ORIGINAL ARTICLES

Salbutamol Improves Diaphragm Force Generation in Experimental Sepsis

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OBJECTIVE: In a high percentage of cases, severe sepsis is accompanied by acute respiratory failure, in which weakness of the respiratory muscles plays an important role. Weakened respiratory muscles that are subjected to an increased mechanical load may develop muscle fatigue, with exacerbation of the respiratory failure. Because β_2-adrenergic drugs increase muscle contraction force, they may play a role in preventing and managing respiratory failure in septic patients. Our aim was to study the effects of salbutamol on diaphragm function in an animal model of peritoneal sepsis.

MATERIAL AND METHODS: The study included 3 groups of animals: *a)* a control group (n=7), in which the animals underwent a median laparotomy without visceral manipulation; *b)* a septic group (n=10), in which peritoneal sepsis was induced by cecal ligation and puncture (CLP); and *c)* a salbutamol group (n=7), in which peritoneal sepsis (CLP) was treated with salbutamol. Hemodynamic parameters and blood gases were measured in vivo. Diaphragm function was evaluated in vitro.

RESULTS: Salbutamol increased aortic blood flow and heart rate while it reduced mean arterial pressure in the animals with peritoneal sepsis ($P<.05$). Sepsis produced a significant drop in diaphragmatic force both before and after the application of a muscle-fatigue protocol. Treatment with salbutamol improved muscle contraction force before and after application of the protocol ($P<.05$).

CONCLUSIONS: The use of β_2-adrenergic drugs such as salbutamol improves diaphragm function in experimental sepsis. The mechanisms that produce this improvement require further study.

Key words: *Respiratory muscles. Sepsis. Diaphragmatic dysfunction. β_2-adrenergic drugs.*

El salbutamol mejora la fuerza diafragmática en la sepsis experimental

OBJETIVO: La sepsis grave se acompaña en un alto porcentaje de casos de insuficiencia respiratoria aguda, donde la debilidad de los músculos respiratorios desempeña un papel importante. Los músculos respiratorios debilitados y sometidos a una carga mecánica aumentada pueden evolucionar a fatiga muscular con agravamiento de la insuficiencia respiratoria. Los fármacos adrenérgicos β_2, al mejorar la fuerza de contracción muscular, podrían ser de utilidad en la prevención y el manejo de la insuficiencia respiratoria de pacientes con sepsis. El objetivo de este trabajo ha sido estudiar los efectos del salbutamol en la función diafragmática en un modelo animal de sepsis peritoneal.

MATERIAL Y MÉTODOS: Se estudiaron 3 grupos de animales: *a)* grupo control (n = 7), al que se realizó laparotomía mediana sin abordaje visceral; *b)* grupo sepsis (n = 10), al que se indujo sepsis peritoneal por ligadura y punción cecal (LPC), y *c)* grupo salbutamol (n = 7), en el que la sepsis peritoneal se trató con salbutamol (LPC + salbutamol). Los parámetros hemodinámicos y los gases sanguíneos se midieron in vivo. La función diafragmática se evaluó in vitro.

RESULTADOS: El salbutamol aumentó el flujo aórtico y la frecuencia cardíaca a la vez que disminuyó la presión arterial media en la sepsis peritoneal ($p < 0,05$). La sepsis determinó una caída significativa de la fuerza diafragmática tanto antes como después de un protocolo de fatiga muscular. El tratamiento con salbutamol mejoró la fuerza de contracción muscular en ambos casos ($p < 0,05$).

CONCLUSIONES: El uso de agentes adrenérgicos β_2 como el salbutamol mejora la función diafragmática durante la sepsis experimental. Los mecanismos de esta mejoría deben estudiarse en mayor profundidad.

Palabras clave: *Músculos respiratorios. Sepsis. Disfunción diafragmática. Adrenérgicos β_2.*

Introduction

Severe sepsis continues to be one of the leading causes of death in critical patients.[1] In a high percentage of cases, severe sepsis is accompanied by acute respiratory failure with varying degrees of abnormal gas exchange in the lungs due to acute lung injury or acute respiratory distress syndrome. Pulmonary edema determines important

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changes in respiratory mechanics, causing reduced functional residual capacity and lung elasticity and thereby increasing the load on the respiratory muscles and possibly leading to muscle fatigue.[2] This situation is exacerbated by muscle weakness, which manifests as a reduction in the contraction force of the respiratory muscles. Depression of muscle function during sepsis has been shown in different ways. Hence, muscle weakness reduces stamina and contributes to exacerbating respiratory failure. Until sepsis improves, patients may require mechanical ventilation to maintain gas exchange and rest the respiratory muscles. The causes that determine this respiratory failure are the subject of debate, though mechanisms similar to those that cause failure in other organs have been implicated.

Some studies have shown that β_2-adrenergic drugs improve the force of the respiratory muscles.[4-8] It can be conjectured that improved muscle function may help to maintain spontaneous ventilation in cases of sepsis where the function of the respiratory muscular pump is at risk. Furthermore, drug therapy of this nature may contribute to conserving or re-establishing muscle force with the aim of reducing mechanical ventilation times or facilitating the withdrawal of mechanical ventilation altogether.

The aim of this study in vivo and in vitro was to ascertain the effects of salbutamol on diaphragm function in an animal model of peritoneal sepsis.

Material and Methods

The experimental protocol for this study complied with the recommendations of the animal experimentation ethics committee (Comisión Honoraria de Experimentación) of the Universidad de la República Honorary. The study used Wistar Kyoto rats weighing between 300 g and 400 g. The rats were anesthetized with intraperitoneal sodium pentobarbital at a dose of 50 mg/kg. Sepsis was induced by means of cecal ligation and puncture (CLP).[9] The cecum was briefly exposed by means of a median laparotomy and ligated below the ileocecal valve. The cecum was then punctured twice using a 16-gauge needle and gently squeezed to extrude the fecal content. Finally, the cecum was returned to the peritoneal cavity and the abdominal wall was sutured in 2 planes. Immediately after surgery, the rats were injected with intravenous saline solution at a dose of 3 mL/100 g of body weight as an initial hemodynamic support measure and to compensate for possible volume loss during surgery. The animals were allowed to recover in individual cages with water and food available ad libitum for 48 hours,

The animals were then assigned randomly to 1 of the following 3 groups:

1. Control group (n=7). The animals underwent laparotomy and examination of the cecum only.

2. Sepsis group (n=10). The animals underwent laparotomy followed by CLP as described above.

3. Salbutamol group (n=7). The animals underwent CLP and were subsequently treated with intravenous salbutamol at a dose of 25 μg/kg administered 48 after the appearance of sepsis.

For the final evaluation, the rats were again anesthetized using sodium pentobarbital and underwent a tracheostomy under local anesthetic (1% lidocaine administered subcutaneously). A tracheal cannula connected to a T-tube with supplementary oxygen supply was placed to allow spontaneous respiration. The superior vena cava was catheterized via the jugular vein. Another catheter was placed in the femoral artery for hemodynamic monitoring and to obtain samples of arterial blood. In all animals, heart rate and mean arterial pressure were measured by a hemodynamic monitor (Life Scope 8, Nihon Kodhen, Tokyo, Japan). An ultrasound flow sensor (T206, Transonic Systems Inc, Ithaca, New York, USA) was placed around the abdominal aorta following careful dissection above the left renal artery in order to measure aortic blood flow. All measurements were taken after a stabilization period of 20 to 30 minutes following the vascular interventions. Aortic impedance was calculated as the ratio between systolic arterial pressure and aortic blood flow (mm Hg/mL/s). Arterial blood samples were obtained for blood gas analysis (ABL520, Radiometer A/V, Brønshøj, Denmark). The animals were finally sacrificed by administering an overdose of sodium pentobarbital.

The left half of the diaphragm was extracted immediately after sacrifice. Strips of muscle tissue were then cut from the diaphragm and mounted in a glass chamber. The diaphragm was perfused with a Krebs solution through which a mixture of 95% oxygen and 5% carbon dioxide had been bubbled at a constant temperature (37°C) and pH (7.40) (Myobath, World Precision Instruments Inc, Sarasota, Florida, USA). Salbutamol was added to the suspension bath in the animals from the salbutamol group to obtain a concentration of approximately 20 μg/L. The strips of diaphragm were connected at both ends to a force transducer (Fort 100, World Precision Instruments Inc, Sarasota, Florida, USA) and were electrically stimulated using platinum electrodes (S10DSLMA Somatosensory Stimulator, Grass Instruments Co, Quincy, Massachusetts, USA). Contractile function was evaluated by means of the maximum peak tension (MPT) that developed and by studying the characteristics of the force-frequency curve. These parameters were recorded using a data acquisition system (CVMS Data Acquisition System, World Precision Instruments Inc, Sarasota. Florida, USA). The force-frequency curves were obtained by applying a sequence of 1-second stimuli at frequencies of 10, 20, 30, 50, and 100 Hz. Force-frequency curves were constructed before and after applying a muscle-fatigue protocol. Diaphragm muscle fatigue was assumed when the force developed by the muscle fell to 50% of the initial value due to the effects of a series of supramaximal stimuli at 20 Hz. MPT, expressed in N/cm^2, was measured as the response to a single stimulus, before and after muscle fatigue was induced. Measurements of muscular forces were indexed by area of cross-section of the muscle strips and expressed in N/cm^2.

Statistical Analysis

All the analyzed variables showed a normal distribution. The values for each parameter of the study population were expressed as a mean (SE). Between-group differences were determined by means of analysis of variance for multiple comparisons. Post hoc analysis was performed using the Scheffé test. Values of $P<.05$ were considered to be statistically significant. Statistical analysis was performed using the SPSS statistical software package version 12.0.1 (SPSS Inc, Chicago, Illinois, USA).

Results

Macroscopic findings of the peritoneal cavity were recorded during the repeat abdominal surgery. The animals from both septic groups (sepsis group and salbutamol group) presented a moderate amount of purulent fluid and no significant between-group differences were observed. The control group showed no signs of inflammatory activity in the peritoneal region.

Physiological Variables in the Study Groups[a]

	Control Group (n=7)	Sepsis Group (n=10)	Salbutamol Group (n=7)
Mean arterial pressure, mm Hg	132 (5.7)	128 (19.3)	103 (9.1)[b]
Heart rate, beats/min	410 (25.8)	418 (45.2)	501 (28.5)[b]
Aortic blood flow, mL/min	33.2 (3.98)	26.0 (6.5)	37.2 (8.4)[c]
Aortic impedance, mL/min/cm H_2O	4.7 (0.5)	5.9 (1.2)[b]	3.8 (0.8)
PaO_2/FiO_2	417 (143.6)	251 (99.2)	431 (107)
Arterial blood pH	7.40 (0.02)	7.41 (0.03)	7.34 (0.08)[b]
Arterial blood bicarbonate, mEq/L	26.4 (2.4)[b]	22.7 (2.6)	24.0 (1.9)

Abbreviation: FiO_2, inspired oxygen fraction.
[a]Values are expressed as mean (SE).
[b]$P<.05$ in the comparison with the other 2 groups.
[c]$P<.05$ in the comparison with the sepsis group.

Physiological Parameters

The table shows the mean (SE) values for the main hemodynamic and blood-gas parameters. Aortic impedance was significantly higher in the sepsis group than in the other 2 groups ($P<.05$). The ratio between $PaCO_2$ and inspired oxygen fraction tended to decrease in the sepsis group, though this decline was not statistically significant. In terms of metabolism, arterial bicarbonate was significantly lower in both the sepsis group and the salbutamol group when compared with the control group ($P<.05$). Mean arterial pressure was significantly lower and heart rate was significantly higher in the salbutamol group than in the other groups ($P<.05$). Aortic blood flow was significantly higher in the salbutamol group than in the sepsis group ($P<.05$).

Diaphragm Function

Figure 1 shows the MPT for the 3 study groups before and after application of the muscle-fatigue protocol. Diaphragmatic force was significantly lower in the sepsis group than in the other groups ($P<.05$). MPT values for the salbutamol group were comparable to those for the control group. Figure 2 shows the force-frequency curves before and after application of the diaphragm muscle fatigue protocol. The curve was significantly lower for the animals in the sepsis group ($P<.05$), whereas the curve for the salbutamol group was not significantly different from that of the control group.

Discussion

Administration of salbutamol determined characteristic hemodynamic changes linked to stimulation of β_2 receptors and showed clear improvement of muscle function both before and after muscle fatigue in our model of experimental sepsis.

The effects of β_2-adrenergic drugs on muscle force have been studied in different clinical and experimental contexts. Salbutamol increases diaphragmatic force in vitro.[6,7] Oral administration of salbutamol to athletes determines greater resistance of muscles to fatigue.[10] Other β_2-adrenergic agonists such as isoproterenol[4] and terbutaline[8] improve diaphragmatic contractility in peritoneal sepsis.

We found that peritoneal sepsis severely compromises diaphragm function, consistent with reports from other authors. Oxygen and nitrogen free radicals, mitochondrial dysfunction, altered mechanisms of intracellular energy production, and the ubiquitin-proteasome pathway have been implicated in the genesis of this disorder.[11-17] The hemodynamic changes induced by salbutamol became evident in our model as systemic administration increased heart rate and aortic blood flow and reduced mean arterial pressure and aortic impedance. These changes demonstrate their inotropic and chronotropic effects on the myocardium and their vasodilator effects on systemic circulation. An improvement in the functional state of the diaphragm—

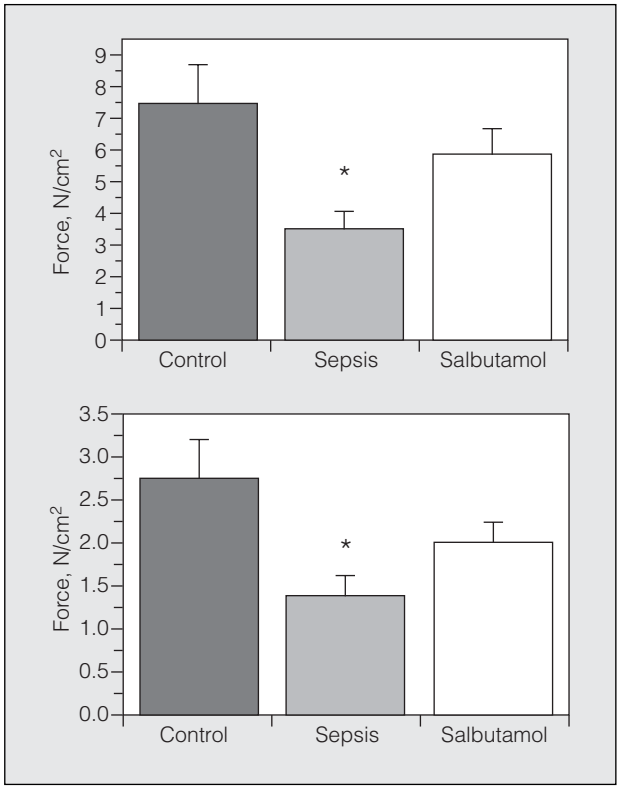

Figure 1. Maximum peak tension (N/cm^2) before (left) and after (right) application of a muscle-fatigue protocol.
[a]$P<.05$ in the comparison between the sepsis group and the control group.

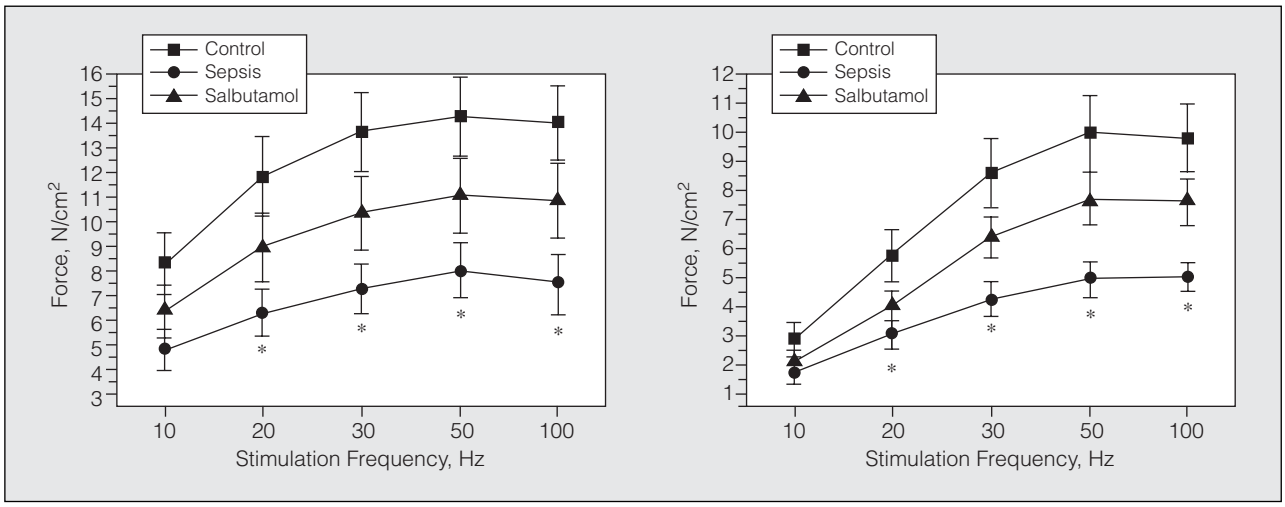

Figure 2. In vitro force-frequency curves (N/cm²) in diaphragms of animals in the 3 groups studied. Curves shown are before (left) and after (right) application of a muscle-fatigue protocol.
[a]*P*<.05 in the comparison between the sepsis group and the control group.

measured by both MPT and the force-frequency curves—was also observed. From a systemic perspective, the trend toward reduced arterial pressure, aortic blood flow, and arterial oxygenation in the sepsis group was not statistically significant. These changes do not, therefore, explain the depression of diaphragm muscle force found in this group. Systemic salbutamol significantly improved aortic blood flow and arterial oxygenation. The drug therapy probably benefitted systemic oxygen levels although it did not ensure improved perfusion and oxygenation of the diaphragm tissue. Because of the experimental design, we cannot establish whether the improvement in muscle force was a result of the cardiovascular changes induced by the drug or of its direct effects on the muscle tissue. Although there was an association between hemodynamic improvement and diaphragmatic force, other possible mechanisms should be studied.

Some studies have reported an ergogenic effect of β_2-adrenergic drugs on skeletal muscle, causing increased anabolism and reduced catabolism of protein,[5,10] and hypertrophy of diaphragm fibres.[18,19] Clenbuterol restores the loss of diaphragm muscle force and mass associated with aging[20] and treatment with corticosteroids.[21] Other authors have suggested that the inotropic effect of salbutamol is mediated by an increase in cyclic adenosine monophosphate and the liberation of calcium from the sarcoplasmic reticulum.[6,7]

Many in vitro and in vivo studies have shown that oxidative and nitrosative stress have important effects on diaphragm contractility. Supinski et al[22] showed that in vivo administration of free radical scavengers reduces diaphragm fatigue at low frequencies. Similarly, Barreiro et al[14] showed that the structural and functional damage to the diaphragm seen in sepsis can be avoided with therapies aimed at preventing oxidative and nitrosative stress.

Salbutamol was able to attenuate depression of contractility before and after diaphragm fatigue in vitro. Similar effects to these have been reported with the use of an oxygen free radical scavenger. Because both β_2-agonists and oxygen and nitrogen free radicals improve diaphragm contractility, it can be conjectured that these drugs share a common metabolic pathway. In fact, the literature has brought to light the antioxidant activity of adrenergic agonists.[23-25] An inhibitory effect on the production of oxygen free radicals by inflammatory cells mediated by the β_2 receptor has also been shown.[26]

While the mechanism by which β_2-adrenergic agonists affect the balance of protein metabolism is not fully understood, there is evidence that they may inhibit the ubiquitin-proteasome pathway, which is responsible for the breakdown of muscle proteins.[27,28] Finally, the effect of β_2-adrenergic agonists on the inflammatory response may be another mechanism implicated in our results. These drugs reduce the production of proinflammatory cytokines such as tumor necrosis factor-α and interleukin 18, induced by sepsis.[29,30]

The antioxidant and anti-inflammatory effects of β_2-adrenergic agonists and their ability to inhibit muscle proteolysis by acting on the ubiquitin-proteasome pathway require further research.

In summary, we have shown that salbutamol improves diaphragm contractility in vitro during peritoneal sepsis. Although this improvement in muscle function is associated with hemodynamic changes and changes in arterial oxygenation, we cannot affirm that this led to improved tissue oxygenation. The mechanisms implicated in these functional changes require further study.

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