β₂-Adrenoceptor agonists reduce the decline of rat diaphragm twitch force during severe hypoxia

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Van der Heijden, H. F. M., L. M. A. Heunks, H. Folgering, C. L. A. van Herwaarden, and P. N. R. Dekhuijzen. β₂-Adrenoceptor agonists reduce the decline of rat diaphragm twitch force during severe hypoxia. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L474–L480, 1999.—The aim of the present study was to investigate the in vivo effects of the short-acting β₂-adrenoceptor agonist salbutamol and the long-acting β₂-adrenoceptor agonist salmeterol on hypoxia-induced rat diaphragm force reduction. In vitro diaphragm twitch force (P_t) and maximal tetanic force (P_o) of isolated diaphragm muscle strips were measured for 90 min during hyperoxia (tissue bath PO₂ 83.8 ± 0.9 kPa and PCO₂ 3.9 ± 0.1 kPa) or severe hypoxia (PO₂ 7.1 ± 0.3 kPa and PCO₂ 3.9 ± 0.1 kPa) in the presence and absence of 1 µM salbutamol or 1 µM salmeterol. During hyperoxia, salbutamol and salmeterol did not significantly alter the time-related decreases in P_t and P_o (to ~50% of initial values). Salbutamol had no effects on P_o or the P_t-to-P_o ratio. Salmeterol treatment significantly reduced P_t and increased the P_t-to-P_o ratio during hypoxia (P < 0.05 compared with control value). Hypoxia resulted in a severe decrease in P_t (to ~30% of initial value) and P_o after 90 min. Both salbutamol and salmeterol significantly reduced the decline in P_t during hypoxia (P < 0.05). The reduction in P_o was not prevented. Salbutamol increased P_t rapidly but transiently. Salmeterol had a delayed onset of effect and a longer duration of action. Addition of 1 µM propranolol (a nonselective β-adrenoceptor antagonist) did not alter P_t, P_o, or the P_t-to-P_o ratio during hypoxia but completely blocked the inotropic effects of salbutamol and salmeterol, indicating that these effects are dependent on β₂-adrenoceptor agonist-related processes.

CONCLUSION

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METHODS

Study design. The effects of treatment with β₂-adrenoceptor agonists on in vitro rat diaphragm contractile properties were studied either during standard optimal in vitro conditions (hyperoxia) or during severe hypoxia. In both conditions, the effects of salbutamol or salmeterol treatment were studied and compared with those of the untreated control group.

General procedures. Adult male outbred Wistar rats aged 16–18 wk and with a mean weight of 376 ± 5 (SE) g were
used. The animals were housed under standard conditions and were fed ad libitum.

The rats were anesthetized with pentobarbital sodium (70 mg/kg ip; Narcovet, Opharma, Arnhem, The Netherlands). A tracheotomy was performed, and a polyethylene cannula was inserted. The animals were mechanically ventilated with 100% oxygen. The diaphragm and adherent lower ribs were quickly excised after a combined laparotomy and thoracotomy, and they were immediately submerged in cooled, oxygenated Krebs solution at pH 7.4. This Krebs solution consisted of 137 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1 mM KH₂PO₄, 24 mM NaHCO₃, 7 mM glucose, and 25 µM D-tubocurarine (Sigma, Bornem, Belgium).

From the central costal region of the right hemidiaphragm, two rectangular strips were dissected parallel to the long axis of the muscle fibers. Silk sutures were tied firmly to both ends. The strips were suspended in two tissue baths containing Krebs solution, maintained at 37°C, and perfused with a 95% O₂-5% CO₂ mixture. The central tendon end was connected to an isometric force transducer (Sensotec model 31/1437-10, Columbus, OH) mounted on a micrometer. Two large platinum stimulating electrodes were placed parallel to the bundles. Stimuli were applied with a pulse duration of 0.2 ms and a train duration of 400 ms and were delivered by a stimulator (1D-electronics, University of Nijmegen, Nijmegen, The Netherlands) activated by a personal computer. To ensure supramaximal stimulation, the strips were stimulated at ~20% above the voltage at which maximal forces were obtained (~6 V through the stimulation electrodes). Data acquisition and storage of the amplified signal were performed via a Dash-16 interface on a personal computer (Twist-trigger software, 1D-electronics). Both strips were placed at their optimal length, defined as the length at which peak twitch force (P₁) was obtained. After a 15-min thermoequilibration period (i.e., before the start of treatments), maximal P₁ and maximal tetanic force (P₀) were determined under normal (hyperoxic) conditions.

Treatment groups. The diaphragm strips were randomly allocated to the treatment groups, and different treatments were given to each of the two muscle strips obtained from one animal. Immediately after the thermoequilibration period, perfusion of the tissue baths with the hypoxic gas mixture significantly reduced the PO₂ in the Krebs solution to ~7 kPa, compared with ~84 kPa in the hyperoxic group (P < 0.001; Table 1). No differences were found in pH or PCO₂ between hypoxia and hyperoxia, and no differences were found in PO₂, PCO₂, or pH between treatment groups.

Table 1. Tissue bath Krebs solution pH, PCO₂, and PO₂

<table>
<thead>
<tr>
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<th>Hyperoxia</th>
<th>Hypoxia</th>
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<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>38</td>
</tr>
<tr>
<td>pH</td>
<td>7.45</td>
<td>7.44</td>
</tr>
<tr>
<td>PCO₂ kPa</td>
<td>3.92</td>
<td>3.96</td>
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<tr>
<td>PO₂ kPa</td>
<td>83.75</td>
<td>7.07</td>
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Values are means ± SE after 90 min of tissue bath perfusion; n, no. of diaphragm muscle strips. *Significantly different from hyperoxic group, P < 0.001 by Student’s t-test.
23.0 ± 0.4 mm. Diaphragm muscle strip weight ranged from 39.7 ± 1.4 to 49.5 ± 3.4 mg. No significant differences were found between treatment groups for either strip length or strip weight (one-way ANOVA with subsequent Student-Newman-Kuels post hoc test). The initial $P_t$ and initial maximal $P_o$ are listed in Table 2. These measurements were performed at the end of the thermoequilibration period, before the start of treatment and under standard hyperoxic conditions. No differences were found between any treatment groups in the pretreatment period (one-way ANOVA).

Effects of $\beta_2$-adrenoceptor agonist treatment during hyperoxia. Salbutamol treatment did not significantly alter repetitive diaphragm $P_t$ during hyperoxia. Also, when expressed as a percentage of the initial $P_t$ no significant overall effect was found (Fig. 1, Table 2). Salbutamol did not affect $P_o$ and the $P_t$-to-$P_o$ ratio either during hyperoxia (Table 2). However, significant interactions were found between salbutamol treatment and time by repeated-measures analysis of $P_t$ and $P_t$-to-$P_o$ ratio ($P < 0.01$). This may indicate that salbutamol initially increased $P_t$ (and $P_t$-to-$P_o$ ratio) but that this effect was not sustained throughout the experiment (Fig. 1).

Salmeterol did not significantly alter repetitive $P_t$ generation during hyperoxia (Fig. 1). However, $P_o$ was significantly reduced by salmeterol treatment, both expressed as specific force (Table 2) and as a percentage of initial $P_o$. However, a significant interaction was found between salbutamol treatment and time for repeated $P_t$ measurements, indicating that the effect varied throughout the experiment (Table 2).

Table 2. Diaphragm $P_t$, $P_o$, and $P_t$-to-$P_o$ ratio at 30-min intervals during hyperoxia and hypoxia in vitro

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment</th>
<th>30 Min</th>
<th>60 Min</th>
<th>90 Min</th>
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<tbody>
<tr>
<td></td>
<td>Hyperoxia</td>
<td>Hypoxia</td>
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<td>Baseline</td>
<td>+ Salmeterol</td>
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<td>Hyperoxia</td>
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<tr>
<td></td>
<td>Baseline</td>
<td>+ Salmeterol</td>
<td>+ Salmeterol</td>
<td>+ Salmeterol</td>
</tr>
<tr>
<td>$P_t$, N/cm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.2 ± 0.4</td>
<td>8.2 ± 0.2</td>
<td>8.9 ± 0.4</td>
<td>8.9 ± 0.4</td>
</tr>
<tr>
<td>%Baseline</td>
<td>29.5 ± 1.1</td>
<td>27.5 ± 0.5</td>
<td>30.4 ± 1.7</td>
<td>29.5 ± 1.1</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>7.9 ± 0.5</td>
<td>8.3 ± 0.2</td>
<td>8.0 ± 0.2</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>%Baseline</td>
<td>7.9 ± 0.5</td>
<td>8.3 ± 0.2</td>
<td>8.0 ± 0.2</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>9.0 ± 0.3</td>
<td>8.5 ± 0.2</td>
<td>7.9 ± 0.4</td>
<td>7.9 ± 0.4</td>
</tr>
<tr>
<td>%Baseline</td>
<td>9.0 ± 0.3</td>
<td>8.5 ± 0.2</td>
<td>7.9 ± 0.4</td>
<td>7.9 ± 0.4</td>
</tr>
<tr>
<td>$P_o$, %Baseline</td>
<td>59.4 ± 4.8</td>
<td>76.3 ± 4.9</td>
<td>76.3 ± 4.9</td>
<td>76.3 ± 4.9</td>
</tr>
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Values are means ± SE. During hyperoxia, twitch force ($P_t$) was not significantly altered (Fig. 1); however, during hypoxia, $P_t$ (both in N/cm² and as %baseline) was significantly increased in salbutamol- and salmeterol-treated diaphragm strips ($P < 0.01$ by repeated-measures ANOVA; Figs 1 and 2). Propranolol reduced $P_o$ when expressed as %baseline during hyperoxia ($P < 0.05$). In salbutamol plus propranolol- and salmeterol plus propranolol-treated strips, $P_t$ (in N/cm²) was significantly reduced during hyperoxia ($P < 0.05$). *Significant difference compared with control treatment found in post hoc analysis, $P < 0.05$. During hyperoxia, twitch force ($P_t$) was significantly reduced in salmeterol-treated strips ($P < 0.05$ by repeated-measures ANOVA). During hypoxia, twitch force was significantly increased in salmeterol- and salmeterol plus propranolol-treated strips only when expressed as %baseline ($P < 0.05$). *Significant difference compared with control treatment found in post hoc analysis, $P < 0.05$. During hyperoxia, $P_t$-to-$P_o$ ratio was significantly altered in salmeterol-treated strips ($P < 0.01$ by repeated-measures ANOVA). During hypoxia, significant effects were found for $P_t$-to-$P_o$ ratio in salbutamol ($P < 0.01$), salmeterol ($P < 0.01$), and salmeterol plus propranolol-treated strips ($P < 0.05$; all by repeated-measures ANOVA). * Significant difference compared with control group found in post hoc analysis, $P < 0.05$. During hyperoxia, $P_t$-to-$P_o$ ratio was significantly increased in salbutamol- and salmeterol plus propranolol-treated strips ($P < 0.01$; Figs 1 and 2). A similar effect was found when $P_t$ was expressed as a percentage of initial $P_t$. During hyperoxia, $P_o$ was significantly lower in salbutamol-treated strips compared with those from control animals (Table 2). The $P_t$-to-$P_o$ ratio was increased compared with that in control strips ($P < 0.001$; Table 2). Again, a significant interaction was found between salbutamol treatment and time ($P < 0.001$). Post hoc analysis showed that the $P_t$-to-$P_o$ ratio was significantly increased at all time points during the experiment (Table 2).
During hypoxia, salmeterol treatment increased $P_t$ compared with that in control strips ($P < 0.01$; Fig. 2). $P_o$ and the $P_t$-to-$P_o$ ratio were not affected by the coadministration of propranolol (Table 2).

### DISCUSSION

The present study shows that both short-acting and long-acting $\beta_2$-adrenoceptor agonists improve diaphragm muscle $P_t$ generation during severe hypoxia in vitro. This partially restored the hypoxia-induced decline in $P_t$. The significant interactions found between treatment and time in these repeated force measurements indicate that the magnitude of these effects varied during the experiment. Salbutamol had a more rapid onset of action compared with salmeterol, but salmeterol had a longer duration of action. Toward the end of the protocol, salbutamol reduced $P_o$ during hypoxia and the decline in $P_t$ appeared to be stronger. In contrast, neither salbutamol nor salmeterol affected $P_t$ during hyperoxia. Salmeterol decreased $P_o$ during hyperoxia, whereas salbutamol had no effect on $P_o$. Finally, the inotropic effects of both salmeterol and salbutamol were blocked by proprano-

![Fig. 1. Diaphragm twitch force ($P_t$) during hyperoxia (open symbols) and hypoxia (closed symbols). Circles, control group; triangles, salbutamol treatment; squares, salmeterol treatment (all concentrations 1 µM). For clarity, means $\pm$ SE are indicated separately at start and end of curves only. Control diaphragm $P_t$ was significantly lower during hypoxia compared with hyperoxic control value ($P < 0.01$). Because an overall repeated-measures ANOVA analysis resulted in multiple significant interactions between the different factors (hypoxia, treatment, and repeated measurements of force), statistical analysis was performed within hypoxic and hypoxic groups subsequently. No significant treatment effects were found within hypoxic group. However, during hypoxia, both salbutamol and salmeterol significantly increased $P_t$ curve compared with hypoxic control value ($P < 0.01$ by repeated-measures ANOVA).](http://ajplung.physiology.org/)

![Fig. 2. Diaphragm $P_t$ during hypoxia. ●, Control group; ▲, salbutamol treatment; ■, salmeterol treatment; ○, propranolol treatment (1 µM); △, salbutamol plus propranolol treatment; □, salmeterol plus propranolol treatment (all concentrations 1 µM). For clarity, means $\pm$ SE are indicated separately at start and end of curves only. Both salbutamol and salmeterol significantly increased $P_t$ curves compared with control values during hypoxia ($P < 0.01$ by repeated-measures ANOVA). In salbutamol plus propranolol- and salmeterol plus propranolol-treated groups, $P_t$ curves were significantly decreased compared with control values ($P < 0.05$ by repeated-measures ANOVA). Propranolol treatment did not significantly alter $P_t$ curve during hypoxia compared with hypoxic control value ($P = 0.341$).](http://ajplung.physiology.org/)


Severity of hypoxia. In the present in vitro experiments, Krebs solution PO2 was reduced to ~7 kPa to induce hypoxia compared with ~84 kPa in the hyperoxic group. Because oxygenation of the diaphragm muscle strips in these experiments is dependent solely on diffusion of oxygen into the core region of the strips, this partial pressure can be considered as severe hypoxia. Besides, in vitro experimental conditions similar to our hyperoxia experiments (PO2 ~84 kPa), significant hypoxia may be present in centrally situated muscle fibers when the critical radius of ~0.6 mm is exceeded at 37°C (23). These authors showed that at temperatures higher than 25°C, P1 and P0 in both the soleus and extensor digitorum longus muscles decreased with the duration of in vitro incubation. This was accompanied by a temperature-dependent depletion of glycogen content in the central portions of these muscles (23). However, in contrast to whole muscle preparations, diaphragm strip thickness was well within this critical radius (31, 32). In previous studies of hypoxic effects on diaphragm contractility, similar levels of in vitro PO2 (26) or slightly higher levels of PO2 (~10–20 kPa) were studied in rat (27), hamster (9), and mouse diaphragms (24) and in situ in the dog diaphragm (2). It is difficult to compare those results with the present findings because higher duty cycles and stimulation frequencies will accelerate the decline in force (2, 33). In the hamster diaphragm, hypoxia (~16 kPa) partly reduced the force-frequency curve, and it reduced submaximal tetanic tension (160 ms, 25-Hz stimulation) by ~35% (9). In the mouse diaphragm, isotonic and (to a lesser extent) isometric fatigue resistances were dramatically reduced by hypoxia (~10 kPa) (24). During fatigue, the rat diaphragm relaxation rate was increased (33). The rat diaphragm Pt was reduced to ~10% of initial Pt after 25 min of stimulation at 0.5 Hz and 0.1-ms pulse duration at a PO2 of 4–5 kPa (26). At a PO2 of ~20 kPa, a similar stimulation protocol reduced rat diaphragm Pt by ~60% after 30 min (27). In the present study, a much lower duty cycle was used (with a stimulation frequency of ~0.01 Hz and a pulse duration of 0.2 ms). With this stimulation paradigm, Pt was decreased by ~70% after 90 min at a PO2 of ~7 kPa compared with a reduction of ~50% at a PO2 of ~84 kPa. This again shows that hypoxia has a clear additive effect on the in vitro decline of Pt.

Mechanisms of force decline during hypoxia. Hypoxia affects virtually all physiological processes, and it impairs the energy supply of living cells. In addition, hypoxia is often accompanied by changes in Pco2 and intracellular as well as extracellular pH (9, 26, 27). Hypercapnia reduced the capacity of the unfatigued human diaphragm to generate force during voluntary contractions (16). Both hypoxia and hypercapnia lowered intracellular pH (26, 27), and the combination of hypercapnia, hypoxia, and acidosis had greater detrimental effects than either abnormality alone (9). In the present study, (extracellular) pH and Pco2 were similar in the hypoxic and hyperoxic groups, and only P02 was modified.

The metabolic and structural mechanisms by which skeletal muscle force production is reduced during acute hypoxia have recently been reviewed by Sieck and Johnson (29). These mechanisms include altered membraneous ionic conductance (33) reducing sarcosomal excitability, downregulation of mitochondrial enzymes, increased production of reactive oxygen-derived species (ROS), and effects on muscle blood flow in vivo (29). Hypoxia may alter membrane conduction of K+, Cl−, and possibly Na+ (28, 33), but whether β2-adrenoceptor agonists affect these processes is disputed (4, 5). A reduced sarcosomal excitability may contribute to neuromuscular transmission failure, which may predominantly affect type IIb muscle fibers as a result of their high actomyosin ATPase activity (29). However, in the present in vitro experiments, diaphragm muscle strips were directly stimulated and neuromuscular transmission was blocked by the addition of α-tubocurarine to the Krebs solution. Also, alterations in muscle blood flow in response to acute hypoxia are unlikely to be involved in these in vitro experiments. Therefore, downregulation of oxidative enzymes such as succinate dehydrogenase or cytochrome-c oxidase may be important mechanisms in the reduction of force generation induced by acute hypoxia during the present experiments. This downregulation may be the result of ROS formation and is encountered after repetitive diaphragm stimulation (15, 29). ROS and scavengers of ROS are increasingly implicated in modulating contractile properties in skeletal and respiratory muscles. In skeletal muscles, a low level of ROS is essential for excitation-contraction coupling and is obligatory for optimal contractile function (21). However, whether ROS formation is altered by β2-adrenoceptor agonist treatment is not known.

Effects of β2-adrenoceptor agonist treatment. The present study shows that treatment with short- and long-acting β2-adrenoceptor agonists reduced the hypoxia-induced decline in diaphragm Pt. The exact mechanism by which β2-adrenoceptor agonists exert their inotropic effect on respiratory muscles is not fully understood. These mechanisms may involve excitation-contraction coupling of skeletal muscle. In earlier experiments, the inotropic effect of salbutamol was blocked by ryanodine, which indicates that this effect is most likely mediated by an improvement of sarcoplasmic reticulum (SR) Ca2+ release (31). This is in agreement with previous findings reported by Cairns and Duhlunty (4, 5) and Cairns et al. (6). They further showed that enhancement of sodium-pump activity, dihydroerythroidine-sensitive Ca2+ currents, glycolysis, and altered action potentials are unlikely to be involved in the mechanisms of action β2-adrenoceptor agonists (4, 5). These findings in mammalian skeletal muscle are in line with earlier experiments conducted with frog skeletal muscle, showing that adrenaline treatment potentiated Pt by modulating calcium channels (10).

The decrease in P0 found in the salmeterol-treated hyperoxic and salbutamol-treated hypoxic groups may
The stronger decline in $P_t$ in the salbutamol-involved in this decrease in $P_o$. Because these effects are not consistently found throughout the present study, it is likely that other processes are also involved in this decrease in $P_o$. Because these effects were not evaluated in this study, we do not have a clear explanation for these findings.

The effect of hypoxia on SR Ca$^{2+}$ release or on other mechanisms that may influence the inotropic effects of $\beta_2$-adrenoceptor agonists in hypoxic skeletal muscles is not known. One study has found a small effect of salbutamol treatment on diaphragm $P_t$ in dogs during metabolic acidosis (12), but no studies were performed under hypoxic conditions. Prolonged hypoxia has been shown to reduce the expression of $\beta_2$-adrenoceptors in cardiac muscle and of $\beta_2$-adrenoceptors in pulmonary and systemic arteries (25). In the lung, hypoxia increased the density and binding affinity of $\beta_2$-adrenoceptors (3). Furthermore, the in vitro regulation of the $\beta_2$-adrenoceptor agonist gene in hamster smooth muscle cells was found to be critically dependent on pH during hypoxia (22). Whether such changes are present within the time frame of the present study and what functional consequences such changes may have is not clear.

Coadministration of propranolol plus salbutamol or salmeterol during hypoxia reduced $P_t$. This effect was also found for propranolol alone when $P_t$ is expressed as a percentage of initial $P_t$. This is in agreement with an earlier report (17) in which a selective $\beta_2$-adrenoceptor antagonist reduced force generation in gastrocnemius muscle preparations. It has been suggested that this could be the result of a blockade of endogenous catecholamines (17). However, it is unlikely that such an effect was of importance in the present in vitro experiment. Alternatively, these findings may indicate changes in oxidative enzyme activity (14).

Methodological considerations. In the present study, alteration of tissue bath oxygenation and addition of $\beta_2$-adrenoceptor agonists and/or antagonists were performed simultaneously. These changes do not immediately exert their effect, and all parameters are likely to have different equilibration times. We did not include separate time frames for either hypoxia (diffusion time) or the onset of action of salbutamol or salmeterol in the diaphragm strips. The use of different time frames would certainly have affected force production because at 37°C, a time-related decrease of in vitro force production is present. In such an experimental design, the use of separate time-matched control groups would have been obligatory.

Not surprisingly, the present study shows that salbutamol has a more rapid onset of action and a larger effect on $P_t$ compared with those with salmeterol. This can be explained by differences in lipophilicity and a lower efficacy (partial agonist) of salmeterol compared with salbutamol (11). Furthermore, diaphragm $P_t$ was decreased further in both hypoxia and hyperoxia when salmeterol started to have an effect. This may have reduced the inotropic effect of salmeterol. However, the duration of action of salmeterol was longer compared with that of salbutamol, which is in agreement with its pharmacological properties (11).

In the present study, very high concentrations (1 µM) of salbutamol and salmeterol were used. At these high concentrations, salmeterol may have non-$\beta_2$-adrenoceptor properties due to its high lipophilicity (18). However, the experiments in which propranolol was added to salbutamol or salmeterol treatment show that these non-$\beta_2$-adrenoceptor-related processes did not play a role in the inotropic effects of salmeterol on diaphragm $P_t$ during hypoxia. In earlier studies (3,1,32), salbutamol was shown to have significant inotropic effects at the clinically relevant concentration of 0.05 µM. Preliminary studies with salmeterol showed that lower concentrations had similar but less pronounced effects during hypoxia. To reach maximal effects and to simplify comparison between the two drugs, we chose the high concentration of 1 µM for both compounds.

Clinical relevance. In patients with severe COPD, chronic hypoxia can frequently be found, often in combination with hypercapnia. Furthermore, during exacerbations of their disease, acute hypoxia or acute-on-chronic hypoxia may be present. This is of particular interest because hypoxia may reduce diaphragm contractile properties in situ (2) and in vitro (26) and may reduce fatigue resistance in vitro (24) and in humans (13). Dysfunction of the respiratory muscles frequently occurs in patients with severe COPD (20). Metabolic changes like hypoxia, hypercapnia, and electrolyte disorders are important factors that may impede (the already compromised) respiratory muscle function (3, 9, 16, 26, 27, 29). In this clinical situation, $\beta_2$-adrenoceptor agonists are often used for bronchodilatation. Previous studies have shown that $\beta_2$-adrenoceptor agonists like salbutamol and terbutaline can improve diaphragm contractile properties under optimal in vitro conditions (1, 32) and after fatigue (8, 19, 30). The present study shows that the decrease in diaphragm contractility under hypoxic conditions can be partially prevented by the addition of $\beta_2$-adrenoceptor agonists in vitro. This might be of importance in the treatment of incipient or manifest respiratory muscle fatigue in COPD patients, but clinical studies are recommended to evaluate the effects of $\beta_2$-adrenoceptor agonists in these situations.
β2-AGONISTS AND DIAPHRAGM CONTRACTILITY DURING HYPOXIA

REFERENCES


