Histochemical properties of upper airway muscles: comparison of dilator and nondilator muscles

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ABSTRACT: The upper airway dilator muscles (UADMs) represent a subgroup of muscles in the pharyngeal area which, in addition to their roles in mastication, vocalization, etc., also have an important respiratory function. Failure of these muscles to maintain upper airway patency during sleep is important in the development of the obstructive sleep apnoea syndrome. In the present study, we evaluated the histochemical properties of the UADMs and compared them to those of neighbouring muscles without respiratory functions, and to the diaphragm, to determine whether the UADMs are specifically adapted to their respiratory role.

Our results, both in dogs and rats, indicate that the dilator and nondilator upper airway muscles are similar and differ from the diaphragm. In rats, there were significantly less type I fibres (<12% as compared to 42% for the diaphragm) and more type IIb fibres (39–67% as compared to 27% for the diaphragm). A similar pattern was seen in dogs: type I fibres <38% as compared to 46% for the diaphragm, and type IIb fibres, 29–35% as compared to 10% for the diaphragm.

These findings suggest that the upper airway dilator muscles are not specifically designed for their respiratory role. They may fail in the presence of increased loads, often encountered in patients with obstructive sleep apnoea, unless appropriate adaptive structural changes take place.

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The pharyngeal area is surrounded by a large number of muscles that are involved in a variety of tasks, including mastication, swallowing, vocalization and breathing. One of the more important roles of a subset of these muscles, the upper airway dilator muscles (UADMs), is to improve pharyngeal patency to airflow. These muscles participate in respiration to prevent upper airway collapse when intrapharyngeal pressure becomes negative during inspiration, and their activity increases proportionally with increased respiratory effort [1–5]. UADM hypotony during unconsciousness may cause suffocation, and insufficient force produced by UADM contraction, to overcome collapsing forces during sleep, has been implicated in the pathogenesis of snoring and the obstructive sleep apnoea (OSA) syndrome [1–5].

In view of the central role of the UADMs in breathing, surprisingly little is known about their structure and function in health and disease, and the relevance of their structure in the pathogenesis of OSA. Previous studies have shown that the UADMs are faster and, hence, less fatigue-resistant than the diaphragm [6, 7]. This is appropriate for the usual pattern of activation under normal circumstances, since the UADMs are intensely activated during mastication and swallowing, but generally exhibit only low-intensity activation during the inspiratory phase. It is likely that UADM function and structure plays an important role in the development and severity of OSA. For example, when detrimental anatomical variations are present in the upper airway, as commonly observed in patients with

OSA [8, 9], the respiratory demands on the UADMs are substantially increased [10, 11], and these muscles may approach the limit of their endurance capabilities.

The UADMs whose respiratory role has been best documented are the genioglossus, geniohyoid, sternohyoid and sternohyoid [1, 2]. The purpose of this study was to examine whether the UADMs are specifically adapted to their respiratory role. We hypothesized that, if such adaptation does exist, the UADMs should exhibit some common properties that differ from neighbouring masticatory muscles that are not involved in respiration. Also, unlike nonrespiratory pharyngeal muscles, they should show some similarities to the inspiratory muscles. Therefore, the histochemical properties and fibre size of UADMs were compared to those of neighbouring pharyngeal muscles not involved in respiratory activity and pharyngeal dilatation, and to the diaphragm. In addition, the UADMs were compared to the diaphragm in rats and dogs, to evaluate whether differences observed between dilator and inspiratory muscles may depend on the mode of mastication in herbivores and carnivores.

Methods

Muscles

The study was performed on muscles taken from eight mongrel dogs and from eight 6–9 month old Wistar rats. In the dogs, samples of the costal diaphragm and the following UADMs were used: sternohyoid (SH), geniohyoid (GH) and genioglossus (GG). In the rats, a strip

of the costal diaphragm, the whole SH, and all of the mouth-floor muscles were extracted *en bloc*, including UADM (GH and GG), and additional muscles which do not dilate the pharynx: the extrinsic tongue muscles (T), mylohyoid (MH) and digastricus (DG) (fig. 1).

Histochemistry

All muscles were embedded in Tissue-Tec mounting medium (Miles Scientific, Naperville, IL, USA) and deep-frozen immediately after sampling, in isopentane at -70°C cooled in liquid nitrogen. Tissue was stored in a -70°C freezer and sectioned ($8\text{ }\mu\text{m}$ thick) on a cryostat (-40°C). Serial sections were stained for myosin adenosine triphosphatase (ATPase) after alkaline pre-incubation (pH 9.4) and for succinate dehydrogenase (SDH) [12, 13]. To determine myosin ATPase activity, slides were incubated in formalin buffer for 2 min, rinsed with distilled water and preincubated in a CaCl_2 -glycine solution (pH 9.4) for 10 min. After rinsing with distilled water, slides were incubated for 30 min in an adenosine triphosphatase (ATP) substrate solution (pH 9.4, 37°C). After rinsing, slides were washed in 1% CaCl_2 (3 min), and CoCl_2 (3 min), rinsed with distilled water and dipped in 10% ammonium sulphide (1 min), rinsed, dehydrated, and mounted under coverslips with glycerine jelly. To determine SDH activity, slides were incubated at 37°C for 25 min in a solution containing Tris buffer (pH 7.4), sodium succinate and nitro blue tetrazolium. After thorough rinsing, slides underwent fixation in 10% formalin solution.

Muscle fibres were classified according to conventional criteria (type I: SDH++, ATPase-; type IIa: SDH+, ATPase+; type IIb: SDH-, ATPase++). Scoring was performed using photographs taken from equal areas of neighbouring cross-sections, and 200 fibres were counted for each muscle. The scores were then pooled, and the relative distribution of the fibre types was calculated.

Fig. 1. — Histochemical localization of succinate dehydrogenase (SDH) activity in transverse section of the tongue and sublingual region in rat. a: glands; b: tongue muscles; c: genioglossus; d: geniohyoid; e: mylohyoid; f: digastricus.

ed. Mean cross-sectional area (mean of 50 muscle fibres) for each fibre type was determined using a computer-based image analysis system (Olympus Cve-2 Image Analysis System; Olympus Corp., Lake Success, NY, USA) with Morphometry software.

Data analysis

Data are presented as mean $\pm$ SEM. Significance of differences between muscles and between groups was examined by analysis of variance (ANOVA). A Bonferroni correction was applied for repeated comparisons. A p-value less than 0.05 was considered statistically significant.

Results

Muscle fibres

The distribution of muscle fibres among the various muscles in the dogs is presented in table 1. The GG and GH differed significantly from the diaphragm, containing approximately half as many type I and three times as many type IIb fibres. The SH was intermediate in its composition, with each fibre type constituting about a third of the total.

A similar pattern of distribution was observed for the corresponding rat muscles (table 2). Common to all rat upper airway muscles, there was a significantly lower type I (5–12%) and higher type IIb fibre content (39–67%) when compared to the diaphragm (42 and 27%, respectively). There were no special characterizing features of the upper airway dilator muscles (GG, GH and SH) when compared to those upper airway muscles that

Table 1. — Distribution of fibre types in various muscles in dogs

	Distribution %		
	I	IIa	IIb
Dilators			
Genioglossus	24 $\pm$ 3 ^{bc}	47 $\pm$ 3 ^b	29 $\pm$ 3 ^c
Geniohyoid	24 $\pm$ 2 ^{bc}	41 $\pm$ 2	35 $\pm$ 2 ^c
Sternohyoid	38 $\pm$ 4	31 $\pm$ 5	31 $\pm$ 3 ^c
Diaphragm	46 $\pm$ 2	44 $\pm$ 2	10 $\pm$ 1

Values are presented as mean $\pm$ SEM. I: type I fibres; IIa: type II fibres; IIb: type IIb fibres. Letters indicate significant difference ($p < 0.05$) compared to other muscles. b: compared to sternohyoid; c: compared to diaphragm.

Table 2. — Distribution of fibre types in various muscles in rats

	Distribution %		
	I	IIa	IIb
Dilators			
Genioglossus	11 $\pm$ 2 ^c	50 $\pm$ 2 ^{abd}	39 $\pm$ 2 ^{abc}
Geniohyoid	12 $\pm$ 2 ^c	35 $\pm$ 2 ^{ef}	54 $\pm$ 3 ^{bef}
Sternohyoid	5 $\pm$ 2 ^d	28 $\pm$ 2 ^{def}	67 $\pm$ 2 ^{cdef}
Nondilators			
Tongue	11 $\pm$ 1	44 $\pm$ 2	45 $\pm$ 2
Mylohyoid	7 $\pm$ 2	49 $\pm$ 2	44 $\pm$ 2
Digastricus	8 $\pm$ 1	52 $\pm$ 2	40 $\pm$ 3
Diaphragm	42 $\pm$ 1 ^{def}	31 $\pm$ 2 ^{def}	27 $\pm$ 2 ^{def}

Values are presented as mean $\pm$ SEM. Letters indicate significant difference ($p < 0.05$) compared to other muscles. d: compared to tongue; e: compared to mylohyoid; f: compared to digastricus. See legend to table 1 for further definitions.

Table 3. — Cross-sectional area of fibre types in various muscles in dogs

	I	Distribution IIa %	IIb
Dilators			
Genioglossus	1742±221 ^b	1635±207 ^{ab}	2816±421 ^b
Geniohyoid	2318±243 ^b	2998±252 ^b	4102±435 ^b
Sternohyoid	3989±319 ^c	5353±513 ^c	6239±528 ^c
Diaphragm	2304±164	2308±171	3429±262

Values are presented as mean±SEM. Letters indicate significant difference ($p<0.05$) in fibre areas compared to other muscles. See legends to tables 1 and 2 for definitions.

Table 4. — Cross-sectional area of fibre types in various muscles in rats

	I	Distribution IIa %	IIb
Dilators			
Genioglossus	635±25 ^{abcf}	849±40 ^{abcf}	1262±66 ^{abcf}
Geniohyoid	1559±129 ^{cde}	2273±167 ^d	4469±274 ^{def}
Sternohyoid	1721±224 ^{cde}	2481±156 ^d	4165±352 ^{de}
Nondilators			
Tongue	682±45 ^f	930±63 ^f	1379±87 ^f
Mylohyoid	922±140 ^f	1615±325	1480±166 ^f
Digastricus	1702±157	2143±209	3241±258
Diaphragm	2337±107 ^{def}	2476±285 ^d	4390±338 ^{def}

Values are presented as mean±SEM. Letters indicate significant difference ($p<0.05$) in fibre areas compared to other muscles. See legends to table 1 and 2 for definitions.

do not dilate the pharynx (T, MH and DG). The GG was similar to the nondilator muscles, while the GH and SH were characterized by a significantly higher content of type IIb fibres.

Comparison of rat and dog muscles revealed that the upper airway muscles of the dogs were richer in type I but had less type IIb fibres compared to rats. The diaphragm showed a similar distribution of type I and II fibres, but the diaphragm of dogs had more IIa and less IIb fibres as compared to the diaphragm of rats.

Mean cross-sectional area

Muscle fibre areas for the dogs are presented in table 3. As expected, type IIb fibres had the largest area, while type I fibres were similar or smaller than type IIa fibres. Areas of diaphragm fibres were similar to those of GG and GH, while all SH fibres were significantly larger.

Muscle cross-sectional areas for the rats are shown in table 4. Here too, the IIb fibres for all muscles (except MH) were the largest and the type I fibres the smallest. In the rats, however, a wide variability in fibre size was found. The GG, like the T and MH, had the smallest fibres. Type II fibres in all other muscles, including the diaphragm, were similar in size, but the diaphragm had the largest type I fibres.

Comparison of the fibre areas in transverse section of dogs and rats demonstrated that the upper airway muscle fibres, but not the diaphragm, of the dogs, were significantly larger than those of the rats.

Discussion

The results of this study indicate that the main UADMs, which participate regularly in respiratory activity, differ significantly in their histochemical properties from the main respiratory muscle, the diaphragm, both in dogs

and rats. In fact, these muscles are structurally identical to those of neighbouring upper airway muscles that do not dilate the pharynx and have no respiratory role.

The role of the UADMs in promoting pharyngeal patency has received much attention, since recognition of their importance in the pathogenesis of snoring and OSA [3, 4]. OSA is a common condition [14], which is associated not only with disordered breathing and chronic fatigue, but also with hypertension and excess cardiovascular mortality [15, 16]. Although a large number of anatomical deformities have been implicated in the pathogenesis of OSA [8, 9, 14], the importance of the functional, neuromuscular component is obvious, since this disorder occurs only during sleep, and is promptly terminated by arousal.

The pharynx is the only portion of the large airways which lacks rigid support, as its anterior wall is surrounded only by soft tissue. The negative pressure generated during inspiration tends to narrow and collapse the pharynx. The UADMs counteract these forces by promoting pharyngeal dilation and stability [1–5, 17, 18]. The activity of the UADMs is modulated by the respiratory cycle and is enhanced by chemical and mechanical perturbations that stimulate breathing [1, 2, 19], thus facilitating increased airflow. During sleep or unconsciousness, however, the decrease in striated muscle tone reduces the muscular support of the pharyngeal area, allowing passive prolapse of the tongue and the soft palate, and, hence, obstruction to airflow.

The respiratory muscles, both the diaphragm and the intercostals, are mixed muscles, characterized by a relatively high percentage of type I fibres [20, 21]. Type I fibres are "slow oxidative" fibres, characterized by high mitochondrial, myoglobin and oxidative enzyme density, and low glycolytic activity. Their metabolism is mainly aerobic, resulting in slow twitch response and low force production, but high endurance and resistance to fatigue. These capacities are important for the diaphragm, whose inspiratory function is not only to lower the central aponeurosis but also to expand the lower rib cage.

The need to continuously overcome elastic and resistive loads during breathing dictates an appropriate muscle composition with a sufficiently high type I fibre ratio for the respiratory muscles. This histochemical profile is absent in the UADMs. Their relatively fast histochemical profile is common both for dogs and rats. This indicates that the difference between UADMs and the diaphragm is not related to a specific mode of mastication of carnivores or herbivores, which could be responsible for other differences observed in this study between the UADMs of the two animal species. Apparently, under normal circumstances, UADMs exhibit only low intensity inspiratory activation, and may not be specifically adapted for increased, chronic respiratory loads, which require high endurance.

Our failure to find histochemical characteristics specific to the UADMs does not necessarily indicate that these muscles are unable to perform the increased demands for respiratory work imposed by the anatomical abnormalities of OSA. Firstly, histochemical properties provide only partial characterization of the physiological properties of muscles. A large number of other parameters, including enzyme profile and metabolic properties, are known to affect muscle performance. For example,

electrophoretic and immunochemical techniques have made it possible to identify the fatigue-resistant myosin heavy chain 2X isoforms. A high percentage of these 2X myofibres (which appear as IIB fibres with standard histochemical technique) was found in the GG [22], and this may explain the high endurance reported for this muscle [7] despite its high content of IIB fibres. In addition, skeletal muscles are known to change their fibre composition in response to different functional demands. This adaptive plasticity enables muscle fibres to switch from one type to another, in response to altered chronic mechanical needs. Hence, even if UADMs are not designed *a priori* to overcome increased respiratory loads, they may undergo appropriate adaptive changes in OSA patients.

The increased force production, anaerobic enzyme activity and fibre cross-sectional area observed in the musculus uvulae of OSA patients as compared to snorers was interpreted as being secondary to chronic hyperstimulation [23]. Similarly, a higher proportion of IIA (and lower proportion of IIB) fibres was observed both for the musculus uvulae and the GG in OSA patients compared to snorers [23, 24]. Mechanical, enzymatic and histochemical parameters were found to correlate with upper airway collapsibility, suggesting that contractile properties were modulated by the load of increased upper airway collapsibility in OSA patients [25]. Increase in type IIA fibres in snorers compared to controls was also found for the pharyngeal constrictor muscle [26], and the authors found it difficult to determine whether these changes were primary or secondary to snoring. Similarly, findings observed in the palatum of patients and upper airway muscles of bulldogs with OSA were considered to represent myopathic changes secondary to chronic loading [27, 28].

The findings of the present study corroborate previous observations concerning some of the upper airway dilator muscles [6, 7, 26–29]. In addition, this study is the first to compare respiratory upper airway dilator muscles to neighbouring upper airway muscles not associated with breathing. The marked similarity between all these muscles, as opposed to the diaphragm suggests that the burden of the respiratory task placed upon the upper airway dilator muscles during normal breathing is rather low, compared to other functions common to all peripharyngeal muscles.

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