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Original Article

Salbutamol Improves Diaphragmatic Contractility in Chronic Airway Obstruction

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ABSTRACT

Introduction: Chronic airflow obstruction in conditions such as chronic obstructive pulmonary disease is associated with respiratory muscle dysfunction. Our aim was to study the effects of salbutamol—a β_2-adrenergic agonist known to improve muscle strength in physiologic and pathologic conditions—on diaphragm contractility in an animal model of chronic airway obstruction achieved by tracheal banding.
Materials and Methods: Twenty-four Sprague-Dawley rats were randomized into a control group and 3 tracheal banding groups, 1 that received acute salbutamol treatment, 1 that received chronic salbutamol treatment, and 1 that received nothing. Arterial blood gases, acid-base balance, and in vitro diaphragmatic contractility were evaluated by measuring peak twitch tension, contraction time, contraction velocity, half-relaxation time, relaxation velocity, and force-frequency curves.
Results: The 3 study groups had significantly reduced arterial pH and increased $PaCO_2$ and bicarbonate levels compared to the control group (P<.05). The untreated tracheal banding group had significantly reduced peak twitch tension and contraction velocity, and a significantly lower force-frequency curve in comparison with the other groups (P<.05). The chronic treatment group had a higher relaxation velocity than the untreated study group (P<.05). The mean (SE) peak twitch tension values were 6.46 (0.90) N/cm^2 for the control group, 3.28 (0.55) N/cm^2 for the untreated tracheal banding group, 6.18 (0.71) N/cm^2 for the acute treatment group, and 7.09 (0.59) N/cm^2 for the chronic treatment group.
Conclusions: Diaphragmatic dysfunction associated with chronic airflow obstruction improves with both the acute and chronic administration of salbutamol. The mechanisms involved in respiratory muscle dysfunction warrant further study.

El salbutamol mejora la contractilidad diafragmática en la obstrucción crónica de la vía aérea

Palabras clave:
Diafragma
Obstrucción traqueal extrínseca
EPOC
Salbutamol

RESUMEN

Introducción: Las enfermedades con obstrucción crónica de la vía aérea, como la enfermedad pulmonar obstructiva crónica, asocian alteraciones funcionales de los músculos respiratorios. Los agonistas adrenérgicos β_2 mejoran la fuerza muscular en condiciones fisiológicas y patológicas. Nuestro objetivo ha sido estudiar los efectos del salbutamol sobre la contractilidad diafragmática en un modelo animal de obstrucción crónica de la vía aérea lograda por obstrucción traqueal (OT) extrínseca.
Materiales y métodos: Se aleatorizaron 24 ratas Sprague-Dawley en 4 grupos: *a)* control; *b)* OT; *c)* OT+salbutamol agudo, y *d)* OT+salbutamol crónico. Se estudiaron los gases sanguíneos, el equilibrio ácido-base y la fuerza diafragmática in vitro, a través de las siguientes medidas: tensión máxima ($T_{máx}$), tiempo de contracción (TC), velocidad de contracción ($dT/dt_{máx}$), tiempo de relajación media ($TR_{1/2}$), velocidad de relajación ($-dT/dt_{máx}$) y curvas fuerza-frecuencia.

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Resultados: Los 3 grupos sometidos a OT presentaron una disminución significativa del pH y un incremento de la presión arterial de anhídrido carbónico y del bicarbonato en sangre arterial ($p < 0,05$). El grupo OT experimentó una disminución significativa de $T_{máx}$, de $dT/dt_{máx}$ y de la curva fuerza-frecuencia en comparación con los otros grupos ($p < 0,05$). La $-dT/dt_{máx}$ fue mayor en el grupo OT+salbutamol crónico comparado con el grupo OT ($p < 0,05$). Los valores medios (± error estándar) de $T_{máx}$ fueron: control, 6,46 ± 0,90 N/cm^2; OT, 3,28 ± 0,55 N/cm^2; OT+salbutamol agudo, 6,18 ± 0,71 N/cm^2; OT+salbutamol crónico, 7,09 ± 0,59 N/cm^2.

Conclusiones: La disfunción diafragmática asociada a obstrucción crónica de la vía aérea mejora con salbutamol administrado tanto en forma aguda como crónica. Los mecanismos involucrados en la disfunción muscular deben analizarse más profundamente.

Introduction

The force necessary for adequate ventilation is generated by the respiratory muscles, which include the diaphragm as the main inspiratory muscle. Diaphragmatic dysfunction can cause or exacerbate respiratory insufficiency. Severe structural and functional abnormalities in the diaphragm occur as a consequence of chronic obstruction of the airways,[1-5] a fact which is particularly relevant in the pathophysiology of chronic obstructive pulmonary disease (COPD). It has been demonstrated that the diaphragm in patients with COPD not only undergoes important structural, biochemical, and metabolic alterations, but also has reduced contractile force.[6-12] Little is known, however, about how to prevent or reverse diaphragmatic dysfunction. Diaphragm contractility in both physiological and pathological conditions has been demonstrated to be enhanced by β_2-adrenergic agonists like salbutamol.[13-16] Although improved contractility is fundamentally attributed to the positive inotropic effect of these drugs, their antiinflammatory, antioxidant, anabolic, and antiproteolytic properties may also play a role. The fact that diaphragm function improves in response to inhaled salbutamol in patients with COPD has been attributed to improved diaphragm loading rather than to enhanced contractility.[17] In view of the above, it is clear that the effects of salbutamol on diaphragm contractility warrant further investigation.

Our aim was to study the effects of salbutamol on diaphragm contractility in an animal model of chronic airway obstruction achieved by tracheal banding. Our hypothesis was that salbutamol increases diaphragm force in conditions of chronic airflow limitation.

Materials and Methods

Animals and Groups

A total of 24 male Sprague-Dawley rats, with a mean (SD) weight of 306 g (30 g), were studied in an experimental model of chronic airway obstruction achieved by tracheal banding applied for 7 days. The animals were distributed in 4 groups as follows: *a)* a control group (n = 6); *b)* a tracheal banding group that received no salbutamol treatment (n = 6); *c)* a tracheal banding group that received acute salbutamol treatment immediately before muscle function evaluation (n = 6); and *d)* a tracheal banding group that received chronic salbutamol treatment—administered by implanted continuous-dose miniosmotic pumps—for 7 days before muscle function evaluation (n = 6). These groups will henceforth be referred to as the control group, the untreated group, the acute treatment group, and the chronic treatment group, respectively.

Experimental Protocol

The experimental protocol for this study complied with the recommendations of the animal experimentation committee of the Universidad de la República, Montevideo, Uruguay. On the first day of the study, all the rats were administered intraperitoneal atropine (0.04 mg) to minimize airway secretions and prevent cardiac arrhythmias as a consequence of the tracheal procedures; 30 minutes later they were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). These procedures were performed in sterile conditions on a heated table, with the rats spontaneously breathing mask-administered oxygen with an inspired oxygen fraction of 1. After asepsis and local anesthesia with 2% lidocaine, a mid-anterior cervical incision was made to access the extrathoracic trachea. A 4×8-mm polyethylene cuff was fitted around the trachea of each rat in the 3 study groups, at a point located between the third and sixth cartilaginous rings. As an indirect measure of pleural pressure, esophageal pressure was calculated using a transducer (9731 P23BC; Statham, Hato Rey, Puerto Rico) connected to the lowest third of the esophagus by means of a water-filled polyethylene catheter. The polyethylene cuffs were adjusted until the tidal-volume inspiratory esophageal pressure of ventilatory effort was 50% above baseline, reflecting the significantly increased respiratory loading that occurs in conditions such as severely exacerbated asthma, COPD, and obstructive sleep apnea. Incisions were made in the tracheas of the control group rats, esophageal pressure was measured, and the incisions were closed. Alzet 2001 continuous-dose miniosmotic pumps (Alzet, San Diego, California, USA) were subcutaneously implanted in the interscapular region in rats in the chronic treatment group. These pumps ensured the continuous daily administration of 1 mg/kg of salbutamol—a dose considered to be clinically relevant by experimental studies.[18] The animals recovered from anesthesia—in response to the administration of oxygen—over a period of 30 minutes on the heated table. They were subsequently kept in individual cages for 7 days, during which time they had access to water and food ad libitum. They were then anesthetized again using the same procedure as before, and a catheter was fitted in the left carotid artery through an anterior cervical incision. Rats in the acute treatment group were administered 25 μg/kg of salbutamol intravenously.[15] Following a 5-minute stabilization period, blood samples were taken for arterial blood gas analysis and the rats were euthanized by exsanguination.

Contractile Properties

In order to study diaphragm muscle force, strips of left hemidiaphragm tissue measuring 3 mm to 5 mm wide and conserving costal and tendinous insertions were extracted and preserved, at a temperature of 37°C, in a Krebs-Ringer bicarbonate buffer (pH, 7.40 [0.02]), with content (mmol) as follows: glucose, 11.5; NaCl, 138; KCl, 5.9; $CaCl_2$, 1.4; $MgSO_4$, 0.9; NaH_2PO_4, 1.2; $NaHCO_3$, 25. The buffer was bubbled with 95% oxygen and 5% carbon dioxide to ensure oxygenation and maintenance of pH. The muscle strips were placed in a Krebs-Ringer bicarbonate buffer (as described above) in a tissue bath system designed to measure muscle force (Myobath-2; World Precision Instruments Inc, Sarasota, Florida, USA). Temperature was

thermostatically maintained at 37°C and a continuous flow ensured full buffer replacement every 15 minutes. Salbutamol was added to the acute treatment group tissue bath at a concentration of 20 μg/L, which corresponds to the plasma concentration reached in humans after an oral dose of 4 mg of salbutamol.[15] To record muscle tension, the strips of muscle, connected to a force transducer (Fort 100; World Precision Instruments Inc), were mounted vertically (tendinous end up) on a mobile support. Signals were captured by an amplifier (TBM4 M; World Precision Instruments Inc) and a computerized data acquisition and analysis software (Anadat 5.2; RHT-InfoDat Inc, Montreal, Québec, Canada). The muscle strips were indirectly stimulated on each side using a pair of platinum electrodes (Somatosensory Stimulator S10DSCMA; Grass Instrument Co, Quincy, Massachusetts, USA).

Once the muscle strips had been prepared for recording, maximum stimulation voltage and optimum resting length were determined. Muscle force at optimum resting length was studied for all the electrical stimuli at supramaximal voltage. With a view to studying simple contraction, isolated electrical stimuli were applied to the muscle strips after a 15-minute stabilization period. Simple contraction characteristics were evaluated by measuring peak twitch tension, contraction time (time to peak twitch tension), and half-relaxation time, defined as the time required for the peak twitch tension to fall to half its value. The relationship between contraction velocity and relaxation velocity (maximum tension rise rate and fall rate, respectively) was also analyzed. Tetanic stimulus was subsequently applied—at frequencies of 10, 20, 50, and 100 Hz, with stimulation trains of 1 second separated by 5-second intervals—in order to construct force-frequency curves.

Once stimulation was concluded, length and weight measurements were made for the diaphragm muscle strips when resting length was optimal. The area of the transverse section of the muscle was calculated by dividing muscle mass (g) by the optimum resting length (cm) and multiplying the result by 1.056, a value corresponding to muscle density expressed in g/cm^3. The area of the transverse section was used to calculate muscle tension—the force-area ratio, expressed in N/cm^2—for each muscle strip.

Statistical Analysis

All the variables analyzed showed a normal distribution. The Levene test showed equality of variance across the 4 groups. The force-frequency curves were compared using analysis of variance (ANOVA) for repeated measures. Simple contraction and blood gas parameters were analyzed using 1-way ANOVA. Post hoc analysis using the Student-Newman-Keuls test was performed to identify differences between the 4 groups. Data were expressed as means (SD) and statistical significance was established at $P<.05$. Statistical analyses were performed using the SPSS program, version 11.0 (SPSS Inc, Chicago, Illinois, USA).

Results

Blood Gas Parameters

Comparing the arterial blood of the rats in the 3 study groups with that of the rats in the control group, pH was lower, whereas $PaCO_2$, bicarbonate concentration, and base excess were higher ($P<.05$). There were no statistically significant differences in PaO_2 between the 4 groups. Table 1 summarizes the blood gas values for the 4 groups.

Diaphragm Function

As can be observed in Table 2, there was a significant reduction in peak twitch tension and contraction velocity in the untreated group compared to the control group ($P<.05$), in contrast with a significant increase in these parameters in the acute and chronic treatment groups ($P<.05$). The chronic treatment group had a higher relaxation velocity than the untreated group ($P<.05$). The force-frequency curves (Figure) revealed reduced diaphragm force in the untreated group compared to the control group ($P<.05$), contrasting with significantly increased diaphragm force in the acute and chronic treatment groups (which both had values comparable to the control group) when compared to the untreated group ($P<.05$).

Table 1
Blood Gas and Acid-Base Equilibrium Analyses[a]

	Control	TrB Untreated	TrB Acute SLB	TrB Chronic SLB
pH	7.35 (0.02)[b]	7.12 (0.06)	7.09 (0.06)	7.15 (0.04)
PaO_2, Torr	231 (36)	350 (30)	316 (34)	273 (70)
$PaCO_2$, Torr	51.4 (3.3)[b]	127.5 (20.1)	140.8 (18.7)	134.4 (18.1)
HCO_3^-, mEq/L	27.2 (0.6)[b]	37.2 (1.3)	38.0 (1.5)	38.1 (1.9)
BE, mEq/L	2.1 (0.5)[b]	7.8 (1.8)	9.4 (1.4)	14.1 (1.9)[c]

Abbreviations: BE, base excess; HCO_3^-, bicarbonate concentration; SLB, salbutamol; TrB, tracheal-banded.

[a] Data are shown as means (SE).

[b] $P<.05$ in comparisons with the other groups.

[c] $P<.05$ in comparisons with the tracheal-banded untreated and tracheal-banded chronic salbutamol groups.

Table 2
Simple Contraction Parameter Analysis[a]

	Control	TrB Untreated	TrB Acute SLB	TrB Chronic SLB
PTT, N/cm^2	6.46 (0.90)	3.28 (0.55)[b]	6.18 (0.71)	7.09 (0.59)
CT, ms	30.0 (2.1)	27.0 (1.5)	27.0 (1.7)	30.7 (1.7)
HRT, ms	30.0 (3.0)	27.8 (3.6)	27.5 (1.8)	28.2 (1.6)
CV, N·s/cm^2	818.3 (98.4)	368.4 (40.7)[b]	807.5 (102.3)	1007.3 (155.8)
RV, N·s/cm^2	408.3 (72.0)	206.7 (18.7)	411.3 (62.9)	481.0 (83.4)[c]

Abbreviations: CT, contraction time; CV, contraction velocity; HRT, half-relaxation time; PTT, peak twitch tension; RV, relaxation velocity; SLB, salbutamol; TrB, tracheal-banded.

[a] Data are shown as means (SE).

[b] $P<.05$ in comparisons with the other groups.

[c] $P<.05$ in comparison with the tracheal-banded untreated group.

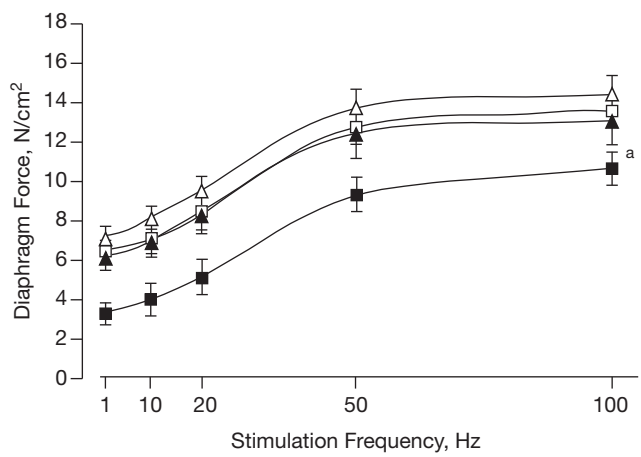

Figure. □ Comparison of force-frequency relationships for animals in the 4 groups: □ control group; ■ tracheal-banded untreated group; ▲ tracheal-banded acute salbutamol group; △ tracheal-banded chronic salbutamol group. Data are shown as means (SE).

[a] $P<.05$ in comparisons with the other groups.

Discussion

The 7-day tracheal banding model results in chronic airway obstruction that reproduces the histologic, biochemical, and contractile abnormalities seen in the diaphragms of patients with COPD.[3-5,19] Abnormal muscle contraction in the untreated group was evident in abnormal peak twitch tension and contraction velocity values and in a deteriorated force-frequency relationship.[2,4] Our study demonstrates that diaphragm abnormalities can be prevented or reversed by pharmacological treatment. Given their bronchodilatory effect, β_2 adrenergic agonists are frequently administered to patients with airflow limitation to improve respiratory loading. In our experimental model of chronic irreversible airway obstruction, the administration of salbutamol was associated with a significant improvement in diaphragm contractility in vitro. Furthermore, this outcome was evident in animals in both the acute and chronic treatment groups. All the animals with tracheal banding, however, developed hypercapnia with chronic respiratory acidosis, irrespective of whether or not salbutamol had been administered, as demonstrated by the intensity and persistence of the resistive load. Although this gas exchange disorder is likely to be caused by more than 1 mechanism, it is probably mostly related to a sharp increase in resistive load—with the ensuing increase in respiratory muscle work and onset of muscle fatigue. As for arterial oxygenation, other studies have demonstrated that the tracheal banding model results in hypoxemia during spontaneous breathing of room air.[3,5,19] Although the use of an inspired oxygen fraction of 1 might have favored the development of atelectasis secondary to denitrogenation, this was not translated into significant changes in PaO_2 in the different groups in our study.

As for the possible mechanisms involved in muscle dysfunction, it should be pointed out that hypercapnia itself has been reported to contribute to a deterioration in contractility,[12,20] and has also been mentioned as a determining factor in muscle dysfunction in patients with COPD.[21] In vitro studies conducted in conditions of hypoxia and hypercapnia have demonstrated that abnormal contractile patterns can be reversed with salmeterol.[22] In our study, hypercapnia may have contributed to the greater deterioration in muscle force observed in the untreated animals, although–as also observed in the aforementioned study–contractility improved with the administration of salbutamol.

It has been reported that inflammatory infiltration and proinflammatory cytokine production increase in the diaphragm in response to respiratory overloading.[3,23] Tumor necrosis factor α for example, has been shown to reduce diaphragm contractility in vitro.[24] In similar animal models to ours, an association has also been observed between reduced diaphragm force and protein oxidation mediated by reactive oxygen species[4]; Barreiro et al[9] observed, for example, that the severity of airway obstruction in patients with COPD was associated with increased protein oxidation in the diaphragm. Other studies comparing the diaphragms of patients with COPD with those of control subjects have confirmed reduced contractility in vitro, lower myosin heavy chain content per muscle fiber, and increased ubiquitin-proteasome pathway activity.[10,11]

Although it has been previously demonstrated that β_2 adrenergic agonists enhance diaphragm force in vitro in both physiological and pathological conditions,[13-16,22] as far as we are aware, ours is the first study to demonstrate that salbutamol improves diaphragm contractility in a chronic airway obstruction model. It has been reported that the inotropic effect of salbutamol is associated with increased cyclic adenosine monophosphate concentrations and Ca^{2+} release from the sarcoplasmic reticulum, resulting in enhanced excitation-contraction coupling.[14,15] We venture to suggest that our results are essentially explained by this particular mechanism operating in both the acute and chronic salbutamol treatment models. Chronic administration of salbutamol might evoke other muscle protection mechanisms; it has been shown, for example, that salbutamol reduces the production of proinflammatory cytokines such as tumor necrosis factor α,[25,26] has antioxidant effects (demonstrated in vitro) that trap free radicals and scavenge reactive oxygen species,[27-29] and also has anabolic effects.[18] A certain capacity for reducing ubiquitin-proteasome pathway activity has also been demonstrated for β_2 adrenergic agonists, which inhibit the protein catabolism associated with respiratory overload.[30-32] On the basis of these studies, we also venture to suggest that, although chronic administration of salbutamol might contribute to improving muscle function through anabolic stimulation and proteolytic activity inhibition, these mechanisms are not very likely to be evoked with acute administration of salbutamol.

In conclusion, our research demonstrates that salbutamol corrects the decrease in diaphragm contractility resulting from chronic resistive respiratory overload. Nonetheless, the mechanisms responsible for both diaphragm dysfunction and diaphragm recovery associated with salbutamol administration warrant further study.

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