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Effect of almitrine on upper airway muscle contraction in young and old rats

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Abstract

The effects of almitrine on the contractile properties of isolated geniohyoid and sternohyoid muscles were determined in physiological salt solution at 30°C in young and old rats. In young rats, almitrine had no effect on twitch or tetanic tension, twitch:tetanic tension ratio, contractile kinetics, active or passive tension–length relationships or frequency–tension relationship in both muscles. Almitrine significantly increased resistance to fatigue in both muscles. In old rats, almitrine had no effect on twitch or tetanic tension, twitch:tetanic tension ratio, contractile kinetics, active or passive tension–length relationships, frequency–tension relationship or fatigue in both muscles. These results show that almitrine, in both young and old rats, has no effect on most of the contractile properties of isolated geniohyoid and sternohyoid muscles. However, almitrine increases resistance to fatigue in both muscles in young but not in old rats.

Keywords: Almitrine; Geniohyoid; Sternohyoid; Airway, upper; Isolated muscle; Contractile properties; Age; Fatigue

1. Introduction

Almitrine is a potent respiratory stimulant active in a variety of animals including the rat (Dhillon and Barer, 1982; O'Halloran et al., 1996), the rabbit (Rhoumy and Leitner, 1981), the awake (Weese-Mayer et al., 1986) and anaesthetized cat (Maxwell et al., 1988) and dog (Bisgard, 1981). It causes increases in ventilation as a result of stimulation of the peripheral arterial chemoreceptors, the carotid and aortic bodies (Bisgard, 1981; Rhoumy and Leitner, 1981). It has been suggested that almitrine may be useful in the treatment of sleep apnea, i.e. in central apneas that relate to intermittent failure of the central respiratory drive and in obstructive sleep apnea that involves intermittent obstruction of the upper airway during sleep (McNicholas, 1990). A considerable dilative force on the upper airway muscles is necessary to reopen the obstructed airway in patients with obstructive sleep apnea (Hudgel et al., 1987). Muscle tone substantially decreases during sleep and this loss of tone in the upper airway muscles leads to upper airway collapse (McNicholas, 1990). Therefore, a respiratory stimulant such as almitrine might be expected to protect against upper airway occlusion by increasing the ventilatory drive to the upper airway muscles. Krieger et al. (1982) concluded that almitrine given orally to patients suffering from obstructive sleep apnea had no effect in reducing the number of apneic events per hour during sleep, but did reduce the mean duration of these events, especially for obstructive and mixed apneas as compared to central apneas. The cause of this effect was not determined. The patency of the upper airway is determined by a balance between the activity of the upper airway and diaphragm muscles. Contraction of the diaphragm favours collapse since it generates a negative pressure in the upper airway. However, contraction of the upper airway muscles counteracts this by dilating and/or stabilizing the upper airway (Remmers et al., 1978). Almitrine causes a preferential increase in geniohyoid compared to diaphragm electromyographic activity in anaesthetized rats (O'Halloran et al., 1996). A drug that would preferentially increase the inspiratory activity of upper airway muscles compared to the activity of the diaphragm would reduce the tendency for upper airway collapse, and thus would be beneficial in the treatment of pharyngeal obstruction. However, another possible means by which almitrine may affect upper air-

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way patency is through a direct action on muscular force and fatigue. Almitrine has been shown to improve the recovery after fatigue in the diaphragm muscle of rats (McGuire et al., 1997) but its effects on upper airway muscle contractile function have not been studied. Although the mechanism of the effect of almitrine on diaphragm fatigue is unknown, it has been suggested to be due to a free-radical scavenging effect (McGuire et al., 1997). Since antioxidant defence systems are upregulated with ageing (Oh-Ishi et al., 1996) and since the incidence of obstructive sleep apnea has a positive relationship with age (Bliwise, 1994), we also questioned whether there was a difference in the effects of almitrine between young and old rats. Another possible source of difference in the effects of almitrine on young and old rats stems from evidence that it acts on mitochondria (Mottershead et al., 1988) and that there is reduced mitochondrial function with ageing (Ozawa, 1997).

Therefore, in the present investigation, we test the hypothesis that almitrine has direct effects on upper airway muscle function and that these effects are altered by ageing by examining the effect of almitrine on the in vitro contractile properties of two upper airway muscles, the geniohyoid and sternohyoid muscles in young and old rats.

2. Materials and methods

2.1. Surgical procedure

Experiments were performed in twenty 10- to 16-week-old and twenty-four 22- to 24-month-old Wistar rats. Animals were housed at 22°C with a 12-h light/12-h dark cycle and given food and water ad libitum. Procedures were carried out in accordance with the Cruelty to Animals Act, 1876 and EU Directive 86/609/EC. Animals were anaesthetized with pentobarbitone (Sagatal, 60 mg kg^{-1} i.p.) and placed supine on a heating blanket (Harvard Homeothermic Blanket Control Unit, Harvard Apparatus, Fircroft Way, Kent, England). Core temperature was monitored continuously and maintained at 37°C. The animals were tracheostomized and artificially ventilated (Harvard Volume Controlled Rodent Respirator, Model 683, Harvard Apparatus, Pleasant Street, Natick, USA). A femoral artery and vein were cannulated with the aid of a microscope in order to record arterial blood pressure and to administer supplementary anaesthetic, respectively. The digastric muscles were located following a mid-line incision in the neck extended to the mandible. These muscles were carefully separated along a natural division that runs longitudinally to reveal the mylohyoid muscles, which are a layer of muscles that overlie the pair of geniohyoid muscles. The muscles were carefully removed to expose the underlying pair of geniohyoid muscles. These muscles run as two parallel strips of muscle bonded medially by dense connective tissue. Anatomically, the muscles run from the midpoint of the mandible to the hyoid bone. The mandibular ends separate naturally at the top. However, the hyoid bone ends have to be cut apart manually to separate the muscles wholly. Throughout the whole procedure, all of the muscles were flushed with Krebs solution to remain viable. The pair of geniohyoid muscles, with mandibular tendons relatively intact, were separated and removed carefully and placed in aerated Krebs solution. For the pair of sternohyoid muscles, they were carefully separated from the underlying tissue and from each other along a natural division. Again, throughout the dissection procedure, the muscle pair was flushed with Krebs solution. Each sternohyoid muscle was removed and placed in aerated Krebs solution.

2.2. Isolated muscle preparation

Muscles were rapidly placed in a 200 ml bath of continuously aerated Krebs solution. Longitudinal strips of approximately 1–2 mm wide were suspended vertically between a pair of platinum electrodes, with the base fixed to an immobile hook and the other end tied to a highly sensitive force transducer (Dynamometer UF1, Pioden Controls), the position of which could be adjusted using a micropositioner, thus varying the length of the muscle strip. This electrode-muscle preparation was then placed in a 125-ml, water-jacketed bath in warmed (30°C), oxygenated (95% O_2–5% CO_2) Krebs solution (pH 7.4) containing (in mM): NaCl 120, KCl 5, Ca gluconate 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.5. It has been shown that muscle strips of this width at 30°C remain reasonably stable (Segal and Faulkner, 1985). Stability is better at 25°C and poor at 37°C so that we chose 30°C as a compromise between the in vivo temperature of 37°C and the ideal in vitro temperature of 25°C. The platinum electrodes were connected to a Grass Square Pulse Stimulator (Model S48, Astro-Med, Astro-Med Industrial Park, Warwick, RI 028393). Using field stimulation (supramaximal voltage, 1 ms duration) to elicit contractions, isometric twitch tension, tetanic tension, twitch:tetanic tension ratio, contraction time, half-relaxation time, the frequency–tension and tension–length relationships and fatigue were measured using a data acquisition system (MacLab/2e, AD Instruments, NSW, Australia) and stored for later analysis on a microcomputer (Apple Macintosh LC 3, Apple Computer, CA, USA).

2.3. Protocol

Supramaximal voltage was determined by increasing the stimulation voltage by increments of 10 V up until a point where the tension generated by the muscle stopped increasing. Supramaximal voltage was 10 V above this point. An equilibration period in the bath of 45 min was allowed before any measurements were made. To determine the tension–length relationship, muscle length was

Table 1
Effects of almitrine on the contractile properties of the geniohyoid and sternohyoid muscles in 10- to 16-week-old rats

	Pt (N cm^{-2})	Po (N cm^{-2})	1/2-R.T. (s)	C.T. (s)	Pt/Po	C.S.A. (cm^2)
Geniohyoid						
Control	0.75 (0.36)	2.84 (1.89)	0.036 (0.008)	0.026 (0.003)	0.21 (0.08)	0.031 (0.010)
Almitrine	0.83 (0.27)	3.12 (1.29)	0.032 (0.006)	0.025 (0.005)	0.21 (0.06)	0.032 (0.009)
Sternohyoid						
Control	1.24 (0.81)	4.67 (1.87)	0.038 (0.003)	0.026 (0.003)	0.21 (0.05)	0.031 (0.004)
Almitrine	1.90 (0.42)	6.01 (2.02)	0.039 (0.006)	0.027 (0.003)	0.24 (0.06)	0.031 (0.005)

Values are mean (SD) for control and after addition of almitrine. Pt, twitch tension; Po, tetanic tension; 1/2-R.T., half-relaxation time; C.T., contraction time; Pt/Po, twitch:tetanic tension ratio; C.S.A., cross-sectional area.
There were no significant differences due to almitrine (ANOVA, $P < 0.05$).

changed in increments of 1 mm using the micropositioner. The passive tension–length relationship was established by recording the change in the baseline tension as the muscle length was changed. The active tension–length relationship was established by eliciting a twitch at each length and recording the tension. The optimal length of the muscle strip was taken as the length producing maximal twitch tension and the muscle was held for the remainder of the experiment at this length. This allowed isometric twitch kinetics (contraction time and half relaxation time) to be determined (see data analysis). The muscle was then stimulated at 10, 20, 30, 40, 50, 60, 80 and 100 Hz for 300 ms at each frequency and allowing a 2-min interval between each stimulus, and a frequency–tension relationship was established. Maximum tetanic tension, which is the maximum tension on the frequency–tension curve, was also determined. Ten minutes following the frequency–tension determination, fatigue was induced by stimulation of the muscle strips for a 5-min period. During this period, pulses of 300 ms duration were delivered to the muscle strips every 2 s, at a frequency of 30 Hz.

Control experiments were carried out in normal Krebs for the entire course of the experiment. The almitrine experiments were carried out in Krebs solution containing

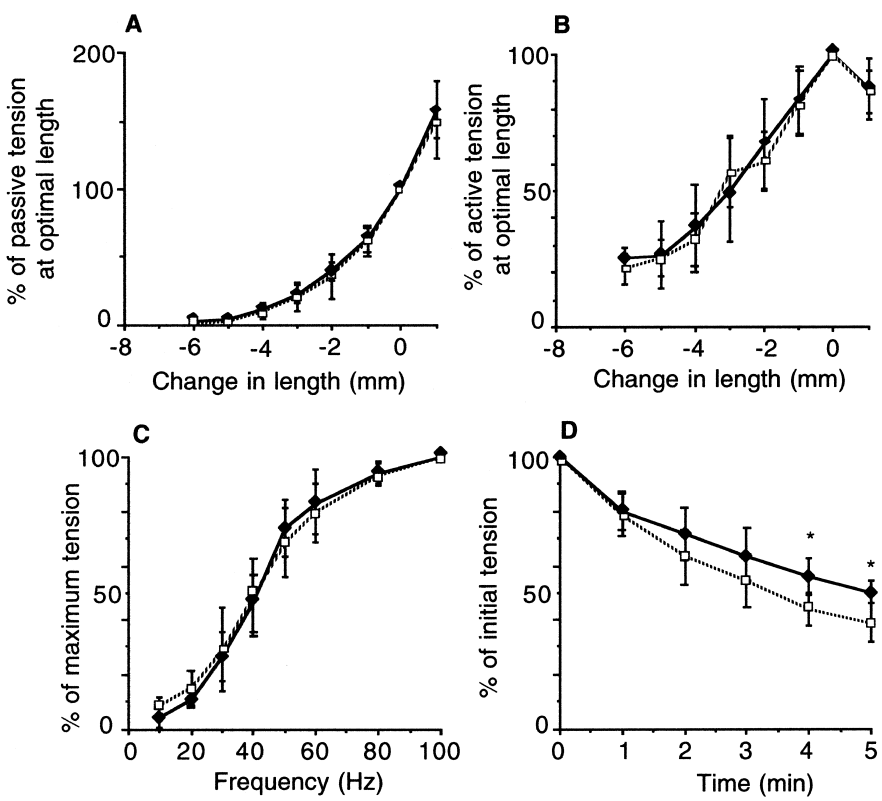

Fig. 1. Effect of almitrine on geniohyoid muscle contractile properties in 10- to 16-week-old rats on passive tension–length relationship (A), active tension–length relationship (B), frequency–tension relationship (C) and fatigue (D). Values are expressed as mean ± SD for control (■) and almitrine (◇).
* indicates significant difference from control (ANOVA, $P < 0.05$).

almitrine, where the almitrine was added into the aerated Krebs solution to achieve a final dose of 10 μg ml^{-1}. Separate muscle strips were used for the control and almitrine experiments. The muscle strips were in the Krebs-almitrine solution for a period of 45 min before any recordings were taken.

2.4. Data analysis

Specific tension was calculated in N cm^{-2} of strip cross-sectional area. Cross-sectional area was calculated by weighing the muscle strip (in grams) after removal from the bath and blotting dry and dividing this by the product of optimal length (in mm) and muscle density, which is assumed to be 1.06 mg mm^{-3}. Isometric twitch kinetics were quantified by measurements of the time to peak tension (contraction time) and the time for the peak tension to decay by 50% (half-relaxation time). For the tension–length relationship, tension values were normalized by expressing them at different lengths as a percentage of the tension value at optimum length. For the frequency–tension relationship, tension values were normalized by expressing them at different frequencies as a percentage of the maximal tetanic value. For the fatigue protocol, tension values were normalized by expressing the force generated by the first pulse of the stimulus train at 1, 2, 3, 4 and 5 min as a percentage of the value at 0 min.

These values, plus the absolute values for specific twitch and tetanic tension, and the twitch:tetanic tension ratio were expressed as mean ± SD and used to compare statistically the control muscle strips and the muscle strips with almitrine using analysis of variance (ANOVA) and Fischer's least significant difference test, with $P < 0.05$ selected as the threshold for statistical significance.

3. Results

3.1. 10- to 16-week-old group

There was no significant difference between the control and almitrine groups in muscle strip cross-sectional area for either the geniohyoid or sternohyoid muscle (Table 1). There was no significant difference between the control and almitrine groups for twitch or tetanic tension, twitch:tetanic tension ratio, contractile kinetics (Table 1), active or passive tension–length relationships or fre-

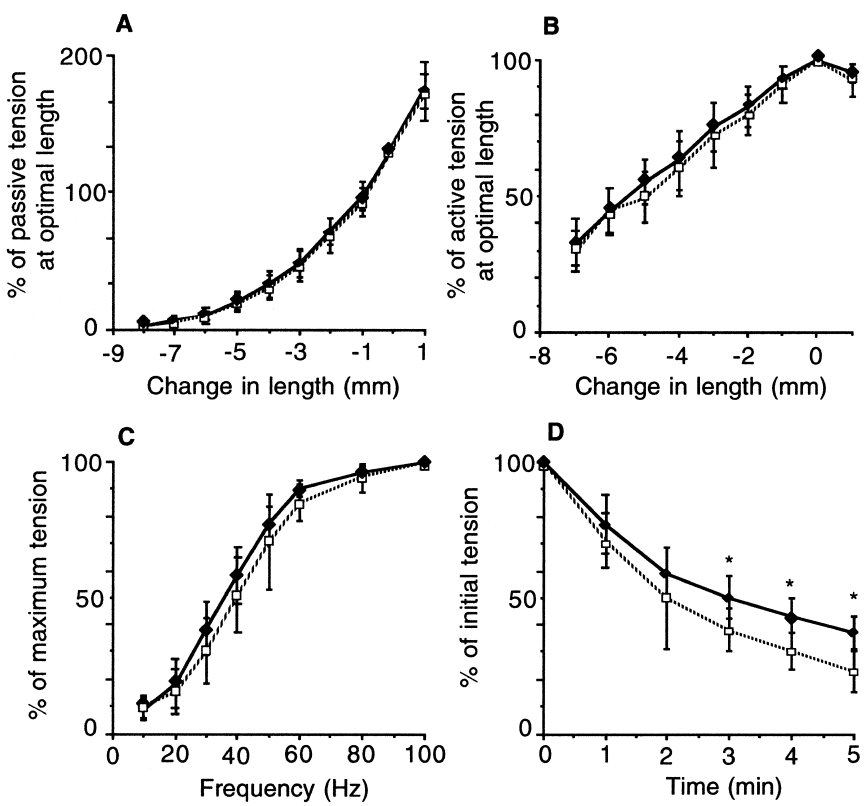

Fig. 2. Effect of almitrine on sternohyoid muscle contractile properties in 10- to 16-week-old rats on passive tension–length relationship (A), active tension–length relationship (B), frequency–tension relationship (C) and fatigue (D). Values are expressed as mean ± SD for control (■) and almitrine (◇). * indicates significant difference from control (ANOVA, $P < 0.05$).

Table 2
Effects of almitrine on the contractile properties of the geniohyoid and sternohyoid muscles in 22- to 24-month-old rats

	Pt (N cm^{-2})	Po (N cm^{-2})	1/2-R.T. (s)	C.T. (s)	Pt/Po	C.S.A. (cm^2)
Geniohyoid						
Control	0.94 (0.51)	3.88 (1.90)	0.038 (0.004)	0.027 (0.004)	0.24 (0.07)	0.030 (0.007)
Almitrine	1.26 (0.70)	5.16 (2.60)	0.038 (0.004)	0.027 (0.006)	0.24 (0.09)	0.029 (0.005)
Sternohyoid						
Control	1.04 (0.51)	4.28 (1.78)	0.038 (0.002)	0.027 (0.002)	0.21 (0.08)	0.035 (0.006)
Almitrine	1.40 (0.52)	5.06 (1.78)	0.038 (0.003)	0.028 (0.002)	0.25 (0.06)	0.034 (0.005)

Values are mean (SD) for control and after addition of almitrine. Pt, twitch tension; Po, tetanic tension; 1/2-R.T., half-relaxation time; C.T., contraction time; Pt/Po, twitch:tetanic tension ratio; C.S.A., cross-sectional area.
There were no significant differences due to almitrine (ANOVA, $P < 0.05$).

quency–tension relationship in both muscles (Figs. 1A,B,C and 2A,B,C).

Almitrine significantly improved the resistance to fatigue (ANOVA, $P < 0.05$) at 4 and 5 min of the fatigue protocol for the geniohyoid muscle (control values of $43.8 \pm 6.1\%$ and $38.9 \pm 7.1\%$, to almitrine values of $56.2 \pm 6.7\%$ and $50.1 \pm 3.8\%$, respectively), and at 3, 4 and 5 min for the sternohyoid muscle (control values of $38.2 \pm 8.0\%$, $30.1 \pm 6.8\%$ and $23.2 \pm 7.8\%$, to almitrine values of $50.2 \pm 8.0\%$, $43.6 \pm 6.3\%$ and $36.7 \pm 6.4\%$, respectively) as shown in Figs. 1D and 2D.

3.2. 22- to 24-month-old group

As in the 10- to 16-week-old group, there was no significant difference between the control and almitrine groups in muscle strip cross-sectional area for either the geniohyoid or sternohyoid muscle (Table 2). There was no

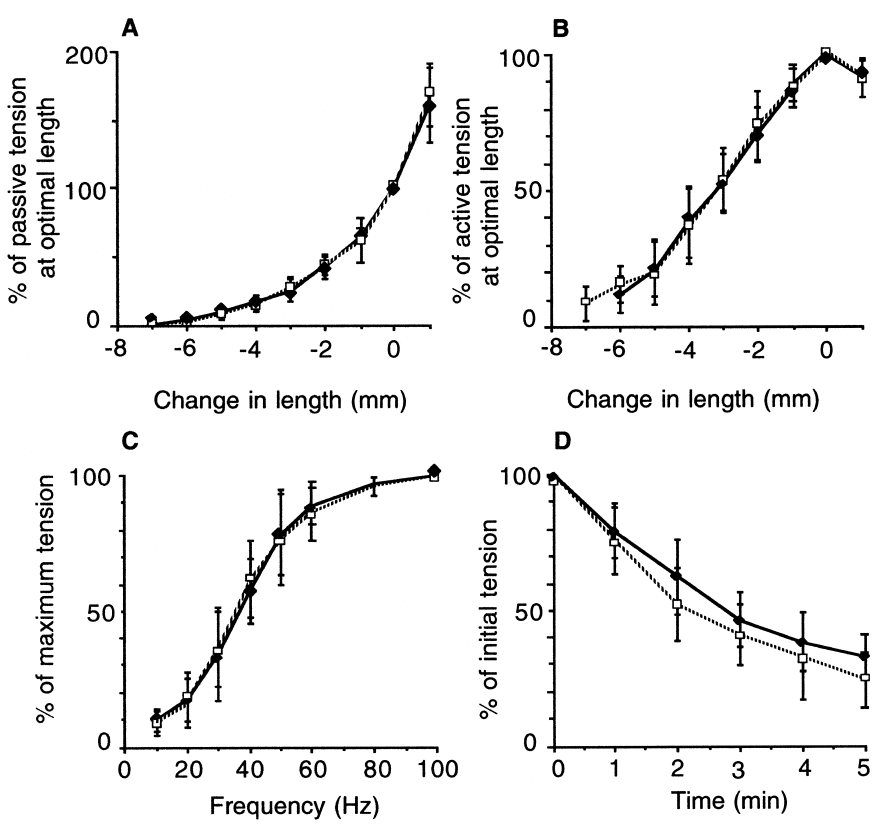

Fig. 3. Effect of almitrine on geniohyoid muscle contractile properties in 22- to 24-month-old rats on passive tension–length relationship (A), active tension–length relationship (B), frequency–tension relationship (C) and fatigue (D). Values are expressed as mean ± SD for control (■) and almitrine (◇). There was no significant difference due to almitrine (ANOVA, $P < 0.05$).

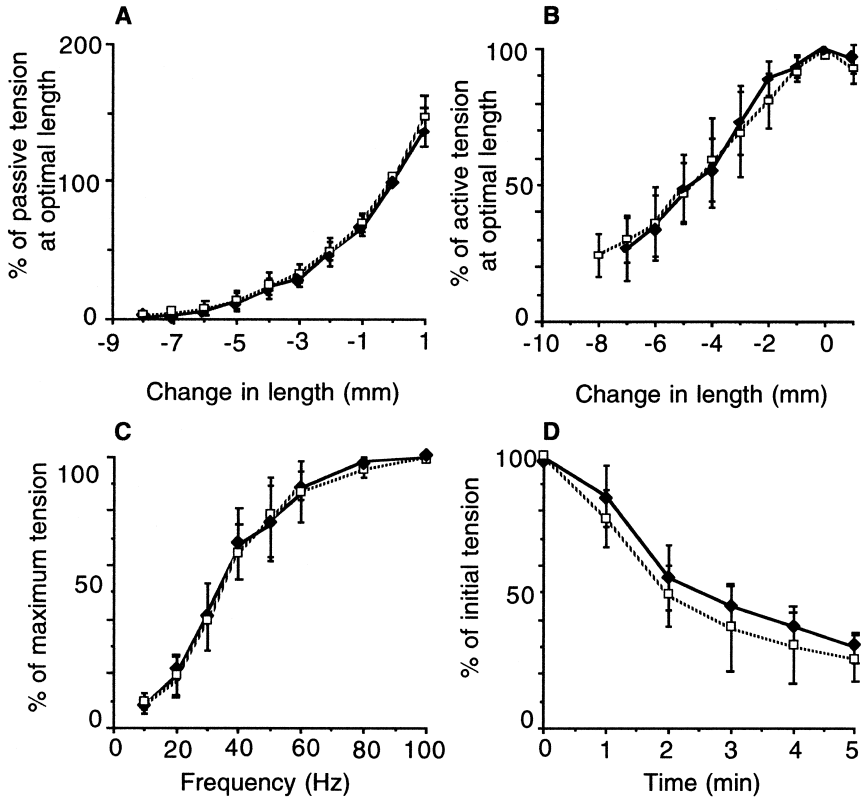

Fig. 4. Effect of almitrine on sternohyoid muscle contractile properties in 22- to 24-month-old rats on passive tension–length relationship (A), active tension–length relationship (B), frequency–tension relationship (C) and fatigue (D). Values are expressed as mean ± SD for control (■) and almitrine (◇). There was no significant difference due to almitrine (ANOVA, $P < 0.05$).

significant difference between the control and almitrine groups for twitch or tetanic tension, twitch:tetanic tension ratio, contractile kinetics (Table 2), active or passive tension–length relationships or frequency–tension relationship in both muscles (Figs. 3A,B,C and 4A,B,C). However, unlike the 10- to 16-week-old groups, almitrine had no effect on fatigue in either muscle in the 22- to 24-month-old groups (Figs. 3D and 4D).

4. Discussion

Almitrine reduces the mean duration of apneic events in obstructive sleep apnea patients (Krieger et al., 1982). The reason for this is unknown but upper airway patency is thought to be due to a balance between the dilative force of the upper airway muscles and the collapsing force produced by the diaphragm (Remmers et al., 1978; Block et al., 1984). Therefore, obstruction of the upper airway occurs when the dilating force of the pharyngeal dilator muscles is insufficient to counterbalance the negative pressure produced by the inspiratory pump muscles (Brouillette and Thach, 1979). Therefore, one possible means by which almitrine may benefit upper airway patency would be by preferential activation of upper airway compared to thoracic musculature. Almitrine causes preferential activation of the geniohyoid muscles as compared to the diaphragm in anaesthetized rats (O'Halloran et al., 1996). However, another possible mechanism by which almitrine may influence upper airway patency is through direct effects on the contractile properties of upper airway muscles. Almitrine has been shown to affect the contractile properties of rat diaphragm muscle and recovery from fatigue (McGuire et al., 1997). Therefore, in the present study, we investigated the possibility that almitrine might directly affect upper airway muscles by examining its effects on the contractile properties of two major upper airway dilator muscles, the geniohyoid and sternohyoid muscles.

Muscle strips were taken from both young (10- to 16-week-old) and old (22- to 24-month-old) rats. We looked at the effects of almitrine on muscle contractile properties from old rats because of the association of obstructive sleep apnea with age. We found that in young rats, while almitrine had no significant effect on the twitch tension, tetanic tension, twitch:tetanic tension ratios, contractile kinetics, tension–length relationships, or the tension–frequency relationships of the geniohyoid or sternohyoid muscles, almitrine did cause a significant increase in the endurance of the both muscles. The lack of effect of almitrine on contractile tension or kinetics is consistent with similar findings in rat diaphragm muscle (McGuire et

al., 1997). In the present investigation, almitrine improved the resistance to fatigue towards the end of each individual 5-min fatigue run for both the geniohyoid and sternohyoid muscles. Again, our results are somewhat consistent with those for rat diaphragm where almitrine improves the rapidity and magnitude of recovery from fatigue (McGuire et al., 1997).

In the old rats, almitrine had no significant effects on any of the contractile properties of either the geniohyoid or sternohyoid muscles. Therefore, the enhanced endurance caused by almitrine in young rats was not observed in old rats. The reason for this difference is difficult to explain since the cellular actions of almitrine are largely unknown. However, there is evidence that it acts directly on the mitochondria (Mottershead et al., 1988) and since there is evidence for reduced mitochondrial function with age (Ozawa, 1997), we speculate that the absence of an effect of almitrine in the old rats may be due to reduced mitochondrial function in the old rats. Alternatively, because reactive oxygen species are involved in the development of fatigue (Reid et al., 1992), the improved endurance in young rats may be due an antioxidant effect of almitrine (Copin et al., 1995). Since ageing is associated with upregulation of antioxidant defence systems in skeletal muscle (Oh-Ishi et al., 1996), such enhanced antioxidant defences might negate an antioxidant effect of almitrine.

Since we did not examine the dose–response relationship for almitrine and since only one dose was used, it is possible that the absence of an effect in the old animals was because an insufficient dose was used. We believe that this is unlikely because the dose of 10 $\mu g\ ml^{-1}$ was chosen to produce maximal effects. In rats, there is no dose–response relationship for the ventilatory response to doses ranging between 1 and 20 mg kg^{-1} i.v. (Dhillon and Barer, 1982). This corresponds to a plasma concentration of approximately 1–20 $\mu g\ ml^{-1}$ (McQueen et al., 1989). The phrenic and hypoglossal nerve responses to almitrine are maximal at 1 mg kg^{-1} i.v. in cats (Weese-Mayer et al., 1985). The carotid chemoreceptor response to almitrine is maximal at plasma levels of approximately 0.7 $\mu g\ ml^{-1}$ with no further response at higher doses (McQueen et al., 1989). Therefore, we considered a dose of 10 $\mu g\ ml^{-1}$ to be more than adequate to evoke maximal responses. At the same time, we did not wish to use higher doses because of the danger of toxicity and because such doses are clinically less relevant. A dose of 37 mg kg^{-1} i.v. was found to be occasionally lethal in rats (Dhillon and Barer, 1982). This corresponds to a plasma level of approximately 50 $\mu g\ ml^{-1}$. In humans, the optimal therapeutic level is approximately 0.3 $\mu g\ ml^{-1}$ (Howard, 1984) and it is well known that almitrine causes neuropathy, which is probably dose-dependent (Gherardi et al., 1987).

In conclusion, these results demonstrate that almitrine improves upper airway muscle endurance of young rats, while having no effect on the endurance of older rats. These results support the suggestion that almitrine may be beneficial in the treatment of obstructive airway conditions (Shepard et al., 1988). It has been suggested that fatigue of the respiratory muscles may be involved in these conditions. The load on the pharyngeal musculature increases with age due to the dimensions of the airway decreasing (Brown et al., 1986). This would increase the vulnerability of the upper airway muscles to fatigue, as would the fact that these muscles are more active in patients with obstructive sleep apnea compared with normal subjects (Surratt and White, 1991). Furthermore, the high proportion of fast fibres in the upper airway muscles makes them inherently vulnerable to fatigue. In the cat, severe hypoxia worsens the endurance of the geniohyoid muscle (Salomone and Van Lunteren, 1991). Hence, the propensity for pharyngeal muscle fatigue to occur may be potentially increased in obstructive sleep apnea patients with severe episodic hypoxia. Therefore, pharmacological compounds that act to inhibit muscle fatigue could be useful in combating these effects. The present results show that almitrine has a direct effect to improve upper airway muscle endurance. However, this potential benefit may have some limitations since almitrine only had this effect in young and not in old animals. Since hypoxaemia occurs during apneic events, it would be of interest to examine the interaction of almitrine and hypoxia on upper airway muscle function.

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References

Bisgard, G.E., 1981. The response of few-fibre carotid chemoreceptor preparations to almitrine in the dog. Can. J. Physiol. Pharmacol. 59, 396–401.

Bliwise, D.L., 1994. Development of sleep disordered breathing and changes in body weight over time in an elderly population. Sleep Res. 23, 234.

Block, A.J., Faulkner, J.A., Hughes, R.L., Remmers, J.E., Thach, B., 1984. Factors influencing upper airway closure. Chest 86, 114–122.

Brouillette, R.T., Thach, B.T., 1979. A neuromuscular mechanism maintaining extrathoracic airway patency. J. Appl. Physiol. 46, 772–779.

Brown, I.G., Zamel, N., Hoffstein, V., 1986. Pharyngeal cross-section area in normal men and women. J. Appl. Physiol. 61, 890–895.

Copin, J.C., Pinteaux, E., Ledig, M., Tholey, G., 1995. 70-kDa heat shock protein expression in cultured astrocytes after hypoxia: regulatory effect of almitrine. Neurochem. Res. 20, 11–15.

Dhillon, D.P., Barer, G.R., 1982. Respiratory stimulation by almitrine during acute or chronic hypoxia/hypercapnia in rats. Bull. Eur. Physiopathol. Respir. 18, 751–764.

Gherardi, R., Baudrimont, M., Gray, F., Louarn, F., 1987. Almitrine neuropathy. Acta Neuropathol. 73, 202–208.

Howard, P., 1984. Almitrine bismesylate (Vectarion). Bull. Eur. Physiopathol. Respir. 20, 99–103.

Hudgel, D.W., Chapman, K.R., Faulks, C., Hendricks, C., 1987. Changes

in inspiratory muscle electrical activity and upper airway resistance during periodic breathing induced by hypoxia during sleep. Am. Rev. Respir. Dis. 135, 899–906.

Krieger, J., Mangin, P., Kurtz, D., 1982. Effects of ventilatory stimulant, almitrine bismesylate, in the sleep apnoea syndrome. Curr. Ther. Res. 32, 697–705.

Maxwell, D.L., Hughes, J.M.B., Nye, P.C.G., 1988. The effect of almitrine bismesylate on the steady-state responses of arterial chemoreceptors to CO_2 and O_2 in the cat. Respir. Physiol. 74, 275–284.

McGuire, M., Carey, M.F., O'Connor, J., 1997. Almitrine and doxapram decrease fatigue and increase subsequent recovery in isolated rat diaphragm. J. Appl. Physiol. 83, 52–58.

McQueen, D.S., Evrard, Y., Gordon, B.H., Campbell, D.B., 1989. Ganglioglomular nerves influence responsiveness of cat carotid body chemoreceptors to almitrine. J. Autonom. Nerv. Syst. 27, 57–66.

McNicholas, W.T., 1990. Sleep apnoea. J. Ir. Coll. Phys. Surg. 19, 53–56.

Mottershead, J.P., Nye, P.C.G., Quirk, P.G., 1988. The effects of almitrine on electron transport in mitochondria isolated from rat liver. J. Physiol. (Lond.) 396, 91.

O'Halloran, K.D., Curran, A.K., Bradford, A., 1996. Effect of almitrine on ventilation and on the diaphragm and geniohyoid muscle activity in the rat. Clin. Sci. 91, 337–345.

Oh-Ishi, S., Toshinai, K., Kizaki, T., Haga, S., Fukuda, K., Nagata, N., Ohno, H., 1996. Effects of aging and/or training on antioxidant enzyme system in diaphragm of mice. Respir. Physiol. 105, 195–202.

Ozawa, T., 1997. Genetic and functional changes in mitochondria associated with aging. Physiol. Rev. 77, 425–464.

Reid, M.B., Haack, K.E., Franchek, K.M., Valberg, P.A., Kobzik, L., West, M.S., 1992. Reactive oxygen in skeletal muscle: I. Intracellular oxidant kinetics and fatigue in vitro. J. Appl. Physiol. 73, 1797–1804.

Remmers, J.E., DeGroot, W.J., Sauerland, E.K., Anch, A.M., 1978. Pathogenesis of upper airway occlusion during sleep. J. Appl. Physiol. 44, 931–938.

Rhoumy, M., Leitner, L.M., 1981. Stimulant effect of almitrine (S2620) on the rabbit carotid chemoreceptor afferent activity. Bull. Eur. Physiopathol. Respir. 17, 255–259.

Salomone, R.J., Van Lunteren, E., 1991. Effects of hypoxia and hypercapnia on geniohyoid contractility and endurance. J. Appl. Physiol. 71, 709–715.

Segal, S.S., Faulkner, J.A., 1985. Temperature-dependent physiological stability of rat skeletal muscle in vitro. Am. J. Physiol. 248, 265–270.

Shepard Jr., J.W., Gafter, W.B., Guilleminault, C., Hoffman, E.A., Hoffstein, V., Hudgel, D.W., Surratt, P.M., McTier, R.F., Wilhoit, S.C., 1988. Upper airway muscle activation is augmented in patients with obstructive sleep apnea compared with that in normal subjects. Am. Rev. Respir. Dis. 137, 889–894.

Surratt, P.M., White, D.P., 1991. Evaluation of upper airway in patients with obstructive sleep apnea. Sleep 14, 361–371.

Weese-Mayer, D.E., Brouillette, R.T., Klemka, L.M., Hunt, C.E., 1985. Effects of almitrine on hypoglossal and phrenic electroneurograms. J. Appl. Physiol. 59, 105–112.

Weese-Mayer, D.E., Brouillette, R.T., Klemka, L.M., Hunt, C.E., 1986. Effects of almitrine on genioglossal and diaphragmatic electromyograms. J. Appl. Physiol. 61, 2122–2128.