm ~5 mm wide,
centered approximately on the point at which the phrenic nerve enters the muscle. We did not find it technically feasible to obtain reliable force recordings from diaphragm strips isolated from animals younger than E18 because of the frailty of the muscle tissue.

Measurements of diaphragm contractile properties. A small piece of aluminum foil, with a short length of silk suture (–5 cm) attached was glued to the central tendon. The ribs, attached to the lateral edge of the muscle strip, were fixed to the Sylgard-coated chamber base with insect pins, and the suture was attached to a force transducer (UF1 Dynamometer, Harvard Instruments, South Natick, MA; 0- to 25-g working range with ±1% accuracy) suspended above the chamber (E18, n = 6; P0–1, n = 5). The phrenic nerve was stimulated with a suction electrode (40–70 µm in diameter) filled with bathing solution. To minimize current spread, the ground wire for antidromic stimulation was wrapped around the tip of the suction electrode. The muscle was stimulated directly by using two needle electrodes, each placed on either side of the muscle strip –4 mm apart. The timing and duration of all stimulation pulses were controlled by a Master-8 (AMPI, Jerusalem, Israel). These timing pulses were then converted to voltage signals by an Isoloflex stimulation unit (AMPI) or a Grass SD9 stimulator (Grass Instruments, Quincy, MA) for nerve and muscle stimulations, respectively. The electrical signal from the force transducer was sent to a Grass model 7D polygraph for preamplification and low-pass filtering (3 kHz). Signals were then transferred (2-kHz sampling rate) to a personal computer running Axotape software (Axon Instruments) via an analog-to-digital interface (TL1; Axon Instruments, Foster City, CA).

Optimal preload length (L0) was established by determining the resting tension at which maximal force was developed in response to a single square-wave voltage stimulus delivered via the phrenic nerve. Muscle length was changed via a micromanipulator (0.01-mm resolution; Mitutoyo, Tokyo, Japan); L0 values were 7.1 ± 0.4 (E18, n = 6) and 10.4 ± 1.1 mm (P0–1, n = 5). The twitch response to single square-wave stimuli was monitored periodically throughout the experiment to ensure that L0 was maintained and to assess tissue viability. Tissue viability was considered acceptable if single twitch responses did not change by >10% during the recording session. The minimum stimulus amplitude and the optimum stimulus duration (tested from 0.2 to 5 ms) required to achieve maximum single-twitch force in response to both phrenic nerve and direct muscle stimulation were also determined. To ensure supramaximal stimulation, an amplitude of 1.5 times the minimum stimulus amplitude was used throughout the experiment. Single twitches were elicited via phrenic nerve and/or direct muscle stimulation, and the peak force, time to peak force, and half relaxation time were determined. The muscle was then stimulated directly and/or via the phrenic nerve with 1-s-long trains at 3, 5, 10, 20, 40, 60, 80, and 100 Hz to determine peak force and fused tetanus frequency. Two minutes of relaxation were allowed between pulse trains. Because direct muscle stimulation could result in an inadvertent activation of intramuscular phrenic nerve branches, the direct muscle stimulation protocol was repeated after nicotinic acetylcholine receptors were blocked with the addition of 10 µM d-tubocurarine to the bathing solution.

Maximal single-twitch or tetanic force was expressed relative to wet and dry weight and protein content. Maximal tetanic force (expressed in N) was normalized to muscle CSA, were all significantly less at E18 compared with P0–1. Because of polyneuronal innervation, >1 EPP response was elicited in the majority of recordings. EPP amplitude was measured from baseline to the maximal value reached. Rise time was calculated from the onset of the EPP until the time when the maximal amplitude was reached. The EPP slope was calculated by a linear fitting of the rising phase of the EPP between 10 and 90% of its maximal amplitude. EPP decay rate was determined by a single exponential fitting of the decay phase measured between maximal amplitude and baseline of the last EPP response.

Statistics. Age-dependent differences were tested by using unpaired t-test (SigmaStat). Differences in EPP amplitude as a function of stimulation frequency were detected by using ANOVA followed by Newman-Keuls test (SigmaStat). P < 0.05 was accepted as statistically significant. Data are presented as means ± SD.

RESULTS

Force measurements. Table 1 lists the values of twitch and maximal tetanic forces generated as a function of muscle weight, protein content, and estimated CSA at E18 and P0–1. Single-twitch force per weight or milligram protein content was less at E18 compared with P0–1, although only values comparing force per wet weight were statistically significant. Maximal tetanic forces, expressed either as force per wet weight, dry weight, protein content, or estimated CSA, were all significantly less at E18 compared with P0–1. The ratios of single-twitch force to maximal
tetanic force were also different at each age (Table 1). The ratios ranged from ~50–70% at E18 compared with 20–40% at P0–1.

Fieldman et al. (12) previously reported the presence of poststimulation contractions (i.e., a second smaller contraction that appeared spontaneously during the relaxation phase of the tetanic contraction) in a subpopulation of neonatal diaphragm preparations. We observed this unexplained phenomenon in a few preparations (2 of 6 E18 preparations, 1 of 5 P0–1 preparations; data not shown).

Twitch characteristics. As demonstrated by the data in Table 1 and Fig. 1A, there were clear age-dependent differences in the characteristics of single muscle twitches. By P0–1, the mean twitch contraction and half relaxation times had decreased by 53 and 67%, respectively, compared with those at E18.

Relationship between force development and stimulation frequency. There were marked age-dependent differences in the relationship between the tetanic force generated by the diaphragm musculature and the frequency of phrenic nerve stimulation. 1) The fused tetanus frequency was much lower at E18 (~15 Hz) compared with P0–1 (~40 Hz; Fig. 1B). 2) There was a striking difference between the embryonic and neonatal muscle with regard to the range of forces generated by modulating the frequency of phrenic nerve stimulation (Fig. 2). At E18, the range was limited to ~65–100% of the maximum tetanic force in response to phrenic nerve stimulations from 1 to 100 Hz. In contrast, at P0–1, tetanic force ranged from ~25 to 100% of maximum in response to the same range of phrenic nerve stimulation. 3) The amount of force produced at the onset of nerve stimulation was less than that produced by direct muscle stimulation at P0–1 at frequencies >60 Hz (Table 1, Figs. 2 and 3; P ≤ 0.05). In contrast, there was no difference between forces achieved at the onset of nerve vs. direct muscle stimulation at E18. 4) The decay time of tetanic force production was much slower at E18 in comparison to P0–1, with the force production being maintained well beyond the termination of nerve stimulation (Fig. 3).

Relationship between declines in diaphragm force levels and nerve stimulation paradigms. There were age-dependent changes in the ability of the muscle to sustain force levels in response to high-frequency nerve stimulation. At E18, the muscle was capable of supporting the same level of force throughout a 1-s pulse of up to 100-Hz phrenic nerve stimulation in all but one of six preparations (Table 1, Fig. 3). In contrast, the force levels declined in all neonatal muscle preparations in a frequency-dependent manner at nerve stimulation rates ≥40 Hz (Table 1, Figs. 1B and 3). The data illustrated in Fig. 3B demonstrate that the decline in force in response to the nerve stimulation could be reversed by stimulating the muscle directly. Moreover, there was no decline in force at either age when the muscle was stimulated directly for 1 s. A further analysis of the E18 preparations (2 of 6 E18 preparations, 1 of 5 P0–1 preparations; data not shown).

Fig. 1. Age-dependent changes in twitch and tetanus characteristics. A: single-twitch contractions generated by embryonic day (E) 18 and postnatal days (P) 0–1 diaphragm musculature after phrenic nerve stimulation (0.5-ms pulse). B: contractile responses of E18 and P0–1 diaphragm musculature in response to 1-s nerve stimulation of varying frequencies. Twitch contraction times decrease and tetanus fusion frequencies increase markedly during perinatal period.

Table 1. Contractile properties of rat diaphragm at embryonic age 18 and postnatal days 0–1

<table>
<thead>
<tr>
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<th>E18 (n = 6)</th>
<th>P0–1 (n = 5)</th>
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<tbody>
<tr>
<td>Twitch force</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/mg wet wt</td>
<td>28 ± 4</td>
<td>51 ± 6*</td>
</tr>
<tr>
<td>mg/mg dry wt</td>
<td>900 ± 149</td>
<td>1,185 ± 228</td>
</tr>
<tr>
<td>mg/mg protein content</td>
<td>72 ± 27</td>
<td>169 ± 51</td>
</tr>
<tr>
<td>Maximal tetanic force</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/mg wet wt</td>
<td>51 ± 11</td>
<td>185 ± 27*</td>
</tr>
<tr>
<td>mg/mg dry wt</td>
<td>1,619 ± 340</td>
<td>4,452 ± 1,093*</td>
</tr>
<tr>
<td>mg/mg protein content</td>
<td>121 ± 42</td>
<td>657 ± 216*</td>
</tr>
<tr>
<td>Normalized force, N/cm²</td>
<td>0.4 ± 0.1</td>
<td>2.1 ± 0.3*</td>
</tr>
<tr>
<td>Single-twitch contraction time, ms</td>
<td>182 ± 20</td>
<td>85 ± 3*</td>
</tr>
<tr>
<td>Single-twitch half relaxation time, ms</td>
<td>156 ± 12</td>
<td>51 ± 3*</td>
</tr>
<tr>
<td>Single-twitch/maximal force ratio</td>
<td>0.64 ± 0.03</td>
<td>0.28 ± 0.02*</td>
</tr>
<tr>
<td>Fused tetanus frequency, Hz</td>
<td>15 ± 2.2</td>
<td>40 ± 0*</td>
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Values are means ± SD; n, no. of rats. E18, embryonic day 18; P0–1, postnatal days 0–1. Single-twitch force was the average of 3 consecutive twitches. Fatigue ratio was calculated as peak tension vs. level of tension at end of 1-s-long nerve stimulation. *P ≤ 0.05 compared with E18.
muscle force production in response to a train of nerve stimulation was performed. We tested whether there would be a drop in force levels if the stimulation were maintained for 1 s. As shown in Fig. 4, when the duration of the stimulus train was increased (10 s), a significant decline in force levels was observed in all E18 preparations. Furthermore, the decline in force levels could be reversed by stimulating the E18 muscle directly.

Measurements of EPPs. To further understand the source of fatigue and to evaluate age-dependent changes in the efficacy of synaptic transmission between the phrenic nerve and diaphragm muscle fibers, EPPs were recorded utilizing embryonic and neonatal in vitro preparations in the presence of d-tubocurarine (4–6 µM). Because of the concern that d-tubocurarine also suppresses presynaptic release of acetylcholine (15), two alternative means of suppressing muscle twitches were attempted. First, we applied µ-conotoxin, which has been shown to block muscle sodium channels (8) and thus paralyze skeletal muscle without affecting the motor nerve or the neuromuscular junction (19). However, there was no visible inhibition of embryonic muscle contractions after adding µ-conotoxin (up to 10 µM) to the bathing medium for 45 min and, as previously reported (5), µ-conotoxin was only partially effective in attenuating neonatal muscle contractions. Thus, presumably due to differences in acetylcholine receptor subunit composition in perinatal and adult muscle (22), µ-conotoxin proved to be an ineffective means of suppressing muscle twitches. Second, we applied 2,3-butanedione,2-monoxime, which has been shown to suppress muscle contractions in adult muscle by reducing calcium release from the sarcoplasmic reticulum and interfering with cross-bridge cycling (14, 32). However, addition of up to 40 µM 2,3-butanedione,2-monoxime was ineffective for blocking embryonic muscle twitches and only partially effective in neonatal muscle preparations.

Figure 5 illustrates typical recordings of EPPs elicited from E18 and P0 muscle fibers in response to phrenic nerve stimulation. The majority of EPPs at E18 (5 of 6) and at P0–1 (8 of 9) obtained in this experiment contained two components. This is thought to be the result of multiple nerve innervation of muscle fibers that occurs during development (7, 9, 36). Figure 5A shows a recording obtained from a P1 muscle fiber in which two components of the EPP could not be separated by modulating the strength of nerve stimulation. This observation suggests that there are at least two axons with similar activation thresholds innervating the muscle fiber.

The characteristics of individual EPPs were not significantly different between embryonic and neonatal muscle. The measured values were as follows: amplitude (1.4 ± 0.6 vs. 1.6 ± 0.5 mV), rise time (2.8 ± 0.4 vs. 3.3 ± 0.6 ms), slope (0.5 ± 0.2 vs. 0.8 ± 0.3 mV/ms), and decay rate (26.8 ± 6.3 vs. 21.1 ± 3.2 ms) for E18 (n = 5) vs. P0 (n = 9). However, it was clear that there were age-dependent differences in the ability to maintain EPP amplitudes in response to a 1-s train of nerve stimulation (Fig. 5, B and C). A significant decline in EPP amplitude beyond the first EPP was found in all E18 preparations. We tested whether there would be a drop in force levels if the stimulation were maintained for >1 s. As shown in Fig. 4, when the duration of the stimulus train was increased (10 s), a significant decline in force levels was observed in all E18 preparations. Furthermore, the decline in force levels could be reversed by stimulating the E18 muscle directly.

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E18 preparations tested (n = 7) in response to ≥20-Hz nerve stimulation (Fig. 5C). In contrast, at P0, a significant decline in EPP amplitude was not apparent until the nerve was stimulated at ≥30 Hz (n = 8; Fig. 5C).

DISCUSSION

PMNs in the perinatal rat undergo major changes in their morphology and electrophysiological properties during the relatively short period spanning the inception of inspiratory drive transmission at E17 through to birth (2, 16, 17, 29). Data from the present study demonstrate that there is a concomitant development of diaphragmatic muscle properties during this period that functionally matches changes in PMN properties. It is also apparent on examination of our data in conjunction with those from previous studies of postnatal diaphragm function (6, 12, 21, 33, 39) that the overall trend for age-dependent changes in contractile force production and fatigue characteristics can be traced back in development through to embryonic and early postnatal stages.

Consideration of in vitro conditions. The experiments in this study were performed at 27°C for two reasons. First, the reduced temperature facilitates tissue viability for the full duration of the experimental procedures (38). Second, from the perspective of understanding the functional ontogeny of the motor unit, it was important to interpret findings from this study of diaphragm contractile properties with those from a parallel study of PMN electrophysiological properties (29). The study of PMN properties was performed by using perinatal cervical splice preparations maintained in vitro at 27°C.
27°C. The Q_{10} for the various neuronal (40) and muscle properties (34, 38) measured will not be identical. However, by studying the neuronal and muscle properties under similar in vitro conditions, reasonable conclusions regarding a functional correlation between the development of the two components of the neuromuscular system can be derived.

Twitch contraction and relaxation times. The twitch contraction and relaxation times were much slower at E18 compared with P0–1. Differences in myosin heavy chain (MHC) content are likely responsible for a significant component of the variations in contraction speed. There are at least six isoforms of MHCs [embryonic, neonatal, slow (or type 1), and fast (2A, 2B and 2X)] that are expressed at various stages of muscle fiber maturation (25, 37). Electrophoretic analyses of MHC expression in the developing rat diaphragm have shown that the mature pattern of MHC isoform expression is not reached during the first month postnatally (43). At E18, the diaphragm is composed largely of the embryonic/neonatal MHC isoforms, which are characterized by slow kinetics and high resistance to fatigue (21, 23, 27, 39, 43). By birth, 30% of the embryonic/neonatal isoform is replaced by the adult slow and fast 2A isoforms, which would, in part, explain the overall increase in contraction speed (21, 35, 41, 44). Differences in the twitch and tetanic relaxation times can be accounted for by age-dependent elaborations of the T-tubule/sarcoplasmic reticulum system and increases in calcium ATPase activity. Both of these mature perinatally, resulting in an increased ability to rapidly sequester calcium from sarcomeres and terminate force production (11, 45).

Force development. The diaphragm musculature at E18 is visibly more fragile and develops less single-twitch or tetanic force per weight or protein content compared with that at the newborn stage. The initial wave of myotube formation (primary myogenesis) in the diaphragm is completed by approximately E17 (coinciding with the time of the inception of inspiratory drive transmission). Subsequently, there is a secondary stage of myogenesis during which the majority of the future diaphragm musculature is formed (1, 3). Thus, between E18 and P0–1 there are substantial increases in the ratio of muscle to connective tissue and the thickness and surface area of the diaphragmatic musculature. Moreover, increases in the density of myofilaments within individual muscle fibers, changes in MHC isoform expression, and the elaboration of the T-tubule/sarcoplasmic reticulum apparatus all lend themselves to enhancing the force-producing capabilities of developing muscle (31, 45).

Force-frequency relationship. At E18, the kinetics of muscle twitches were slow, and thus fused tetanic contractions were achieved at a relatively low frequency (~15 Hz). The frequency at which the diaphragm is driven by the phrenic nerve in utero is not known. However, we have examined the repetitive firing properties of PMN at E18 under in vitro conditions similar to those utilized in this study for measuring muscle properties. As is the case for diaphragm twitch contractions, the duration of PMN action potentials is considerably longer at E18 compared with those at P0–1 (29). Consequently, the slow kinetics of the PMN action potentials at E18 limit the maximum rate of repetitive firing in response to a 1-s depolarizing pulse to ~20 Hz. This is also within the range of cervical motoneuron firing rates observed during inspiratory bursts generated by brain stem-spinal cord in vitro preparations isolated from E18 rats (10). Interestingly, the range necessary to produce the full gradation of tetanic force in the diaphragm musculature spans from ~1 to 20 Hz (Fig. 2). Thus it would appear that there is a functional matching of PMN firing and diaphragm contractile properties at E18. It was also apparent that the actual range of forces generated by the diaphragmatic embryonic musculature is quite narrow; ~60% of the maximum tetanic force is generated at minimal rates of stimulation. Perhaps a fine gradation of force generation in the diaphragm musculature is not an essential component of fetal breathing movements in utero. Rather, given the low rheobase current necessary to activate PMNs (29) and the utilization of the upper end of the force output range for the diaphragm musculature, it seems that the neuromuscular system is designed to ensure breathing movements of adequate magnitude for ribcage expansion, despite a rather weak inspiratory drive transmission present during the early stages after the inception of respiratory rhythmogenesis (10).

By birth, the single-twitch contractions were considerably faster and the tetanic frequency higher relative to those at E18. At P0–1, PMN action potentials are also of shorter duration, and thus the repetitive firing frequency is higher, reaching an approximate maximum of 30 Hz in response to a 1-s depolarizing pulse (29). This range is similar to that observed from recordings of PMNs during inspiratory bursts generated by neonatal rat brain stem-spinal cord preparations (26). Similar to the situation observed at E18, the range of neonatal PMN firing matches those necessary to generate the full range of potential force levels from the diaphragm musculature.

It also is apparent from Fig. 2 that the range of forces generated by the diaphragm at P0–1 in response to graded increments of nerve stimulation is much wider than that at E18. This property would allow for the modulation of diaphragmatic force development to produce tidal volumes that meet the variety of respiratory demands encountered postnatally.

Neuromuscular fatigue. The diaphragm musculature at P0–1 was capable of maintaining a constant level of force in response to a 1-s train of nerve stimulations at 20 Hz. However, at higher frequencies of nerve stimulation, there was an incremental decline in force during the latter phases of the 1-s stimulation. The experiments involving combined nerve and muscle stimulation showed that the force reduction was related to some aspect of transmission between the nerve and muscle. Subsequent analyses of EPP amplitudes deter-
timated that there was a gradual decrement in EPP amplitude during the 1-s period of phrenic nerve stimulation at a rate >20 Hz. Bazzy and Donnelly (6) demonstrated that part of the failure to follow high-frequency stimulation in neonatal phrenic nerve-diaphragm preparations was due to failure at the neuromuscular junction. Fournier et al. (13) also determined that failure of action potential propagation to the motor axon nerve terminals at higher frequencies could contribute to neurotransmission failure in neonatal rats. Both of these processes are thought to mature during the first 2 wk postnatally and then remain unchanged thereafter (13). Regardless, neuromuscular fatigue may not be a significant problem for newborn rats, because the range of repetitive stimulation rates necessary to produce fatigue at P0–1, at least in vitro, is beyond the typical maximum sustained frequency at which PMNs fire under similar conditions (10, 26, 29).

The absence of a decline in the force output of the E18 diaphragm musculature in response to 1-s-long trains of nerve stimulation was, at least initially, a surprising result. We had assumed that the above-mentioned factors contributing to neurotransmission failure at P0–1 would be more pronounced at E18. Indeed, the ensuing measurements of EPPs clearly showed that neuromuscular transmission failure was much more severe at E18 compared with that at P0. The fact that the neuromuscular fatigue did not translate into a failure of force production could be explained by the prolonged maintenance of calcium ions within the sarcomeres of embryonic muscle. The T-tubule-sarcoplasmic reticulum system is underdeveloped prenatally (11, 45). Thus, as is evident from examining the slow decay of individual twitches or tetanic force output (Figs. 1 and 3), the force levels persist well beyond the termination of the synaptic drive from the phrenic nerve. Furthermore, our measurements of force production in response to a prolonged phrenic nerve stimulation at E18 (10 s; Fig. 4) support this hypothesis by showing that the decline in force levels was manifest if sufficient time was allowed for the sequestering of calcium ions. Functionally, these data imply that, in utero, diaphragm contractions persist beyond the period of inspiratory drive from PMNs (despite any neuromuscular transmission fatigue). This would be advantageous for ensuring a prolonged expansion of the ribcage and stretching of the lungs, despite a weak synaptic drive from the PMN pool to the diaphragm. In contrast, by birth, the PMN diaphragm properties are such that there is a more controlled duration and range of diaphragm contractions by motoneuronal inspiratory drive.

Summary. In summary, from the day after the inception of inspiratory drive transmission through to birth, diaphragmatic muscle properties undergo significant development and maturation. Furthermore, the diaphragm contractile and PMN repetitive firing properties develop in concert so that the full range of potential diaphragm force recruitment can be utilized and problems associated with diaphragm fatigue are minimized.

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