Functional, cellular, and biochemical adaptations to elastase-induced emphysema in hamster medial scalene

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Fournier, Mario, and Michael I. Lewis. Functional, cellular, and biochemical adaptations to elastase-induced emphysema in hamster medial scalene. J Appl Physiol 88: 1327–1337, 2000.—The scalene has been reported to be an accessory inspiratory muscle in the hamster. We hypothesize that with the chronic loads and/or dynamic hyperinflation associated with emphysema (Emp), the scalene will be actively recruited, resulting in functional, cellular, and biochemical adaptations. Emp was induced in adult hamsters. Inspiratory electromyogram (EMG) activity was recorded from the medial scalene and costal diaphragm. Isometric contractile and fatigue properties were evaluated in vitro. Muscle fibers were classified histochemically and immunohistochemically. Individual fiber cross-sectional areas (CSA) and succinate dehydrogenase (SDH) activities were determined quantitatively. Myosin heavy chain (MHC) isoforms were identified by SDS-PAGE, and their proportions were determined by scanning densitometry. All Emp animals exhibited spontaneous scalene inspiratory EMG activity during quiet breathing, whereas the scalene muscles of controls (Ctl) were silent. There were no differences in contractile and fatigue properties of the scalene between Ctl and Emp. In Emp, the relative amount of MHCx was 15% higher whereas that of MHC2a was 14% lower compared with Ctl. Similarly, the proportion of type IIa fibers increased significantly in Emp animals with a concomitant decrease in IIx fibers. CSA of type IIX fibers were significantly smaller in Emp compared with Ctl. SDH activities of all fiber types were significantly increased by 53 to 63% in Emp. We conclude that with Emp the actively recruited scalene exhibits primary-like inspiratory activity in the hamster. Adaptations of the scalene with Emp likely relate both to increased loads and to factors intrinsic to muscle architecture and chest mechanics.

IN HUMANS, IT IS WELL ACCEPTED that the scalene muscle functions as a primary inspiratory muscle (4, 5, 9, 35, 44). By contrast, we have recently reported that in the hamster model of elastase-induced emphysema (Emp) remains the most comprehensively documented animal model of chronic hyperinflation in which adaptations of the respiratory muscles, particularly the diaphragm, have been reported (12, 13, 22, 27, 40). We hypothesize that with the chronic respiratory loads and/or dynamic hyperinflation associated with the hamster model of Emp, the scalene will become actively recruited during resting ventilation in phase with the diaphragm and not merely recruited as a reserve system (i.e., subserve a primary-like inspiratory role; this term does not imply differences in the quantity of work between different respiratory muscles, fulfilling our definition). We postulate that, because of its mechanical action on the rib cage (i.e., rostral displacement of the sternum and ribs and increased anteroposterior diameter of the rib cage; Ref. 6), the scalene would compensate, in part, for the mechanical inefficiency of the diaphragm imposed by the chronic state of hyperinflation, despite adaptive changes in the diaphragm of these animals aimed at preserving its function (12, 13, 22, 27, 32, 40). This supposition is further strengthened by studies done in patients with chronic obstructive pulmonary disease (COPD), which have reported increased electromyogram (EMG) activity of the scalene (8) as well as increased firing frequencies of recruited parasternal and scalene motor units in patients with COPD compared with normal subjects (17). This is also in accordance with studies in which the contribution of rib cage and/or neck muscles to pressure generation during inspiratory efforts was increased in hyperinflated patients with COPD at rest or during exercise (29, 31), or reduced 3 mo after lung volume reduction surgery (24).

The aim of the present study was, therefore, to evaluate the effects of elastase-induced Emp in the hamster on the functional, cellular, and biochemical properties of the medial scalene muscle. Specifically, we evaluated the adaptations of the medial scalene with regard to 1) its recruitment profile during resting ventilation, 2) contractile and fatigue properties, 3) expression of myosin heavy chain (MHC) isoforms, 4) fiber type composition, 5) fiber size, and 6) fiber oxidative activity.

METHODS

Experimental Paradigms

Evaluation of the lateral and anterior segments of the medial scalene. In the hamster, the scalenus medius represents the major component of the scalene with >85% of the...
muscle mass (16). However, because the medial scalene tendons originate from four different cervical levels, this multitendon configuration at its origin makes it impossible to assess the physiological properties of the whole intact medial scalene. However, despite this anatomic configuration, advantage can be taken of a natural division of the medial scalene into anterior (i.e., the innermost or anteromedial portion) and lateral (i.e., the outermost or lateral portion) segments made by a slip of the serratus at approximately the middle portion of the medial scalene at the level of the third rib (Fig. 1).

Measurements of contractile properties were performed to assess whether any major differences between the two segments existed in the normal hamster because use of the anterior segment for physiological studies was more technically demanding with a greater risk of fiber injury with dissection. The muscle fiber composition of the medial scalene was also determined by using histochemical and immunohistochemical techniques.

Effects of Emp on the medial scalene properties. In these experiments, the entire medial scalene was analyzed in histochemical, immunohistochemical, and biochemical studies, whereas the lateral segment of the medial scalene was used for physiological experiments.

Experimental Animals

Thirty-four adult male Golden Syrian hamsters were studied. Of these, 16 animals (age = 9 mo; body wt 196.5 ± 13.2 g) were studied to evaluate isometric contractile and fatigue properties of the lateral and anterior segments of the medial scalene, and the remaining 18 hamsters were studied to determine the influences of Emp on the scalene muscle. All animals were housed with controlled ambient temperature (21°C) and 12:12-h light-dark cycle. Food (Purina rodent chow) and water were provided ad libitum. For all procedures, general anesthesia was achieved by using ketamine HCl (200 mg/kg ip) and xylazine (10 mg/kg ip). Additional anesthesia (one-fourth of the initial dose) was given as required to maintain adequate anesthesia, although most procedures were of short duration. During procedures, rectal temperature was monitored (Sensortek) and maintained between 37 and 38°C with radiant heat. At the conclusion of terminal experiments, the animals were euthanized with an overdose of pentobarbital sodium. Approval for all studies was obtained from the Institutional Animal Care and Use Committee of the Cedars-Sinai Medical Center Burns and Allen Research Institute.

Induction and Verification of Emp

Male hamsters (initial body wt 100 g; age 7–9 wk) were divided into two groups: control (Ctl; n = 9) and Emp (n = 9).

Emp was induced under general anesthesia by the single intratracheal instillation of porcine pancreatic elastase (40 IU/100 g body wt in 0.3 ml of sterile normal saline; Sigma Chemical, St. Louis, MO). Ctl animals were given 0.3 ml of normal saline only via the intratracheal route. During the instillation of elastase or saline, the animals were held upright and angulated in various positions to facilitate and diffuse bilateral instillation of the administered solution. The animals were studied 1 yr later, at which time fully evolved Emp was expected. Emp was verified by measuring static pressure-volume relationships of the hamster lungs immediately after excision. Maximum lung volume was defined as the volume at 25 cmH2O pressure with air inflation (27, 37).

EMG Studies

In the Emp and Ctl animals, a short middiaphragm longitudinal skin incision was performed on the right side to expose the medial scalene at the lateral edges of the pectoralis profundis cranialis and rectus abdominis muscles. A pair of electrodes was implanted in the scalene at the level of the second intercostal space immediately rostral to a slip of the serratus ventralis thoracis, which is superficial to the lateral segment of the scalene (see Ref. 16 for details). This muscle slip is intercalated between the lateral and the anterior segment under which it originates on rib 3, thus creating a natural division into two medial scalene segments.

A small incision through the abdominal musculature was made below the right costal margin to expose the abdominal surface of the diaphragm. A pair of electrodes was implanted in the midcostal region of the right hemidiaphragm.

Electrodes were made of fine fluorocarbon-insulated multistranded stainless steel wires (38 gauge; Cooner Wire) that had 1 mm of insulation removed near the tip. The electrodes for each pair were positioned ~3 mm apart within muscles. One additional wire acting as common ground was also implanted subcutaneously. Leads were connected to calibrated differential amplifiers (Dantec), and the signals were band-pass filtered between 10 Hz and 2 kHz, with a 60-Hz filter incorporated, and further amplified (Service Associate).

The same calibration and amplification were used for all experiments. EMG signals were monitored on a storage oscilloscope (Gould), and selected episodes were saved and displayed on a plotter (Hewlett Packard).

EMG activities from the medial scalene and diaphragm muscles were obtained from anesthetized and tracheosto-
mized hamsters in a supine position. Further tests were also made with animals in a prone position or lying on their side. Although differences in these recordings compared with those obtained in the supine position were not systematically studied, none was observed. Records of raw EMG activity of the medial scalene were compared with those of the costal diaphragm to characterize the inspiratory phase. However, no efforts were made to accurately determine differences and/or similarities in onset and offset times for their respective respiratory activities (i.e., relative to each other).

Isometric Contractile and Fatigue Properties

Anatomic dissection of the scalene. After a ventral midline skin incision from the low thoracic level to the neck and an incision along the left costal margin, the pectoral was resected laterally to expose the thoracic portion of the scalene. The dacies were sectioned at the manubrium above the first rib and reflected laterally, exposing the remaining cervical portion of the scalene. Major blood vessels (brachial and jugular) were ligated, and the thoracic cavity was open by sectioning along the lateral edge of the sternum, along the caudal edge of rib 5, and along the axillary line. The section was extended to the neck along the lateroposterior aspect of the scalene. Rapidly, the clavicle was cut and a midline section was made along the cervical vertebrae down to the sternum so that the scalene could be removed en bloc. The muscle was immediately transferred to a preparatory tissue bath containing a Krebs-Henseleit solution constantly aerated with 95% O2-5% CO2 and kept at room temperature. The scalene, including its tendinous attachments to transverse processes, and portions of ribs 1, 3, 4, and 5 (if applicable) were rapidly dissected from remaining tissue and pinned down with some stretch at its in situ length. The lateral and anterior segments of the medial scalene have origins from C2 to C3 and C4 to C5, respectively (with some overlap). Both segments were isolated with their insertion onto rib 4 intact. The small slip of scalene inserting onto rib 3 was peeled off (as well as that inserting onto rib 5 when present), and both segments making strips of ~3 mm wide were prepared for the measurement of their respective physiological properties (Fig. 1).

Physiological Studies. The techniques used for determining physiological properties have been described in detail (26). Plastic clamps were attached to cervical tendons (C2 and C3 or C4 and C5) and rib 4, and the muscle segment was mounted vertically in a tissue bath containing the same aerated solution, but with d-tubocurarine (12 µM/l), and kept at 20°C. The cervical clamp was attached to a calibrated force transducer (Grass FT10), and the rib clamp was secured to a micromanipulator (Kopf). Direct muscle stimulation (Grass S88) of constant current pulses (Mayo Engineering) of 1.5 ms duration using a fine-wire electrode (50 gauge, California Wire) was delivered. Muscle preload was incrementally adjusted until the optimal muscle length for maximum twitch (Pt) response. During the experiment the stimulation paradigm was controlled by a computer program. Pt was measured from series of single pulses from which contraction time (CT; time to peak force) and one-half relaxation time (RT1/2; time for force to fall to half its peak value) were also determined. Force-frequency relationships were measured for stimulation frequencies from 5 to 100 pulses/s in trains of 1-s duration, and maximal tetanic force (Pmax) was determined. Forces were normalized for estimated physiological cross-sectional area (CSA) of the muscle sample by the following formula: CSA = muscle weight/(1.0564 × L0), where 1.0564 g/cm3 is the muscle density. Muscle fatigue resistance was assessed by using a test in which stimuli are presented at 40 pulses/s in trains of 330-ms duration and repeated each second for a 2-min period (2). A fatigue index is measured as the ratio of the final force generated to the initial force.

Histochemical Procedures: Fiber-Type Proportions and CSA

The entire medial scalene was mounted and pinned on cork at approximately in vivo resting muscle length (including rib attachment), frozen in isopentane cooled to its melting point by liquid nitrogen, and stored at ~70°C until analysis. Serial muscle cross sections (10-µm thickness) were cut in a cryostat (Reichert-Jung 2800E) at ~20°C. Sections were stained for myofibrillar ATPase after acid preincubations (pH 4.25 and 4.5) (15) and after alkaline preincubation after tissue fixation (18) with these modifications. Muscle sections were fixed with 2% paraformaldehyde in a 0.1 M cacodylate buffer (pH 7.4) for 2 min at room temperature. Sections were rinsed with 0.18 M CaCl2 and then preincubated for 12 min in 0.1 M 2'-amino 2'-methyl 1'-propanol buffer (pH 9.6) before standard incubation at pH 9.4. This modified step allowed the histochemical identification of muscle fibers as types I, IIA, IIB, IIC, and IIX (15). Selected areas across the entire section were digitized with an image-processing system composed of a Leitz Laborlux S (Leica) microscope, charge-coupled device video camera systems (Optronics model VI-470), high-resolution monitor (Sony model 1343MD), 486 DX 50-MHz personal computer (Truevision) imaging board, and Mocha image analysis software (v 1.2;andel). The imaging system was calibrated for density measurements by use of a set of neutral-density filters and for morphometry with a stage micrometer. Single muscle fibers were outlined, and optical densities for the entire fiber area were measured. The CSA of individual fibers was determined from the number of pixels within outlined fiber boundaries.

Immunohistochemical Procedures

Anti-MHC monoclonal antibodies (38) were used for the indirect immunoperoxidase identification of MHC isoforms in the medial scalene. Serial muscle cryosections (10-µm thickness) matching the ATPase stains were dried at room temperature, fixed in cold acetone for 5 min, washed with PBS, and incubated in 20% horse serum for 15 min at room temperature. Sections were incubated for 1–2 h at room temperature in one of the following antibodies (diluted in PBS): BA-D5 (1:10) reacting with MHC1b, SC-71 (1:10) reacting with MHC2A; BF-F3 (1:10) reacting with MHC2B, and BF-35 (1:20) reacting with all MHCs except MHC2x. These mouse antibodies (lgG1) were generously provided by Regeneron Pharmaceuticals (Tarrytown, NY). A rabbit polyclonal antibody, MY-32 (1:400; Sigma Chemical), reacting with all fast MHCs, was also used to confirm that presumptive type IIX fibers express a fast MHC isoform. Sections were rinsed with PBS and exposed to peroxidase-conjugated secondary antibody (1:200) for 30 min at room temperature. Control sections were exposed only to secondary antibodies. Sections were rinsed with PBS and exposed to ABC (Vector) reagent, and visualization was obtained after diaminobenzidine reaction with nickel amplification.

Fiber SDH Activity

Fiber oxidative capacity was determined by quantifying the activity of succinate dehydrogenase (SDH; a key mitochondrial enzyme in the Krebs cycle) in individual muscle fibers. The methodology employed to quantitate SDH activity has been described in detail in previous reports (1). Briefly, in the histochemical reaction for SDH, the progressive reduction of
The concentration of NBT diformazan (NBT-dfz) deposited within a muscle fiber was calculated by using the Beer-Lambert equation

\[ \text{[NBT-dfz]} = \frac{\text{OD}}{k \times L} \]

where OD was the optical density of the muscle fiber measured at 570 nm (the peak absorbance wave length for NBT-dfz), k was the molar extinction coefficient for NBT-dfz (26,478 mol cm\(^{-1}\)), and L was the pathlength (i.e., 6-µm section thickness) for light absorbance. The OD of muscle fibers was determined by using a microdensitometric procedure implemented on the computer-based image processing system. The video image was then digitized (8-bit gray level resolution) into a matrix of 1,024 pixels (picture elements). The gray levels of the video scanner were calibrated for photometry (OD units) by using a series of neutral-density filters (0.004 to 2.00 OD units; Melles Griot, Irvine, CA). We have previously demonstrated the linearity of the SDH reaction over a period of at least 7–9 min (1). In reactions where succinate was absent from the reaction medium, there was measurable staining (i.e., reduction of NBT), but the OD did not change significantly across the same time periods. The tissue blank OD also corresponded to the OD measured at time zero in reactions where succinate was present in the medium. Based on these data, we justified the use of a single end-point measurement of OD, with a reaction time of 5 min. From these end-point measurements, a rate of SDH reaction was interpolated. Mean SDH activity of individual scalene muscle fibers was determined by averaging the OD of all pixels within outlined muscle fibers. To correct for the nonspecific formation of NBT-dfz, the tissue blank OD for each fiber was subtracted from the OD measured when substrate was added to the incubation medium. From the Beer-Lambert equation, the mean SDH activity of each fiber was expressed as millimoles of fumarate per liter of tissue per minute. Approximately 200–300 fibers were sampled across the medial scalene from each specimen.

Electrophoretic Identification of MHC Isoforms

For the myofibril extraction, 10-mg muscle samples were homogenized by hand on ice in 20 vol of cold buffer (39). The homogenate was centrifuged at 4°C, the supernatant was discarded, and the pellet was resuspended. The final pellet was resuspended in 10 vol of the buffer. Myofibril content was determined by using a microbacinichonic acid protein assay kit (Pierce) and was quantified by using an ELISA reader at 570 nm (the peak absorbance wave length for NBT-dfz). Samples of purified myofibrils were diluted 1:8 in a denaturing sample buffer. Final myofibrillar protein concentration was ~0.125 µg/µl. Proteins were denatured in the same buffer by boiling samples for 2 min before loading. The MHC composition was determined by a SDS-PAGE technique with 8% separating gels (41). Each well was loaded with 0.7–0.9 µg of protein extract. Electrophoresis was performed with a Bio-Rad Mini-Protean II system for a duration of 25 h at constant 80 V with running buffers kept at 4–7°C.

The gels were stained with silver nitrate (Bio-Rad Silver Stain Plus kit). Dried stained gels with duplicate samples were scanned twice by an Ultra-Violet Products Image Store 5000 system, and densitometric measurements were performed with its Gel Documentation and Software System. After background subtraction, the relative contribution of each band within a gel was determined by the ratio of the total gray level within the area of a specific band to that of the cumulative gray level of all the bands present in a sample. The specificity of each band has been demonstrated by immunoblotting identification after electrophoretic transfer (15). This SDS-PAGE method allows the clear separation of MHC isoforms from denatured myofibrils of adult hamster skeletal muscle with the fastest migrating band corresponding to MHC\(_{1b(low)}\), followed in order by MHC\(_{2b}\), MHC\(_{2x}\), and MHC\(_{2a}\). The densitometric analysis of each identified MHC isoform was performed in duplicate on two samples for each muscle, and the average relative content of each MHC isoform was estimated. In some animals the diaphragm and tibialis anterior (TA) were also analyzed for comparison and identification of all adult MHC bands. The TA was used to demonstrate the presence of pure type IIb fibers, which express MHC\(_{2b}\), because no pure type IIb fibers were identified in the scalene. Therefore, the TA results confirm reactivity of our MHC\(_{2b}\) antibody with hamster muscle and can be used to confirm the location of the MHC\(_{2b}\) band in the gel electrophoresis.

Statistical Analysis

For physiological, histochemical, immunohistochemical, and biochemical assays, one-way ANOVA test was used to determine differences between Ctl and Emp animals as well as between lateral and anterior scalene segments. For comparisons of force-frequencies relationships and fatigue resistance, an ANOVA with repeated measures was performed with post hoc analysis using the Newman-Keuls test. Differences were considered significant at the level of P < 0.05. Data reported are presented as means ± SE.

RESULTS

Experimental Protocol A: Evaluation of the Mediastinal Scalene

Physiological properties of lateral and anterior segments. The isometric contractile and fatigue resistance characteristics of the lateral and anterior segments of the medial scalene are depicted in Table 1. As expected, the L\(_{0}\), of the lateral segment, with a more rostral origin, was found to be 10.6% longer than that of the anterior
The RT1/2 of the lateral segment was 16.4% shorter than that of the anterior segment (P < 0.05). There were no differences in the speed-related properties, CT and RT1/2, between the anterior and lateral segments of the scalene. However, the RT1/2 of the lateral segment was 16.4% shorter than that of the anterior segment (P = 0.06). No significant differences between the segments were observed with regard to the specific forces generated by the scalene (i.e., P1 and P∞; Table 1). The force-frequency relationships of the two segments were also similar. With repetitive stimulation, the tetanic forces generated by both anterior and lateral segments decreased to intermediate levels after 2 min (not shown). However, the fatigue resistance of the lateral segment of the scalene, as measured by the fatigue index (Table 1), was 14.6% lower than that of its anterior segment (P < 0.01).

The similar isometric contractile properties between the two segments would suggest that use of either segment would be representative of the whole medial scalene, despite the small difference in their resistance to fatigue. Thus we have selected to analyze the lateral segment of the medial scalene in our physiological assessment of the effect of Emp on the contractile properties of the medial scalene.

Medial scalene histo- and immunohistochemistry. Studies were performed on the whole medial scalene. Gross observation of muscle sections clearly indicated a gradient in distribution of type I fibers decreasing from the anterior toward the lateral aspects of the medial scalene (Fig. 2). Myofibrillar ATPase stains allowed the identification of type I, IIa, and IIx fibers, whereas type IIb fibers were virtually absent in the medial scalene. In contrast, type IIb fibers were simultaneously identified in large numbers in the tibialis anterior (Fig. 3). The total number of scalene fibers analyzed varied from 1,062 to 1,249. In the medial scalene, type I fibers accounted for 16.1 ± 2.7%, type IIa for 30.2 ± 1.7%, and type IIx for 53.1 ± 1.4%. There were also 0.5 ± 0.2% of type IIc fibers and only 0.1 ± 0.1% type IIb fibers. This muscle fiber phenotype was confirmed by immunohistochemical analysis of MHC isoforms expressed within the same fibers. However, hybrid fibers were also occasionally identified. Coexpression of MHC1 and MHC2A isoforms was observed in histochemically typed IIc fibers, whereas some other histochemically typed IIx fibers coexpressed MHC2A and MHC2X isoforms (e.g., Fig. 3) or MHC2X and MHC2B isoforms (not shown).

Experimental Protocol B: Effects of Emp on the Medial Scalene

Body weights and lung volumes. Final body weights were not different between the groups (Emp = 215.0 ± 7.1 g; Ctl = 212.1 ± 7.5 g). Maximum lung volume was significantly increased (182%) in Emp animals compared with Ctl (Emp = 18.6 ± 1.4 ml; Ctl = 10.2 ± 0.2 ml; P < 0.01). The pressure-volume curve was shifted up to the left in Emp animals.

EMG studies. During resting ventilation, although no EMG activity was evident in the medial scalene of Ctrl animals (Fig. 4A), inspiratory EMG activity of the scalene, in phase with the costal diaphragm activity, was consistently documented in all nine Emp animals. The lowest medial scalene EMG activity in an Emp animal is depicted in Fig. 4B, and the highest EMG activity from another Emp animal is depicted in Fig. 4C, thus providing a sense of the range of activities recorded during quiet breathing. Thus primary-like inspiratory activity of the medial scalene was evident in all the Emp animals. No correlation was found between the amplitude of EMG activity of the medial scalene during eupnea in Emp hamsters and the degree of lung hyperinflation.

Contractile and fatigue properties. There was no significant impact of Emp on the L∞ of the scalene muscle. Furthermore, no differences were observed between Emp and Ctl hamsters with regard to twitch characteristics and P∞ of the scalene (Table 2). No differences in the force-frequency relationships were observed between the groups (Fig. 5). The fatigue resistance of the scalene was not affected by the presence of Emp (Table 2).

MHC phenotype. MHC isoforms were identified by SDS-PAGE and determined from the whole medial scalene (Fig. 6). In Emp animals, there was a significant increase (15%) in the relative proportion of MHC2A isoform (P < 0.05), with a concomitant reduction (14%) in the proportion of MHC2X isoform (P < 0.05) in the medial scalene, compared with Ctl (Fig. 7).

Fiber type proportions and fiber CSA. The proportion of type IIa fibers in the medial scalene significantly increased by 6% in Emp hamsters compared with Ctl (P < 0.05; Fig. 8A). In addition, the proportion of type IIx scalene fibers was significantly reduced by 9% with Emp (P < 0.01; Fig. 8A). The CSA of type IIx
scalene fibers of the Emp hamsters was reduced by 23% compared with Ctl animals (P < 0.05; Fig. 8B). A tendency for type IIa fiber atrophy (12%; P = 0.2) was also evident. The combination of fiber atrophy and reduced proportion of type IIx fibers resulted in a significant 13% decrement in their relative contribution to total muscle area (P < 0.01; Fig. 8C). In contrast, the relative contribution of type IIa fibers to total muscle area significantly increased by 8% (P < 0.05; Fig. 8C), whereas there was a trend for a small 5% increase in the contribution of type I fibers to total muscle area (P = 0.06; Fig. 8C).

Fiber SDH activity. The activity of SDH within individual types I, IIa, and IIx fibers of the medial scalene significantly increased by 53, 63, and 54%, respectively, in the Emp group compared with Ctl animals (P < 0.05; Fig. 9).

DISCUSSION

This study examined the functional, cellular, and biochemical impact of elastase-induced Emp on the medial scalene muscle in the hamster. With Emp, the medial scalene exhibited a primary-like inspiratory EMG activity during eupnea, compared with its accessory role in Ctl animals. Although L0 and isometric contractile properties of the scalene were unaffected by Emp, there was evidence of muscle fiber conversion from type IIx to IIa, with the same direction of change noted for MHC isoforms (i.e., increased proportion of MHC2A and decreased MHC2X). The CSA of type IIx scalene fibers of Emp animals was reduced, whereas the activity of SDH was significantly increased for all fiber types.

Functional Activity of the Medial Scalene

With the imposition of the Emp state, the function of the medial scalene changed from an accessory role (16)
to one in which phasic recruitment was now evident during resting ventilation. Thus the muscle now exhibited a primary-like inspiratory function. We interpret this as a response to the chronic persistent loads imposed on the respiratory pump by Emp and/or the increased reliance placed on the chest wall and neck muscles. This increased scalene activation may be due to the mechanical inefficiency of the diaphragm in the presence of hyperinflation with Emp, despite diaphragm adaptations aimed at preserving its function (12, 22, 27). Similarly, increased reliance on scalene activity was observed with unilateral phrenicotomy in Ctl hamsters, as demonstrated by phasic recruitment during eupnea, thus compensating for partial inactivation of diaphragm function (16).

Furthermore, in patients with COPD, inferences have been made of increased contribution of rib cage and/or neck muscles based on gastric and pleural pressure swings at rest or with exercise (29, 31). Gandevia and co-workers (17) provided more direct evidence of inspiratory rib cage and neck muscle recruitment in patients with COPD. Compared with control subjects, the discharge frequencies of single motor units in the scalene and parasternal muscles were significantly increased in patients with COPD, despite enhanced neural drive to the diaphragm, supporting the postulate that mechanical factors impairing diaphragm action place a greater demand on other inspiratory muscles (7). Furthermore, with improvement in diaphragm geometry and performance 3 mo after lung volume reduction surgery for Emp, data implying the reduced need for rib cage and neck muscle recruitment were reported (24).

In the present study, no correlation between the EMG amplitude and the degree of lung hyperinflation was observed. Although intuitively one might expect such a relationship between these variables, a number of technical factors potentially could have influenced the amplitudes of the EMG signals obtained in Emp animals (e.g., absolute distance between implanted EMG electrodes, precise location of implantation and variance in muscle size between animals, etc.). Every attempt was made to control for the above variables. Extreme care was exercised in the placement of EMG electrodes. Sampling of activity was from the same general area in all animals. This was deemed especially important in view of the gradient in the distribution of fibers noted from the anterior toward the lateral aspects of the medial scalene muscle. We previously extensively evaluated the specificity of our EMG signals (16). Specifically, we performed detailed experiments to rule out cross contamination from other rib cage muscles, including selective denervation of the medial and ventral portions of the scalene during provocative challenges shown to promote scalene muscle recruitment in Ctl hamsters (16). On the basis of our

| Table 2. Effect of emphysema on isometric contractile and fatigue properties of the medial scalene |
|---------------------------------------------------|---------------------------------------------------|
|                                         Control |                                         Emphysema |
| L₀, mm                                    | 21.4 ± 0.7                                      | 21.2 ± 0.5                                      |
| CT, ms                                    | 67.4 ± 3.7                                      | 64.5 ± 7.8                                      |
| RT₁₂, ms                                  | 75.0 ± 5.2                                      | 73.9 ± 10.6                                     |
| P₀, N/cm²                                 | 7.4 ± 0.5                                       | 7.1 ± 0.5                                       |
| P₀, N/cm²                                 | 20.9 ± 0.8                                      | 19.2 ± 0.9                                      |
| P₀/P₀                                     | 0.37 ± 0.02                                     | 0.37 ± 0.03                                     |
| FI                                         | 0.42 ± 0.02                                     | 0.47 ± 0.02                                     |

Values are means ± SE.
prior extensive validation studies, we are confident that the EMG signals in the present study are specific for recruitment of the medial scalene muscle in the Emp animals.

Scalene Muscle Adaptations with Emp

$L_0$ and isometric contractile properties. In the present study, $L_0$ of the lateral segment of the medial scalene was unaffected by Emp. This contrasts with a reduction in diaphragmatic $L_0$ reported in Emp hamsters (i.e., length adaptation) (12, 22, 27). In the dog, it was reported that scalene muscle length measured sonomicroscopically at functional residual capacity corresponded to about 85% of $L_0$ (11). With an increment in lung volume to total lung capacity, the scalene shortened only slightly (~5%) compared with length at functional residual capacity. Although the anatomic and geometric configurations of the scalene differ between the dog and the hamster, and the precise degree of shortening in the hamster scalene may well have been greater, it is likely that range of shortening of the scalene in the hyperinflated Emp hamster provided an insufficient stimulus for length adaptation/remodeling (e.g., a reduction in the number of sarcomeres in series).

The contractile and fatigue properties of the scalene were similar between Emp and Ctl animals. This finding differs from changes noted in the diaphragm of Emp hamsters in which reduction in specific force has been reported in some studies (25, 27, 43) together with improved fatigue resistance (13, 25, 27). The contractile data observed in the scalene of Emp animals were not surprising given that the relative contribution of type I fibers to total area of the medial scalene increased by only ~5%. Furthermore, these data demonstrate the discordance between improved oxidative capacity of muscle fibers and fatigue resistance when tested at the whole muscle level (10). This also highlights the limitations and nonspecificity of various fatigue paradigms to assess fatigue resistance. The fatigue test employed in the present study was developed to evaluate mechanical and/or metabolic factors responsible for fatigue, while deemphasizing the influences of neuromuscular junction or axonal branch point failure (2).

Fiber type and MHC phenotype. In the present study, an increased proportion of type IIa fibers were noted in the scalene of Emp animals with a concomitant decrease in the proportions of IIx fibers. Such a change is compatible with an “endurance training” effect in which fiber conversion from lower oxidative (IIb/x) to high-oxidative (type IIa) fibers has been described for both limb muscles (19–21) as well as the diaphragm (28). A similar direction of change was noted for MHC phenotype in the medial scalene of Emp animals. This type of directional change in MHC phenotype was previously reported in analyses of limb muscle biopsies after either endurance or resistive training (23). No alterations in the proportions of MHC1 isoforms or in the proportions of type I fibers were observed in these reports or in the present study, compared with experimental paradigms involving more stringent nonphysiological stimuli, e.g., chronic electrical stimulation (33). In the present study, the overall direction of change for both fiber types and MHC isoform proportions in the medial scalene reflects the increased neuromuscular activation (i.e., EMG activity) of the scalene with Emp. It is of interest to note that similar changes for both fiber-type proportions and MHC phenotype were also observed in the diaphragm of Emp animals compared with Ctl hamsters (unpublished observations).

Fiber size. Atrophy of type IIx fibers of the medial scalene was noted in Emp hamsters with a trend for reduced fiber size for other fiber types. By comparison, most studies evaluating diaphragm adaptations to Emp reported hypertrophy of fibers (22, 27, 42). Several postulates for fiber atrophy in the Emp scalene are of interest. First, the impact of endurance training on muscle fiber size has been variably reported in the...
literature (21) with some studies (e.g., 14) reporting no impact and others reporting atrophy of limb muscle fibers (3). With regard to the respiratory muscles, it is of interest to note that reduced diaphragm fiber CSA was reported in the rat after intense endurance running (34) and in the hamster after swimming (36). The observations of the present study may thus be compatible with an endurance training effect on the scalene muscle in Emp hamsters. Second, scalene fiber atrophy may relate to reduced activity and postural use of the muscle in Emp animals because of breathlessness. However, Mattson and Poole (30) recently reported activity levels in Ctl and Emp hamsters to be similar. This would tend to rule out reduced postural activity of the scalene as an important potential mechanism for fiber atrophy. However, if activity level had decreased, one would have expected a reduction in muscle fiber oxidative potential, not the increase observed in the present study. Lastly, with the transition from type IIx to IIa fibers in the Emp scalene, one would expect a matching reduction in fiber size. Whatever the mechanism(s), we speculate that atrophy may enhance the efficiency of muscle contraction by reducing distances for substrate diffusion.

SDH activity. Significant increments in oxidative capacity were observed for all fiber types of the medial scalene of Emp animals compared with Ctl. Similar changes were previously reported for the diaphragm of Emp hamsters (13, 25, 27). Furthermore, increased SDH activity and capillarity were reported for rat diaphragm fibers after endurance exercise training (34) or in hamsters with Emp (25, 27). The increments in oxidative capacity, mitochondrial proteins, and capillarity are well-established sequelae of endurance training (19, 20). Thus, in keeping with some of the other data discussed above, the increments in SDH activity in scalene fibers of Emp hamsters likely reflect adaptations associated with enhanced recruitment of the scalene muscle in the Emp state.

Fig. 8. Fiber-type proportions (A), cross-sectional areas (CSA; B), and relative contribution of each fiber type to total muscle area (C) in medial scalene of Emp and Ctl animals. Note: Emp animals show a significant increase in proportions of type IIa fibers with a concomitant decrease in type IIx fibers and significant atrophy of type IIx fibers. This resulted in an increased contribution of type IIa fibers to scalene area and in a reduced contribution of type IIx fibers to muscle area in Emp animals. Values are means ± SE. *Significantly different from control valves, P < 0.05.

Fig. 9. Succinate dehydrogenase (SDH) activity within individual fibers of medial scalene in Emp and Ctl animals. Note significant increase in SDH activity in all fiber types with Emp. Values are means ± SE. *Significantly different from control values, P < 0.05.
In summary, with the induction of Emp in the hamster, the functional activity of the scalene muscle changes from subserving solely an accessory role to functioning as a primary-like inspiratory muscle. The transition of fiber types and MHC isoform proportions (type IIX → type IIa; MHC2x → MHC2a), reduction in the CSA of type IIX fibers, and increased activity of SDH in all fibers of the medial scalene of Emp hamsters likely reflects adaptations to enhanced phasic recruitment. However, differences between the adaptations of the diaphragm and scalene muscles with elastase-induced Emp suggest that, in addition to the load compensatory factors described above, factors intrinsic to muscle anatomy, geometric configuration, architecture, and chest mechanics play important roles in the plasticity of respiratory muscles.

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