Effects of Dibutyryl Cyclic Adenosine Monophosphate on Hypercapnic Depression of Diaphragmatic Contractility in Pentobarbital-Anesthetized Dogs

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ABSTRACT

Background: Hypercapnia is associated with diaphragm muscle dysfunction that causes a reduction of diaphragmatic force generated for a constant elective myographic activity. No published data are available concerning hypercapnic depression of diaphragmatic contractility during dibutyryl cyclic adenosine monophosphate (DBcAMP) administration.

Objective: The aim of this study was to assess the effects of DBcAMP on hypercapnic depression of diaphragmatic contractility in pentobarbital-anesthetized dogs.

Methods: This experimental study was conducted from July to December 2008 at the Department of Anesthesiology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan. Adult (aged >5 years) mongrel dogs weighing 10 to 15 kg were randomly divided into 3 equal groups. Hypercapnia (80–90 mm Hg) was induced with 10% carbon dioxide added to the inspired gas. When hypercapnia was established, group 1 was infused with low-dose DBcAMP (0.05 mg/kg/min); group 2 was infused with high-dose DBcAMP (0.2 mg/kg/min); and group 3 received placebo (saline). Study drug was administered intravenously for 60 minutes. Diaphragmatic contractility was assessed by transdiaphragmatic pressure (Pdi) at baseline, induction of hypercapnia, and study drug administration.

Results: Twenty-one dogs were divided into 3 groups of 7. There were no significant differences observed at baseline. In the presence of hypercapnia, Pdi (mean [SD], cm H2O) at low- (20-Hz) and high-frequency (100-Hz) stimulation was significantly decreased from baseline in each group (all, \( P = 0.001 \)). In groups 1 and 2, Pdi at both stimuli was significantly increased during DBcAMP administration compared with hypercapnia-induced values (group 1: 20-Hz, 13.5 [2.2] vs 15.0 [2.4], \( P = 0.001 \); 100-Hz, 21.2 [1.6] vs 22.5 [1.6], \( P = 0.001 \); group 2: 20-Hz, 19.2 [1.7], \( P = 0.001 \); 100-Hz, 27.2 [2.5], \( P = 0.001 \)). The Pdi at both stimuli during DBcAMP administration was significantly higher in group 2 than in group 1 (20-Hz, 19.2 [1.7] vs 15.0 [2.4], \( P = 0.001 \); 100-Hz, 27.2 [2.5] vs 22.5 [1.6], \( P = 0.003 \)). In group 3, Pdi did not significantly change in regard to either stimulus from hypercapnia-induced values.
**Conclusion:** DBcAMP, in a dose-dependent manner, was associated with improved hypercapnic depression of diaphragmatic contractility in these pentobarbital-anesthetized dogs. (*Curr Ther Res Clin Exp*. 2010;71:154–161) © 2010 Excerpta Medica Inc.

**Key words:** muscle, diaphragm, contractility, hypercapnia, dibutyryl cyclic adenosine monophosphate (DBcAMP).

**INTRODUCTION**

Patients with chronic airway obstruction, who are hypercapnic, have decreased performance of respiratory muscles, especially the diaphragm.\(^1\) In healthy human subjects, hypercapnia is associated with diaphragm muscle dysfunction, which causes a reduction in the diaphragmatic force generated for a constant elective myographic activity.\(^2\) Several drugs have been assessed for their effects on diaphragmatic contractility during hypercapnia.\(^3,4\) Aminophylline, neostigmine, milrinone, and olprinone have been effective for improving hypercapnic depression of diaphragmatic contractility; however, isoproterenol did not affect diaphragm muscle dysfunction induced by hypercapnia.\(^3,4\) Dibutyryl cyclic adenosine monophosphate (DBcAMP), a derivative of cAMP, is more membrane permeable and less susceptible to intracellular hydrolysis than cAMP.\(^5\) It has been reported to improve cardiac performance in dogs through its inotropic and chronotropic effects\(^6\) and has been used for the treatment of congestive heart failure.\(^7\) Like cardiac contraction, diaphragmatic contractility of fatigued canine diaphragm was found to be augmented by DBcAMP.\(^8\) However, based on a search of MEDLINE and EMBASE (terms: muscle, diaphragm, contractility, hypercapnia, and DBcAMP; years: 1985–2008), no published data are available concerning hypercapnic depression of diaphragmatic contractility during DBcAMP administration.

The purpose of this experimental study was to assess the effects of DBcAMP on the performance of the diaphragm depressed by hypercapnia in pentobarbital-anesthetized dogs.

**MATERIALS AND METHODS**

This study was conducted at the Department of Anesthesiology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan, from July to December 2008. The protocol was approved by the university’s animal research committee, and animal care was conducted in accordance with guidelines for ethical animal research.\(^4\)

Healthy, adult (aged >5 years) mongrel dogs weighing 10 to 15 kg (provided by Animal Guidance Center, Mashiko, Japan) were used in the study. Animals were randomly assigned, using a computer-generated random-number list, to 1 of 3 groups: group 1 received low-dose DBcAMP (0.05 mg/kg/min); group 2 received high-dose DBcAMP (0.2 mg/kg/min); and group 3 received placebo (saline).

Animal preparation was similar to that previously described.\(^4\) Briefly, anesthesia was maintained with pentobarbital 2 mg/kg/h IV. This dose has been found to have no effect on diaphragmatic contractility.\(^8\) No muscle relaxants were used. Each dog’s trachea was intubated, and ventilation was mechanically controlled with a mixture of oxygen (O\(_2\)) and air (fraction of inspired O\(_2\), 0.4) to maintain partial pressure of O\(_2\) in
arterial blood (PaO₂) >100 mm Hg, partial pressure of carbon dioxide in arterial blood (PaCO₂) 35 to 40 mm Hg, and partial pressure of hydrogen in arterial blood (pHa) 7.35 to 7.45. The right femoral artery was cannulated for monitoring arterial blood pressure. The right femoral vein was cannulated for administering maintenance fluids (lactated Ringer’s solution 10 mg/kg/h) and pentobarbital. The left femoral vein was cannulated to administer the study drug. Rectal temperature was monitored continuously and maintained at 36.5°C to 37.5°C using a heating pad.

Transdiaphragmatic pressure (Pdi) was measured as previously described. Briefly, 2 thin-walled latex balloons were used: 1 positioned in the stomach and 1 in the middle third of the esophagus. Balloons were connected to a differential pressure transducer (TP604T, Nihon Koden, Tokyo, Japan) and an amplifier (model 1257, Nihondenki San-ei, Tokyo, Japan). Phrenic nerves were exposed bilaterally at the neck, and stimulating electrodes were placed around them. Supramaximal electrical stimuli (10–15 V) of 0.1-millisecond duration were applied for 2 seconds at low- (20-Hz) and high-frequency (100-Hz) stimulation. Diaphragmatic contractility was evaluated by measuring the maximal Pdi generated by test stimuli after airway occlusion at functional residual capacity (FRC). Electrical activity of the diaphragm was recorded by using 2 pairs of fishhook electrodes, and its signal was rectified and integrated with an integrator with a time constant of 0.1 second. This was regarded as the integrated electrical activity of the crural (Edi-cru) and costal (Edi-cost) parts of the diaphragm.

Dogs were allowed to stabilize for ≥30 minutes before study start. Baseline measurements of heart rate (HR), mean arterial pressure (MAP), pHa, PaCO₂, PaO₂, Pdi, Edi-cru, and Edi-cost were recorded in each group. Hypercapnia (PaCO₂ 80–90 mm Hg) was produced by adding 10% CO₂ to the inspired gas. Hypercapnia was established at 2 hours after the initiation of CO₂ administration. At that point, group 1 was infused with low-dose DBcAMP (0.05 mg/kg/min); group 2 was infused with high-dose DBcAMP (0.2 mg/kg/min); and group 3 received placebo (saline). An infusion pump was used to administer the study drug intravenously for 1 hour. HR, MAP, pHa, PaCO₂, PaO₂, Pdi, Edi-cru, and Edi-cost were then measured. Saline was administered at a rate of 10 mL/h. Percentage changes from baseline in Edi-cru (%Edi-cru) and Edi-cost (%Edi-cost) were determined.

**Statistical Analysis**

An a priori power analysis indicated that 7 dogs per group would be required to detect a significant difference in Pdi to each stimulus between baseline, hypercapnia, and treatment periods with a power (1-β) of 0.8 (α = 0.05). Values are reported as mean (SD). Statistical analyses were performed by using ANOVA for repeated measurements, followed by the Bonferroni-Dunn test for multiple comparisons and t test, where appropriate. P < 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 8.0 (SPSS Inc., Chicago, Illinois).

**RESULTS**

Twenty-one adult mongrel dogs (15 males and 6 females; mean [SD] weight, 12.5 [1.5] kg) were included in the study; 7 dogs were assigned to each treatment
group. No significant differences in HR, MAP, pHa, PaCO₂, PaO₂, Pdi, %Edi-cru, and %Edi-cost were observed between the 3 groups at baseline (Tables I and II).

When hypercapnia was established, HR (mean [SD], bpm) and MAP (mean [SD], mm Hg) were significantly increased compared with baseline in each group (group 1: HR, 149 [3] vs 140 [3], \( P = 0.001 \), MAP, 130 [6] vs 121 [6], \( P = 0.001 \); group 2: HR, 148 [4] vs 139 [5], \( P = 0.001 \), MAP, 131 [7] vs 121 [5], \( P = 0.001 \); group 3: HR, 151 [7] vs 140 [6], \( P = 0.001 \), MAP, 134 [5] vs 123 [6], \( P = 0.001 \) (Table I). After the administration of DBcAMP, HR was significantly increased in groups 1 and 2 and MAP was significantly decreased in group 2 compared with values obtained during hypercapnia (group 1: HR, 155 [3] vs 149 [3], \( P = 0.001 \); group 2: HR, 166 [5] vs 148 [4], \( P = 0.001 \), MAP, 117 [6] vs 131 [7], \( P = 0.001 \).

By administrating CO₂ to induce hypercapnia, a significant increase in PaCO₂ (mean [SD], mm Hg) and a significant decrease in pHa (mean [SD]) were observed in each group (group 1: PaCO₂, 85.0 [3.5] vs 37.4 [1.9], \( P = 0.001 \), pHa, 7.12 [0.03] vs 7.41 [0.01], \( P = 0.001 \); group 2: PaCO₂, 84.7 [3.9] vs 36.5 [0.7], \( P = 0.001 \), pHa, 7.13 [0.02] vs 7.42 [0.01], \( P = 0.001 \); group 3: PaCO₂, 84.4 [3.3] vs 36.2 [0.4], \( P = 0.001 \), pHa, 7.13 [0.01] vs 7.40 [0.01], \( P = 0.001 \) (Table I). No significant difference in PaO₂ was observed.

In the presence of hypercapnia, Pdi (mean [SD], cm H₂O) at low- (20-Hz) and high-frequency (100-Hz) stimulation significantly decreased from baseline in each group (group 1: 20 Hz, 15.1 [2.6] vs 13.5 [2.2], \( P = 0.001 \), 100 Hz, 23.2 [1.6] vs 21.2 [1.6], \( P = 0.001 \); group 2: 20 Hz, 15.2 [1.4] vs 13.7 [1.4], \( P = 0.001 \), 100 Hz, 23.1 [2.4] vs 21.0 [2.4], \( P = 0.001 \); group 3: 20 Hz, 15.0 [2.3] vs 13.4 [2.7], \( P = 0.001 \), 100 Hz, 23.1 [2.8] vs 20.8 [2.9], \( P = 0.001 \) (Table II). In group 3, Pdi to each stimulus did not change from hypercapnia-induced values. In groups 1 and 2, during DBcAMP administration, Pdi at both stimuli frequencies significantly increased compared with the hypercapnia-induced values (group 1: 20-Hz, 13.5 [2.2] vs 15.0 [2.4], \( P = 0.001 \), 100-Hz, 21.2 [1.6] vs 22.5 [1.6], \( P = 0.001 \); group 2: 20-Hz, 13.7 [1.4] vs 19.2 [1.7], \( P = 0.001 \), 100-Hz, 21.0 [2.4] vs 27.2 [2.5], \( P = 0.001 \)). The Pdi at both stimuli frequencies during DBcAMP administration was significantly higher in group 2 than that in group 1 (20 Hz, 19.2 [1.7] vs 15.0 [2.4], \( P = 0.001 \); 100 Hz, 27.2 [2.5] vs 22.5 [1.6], \( P = 0.004 \)). No changes in %Edi-cru and %Edi-cost were observed throughout the experiment in any group (Table II).

### DISCUSSION

The major findings of this study were as follows: (1) when hypercapnia was established, Pdi at 20-Hz and 100-Hz stimulation significantly decreased compared with baseline values; and (2) DBcAMP was associated with increased Pdi at both stimuli frequencies in a dose-dependent manner. These findings are suggestive of the positive inotropic effects of DBcAMP on the diaphragmatic contractility in patients during hypercapnia, which may contribute to the development of diaphragm muscle dysfunction.

Diaphragmatic contractility is assessed by force-frequency characteristics\(^9\)–\(^11\) and is often evaluated by the measurement of Pdi, which is affected by the length and geometry of the diaphragm during precontracted conditions.\(^12\) A major determinant
of the diaphragmatic length and geometry is lung volume. Conceivably, the changes in Pdi may be secondary to changes in the end-expiratory lung volume. In this study, however, the airway was occluded at the end-expiratory lung volume (ie, FRC) during measurements.

Hypercapnia impairs diaphragmatic contractility in vivo and in vitro. Previous research has found hypercapnic depression of diaphragmatic contractility in pentobarbital-anesthetized dogs. In that study, Pdi at 20- and 100-Hz stimulation significantly decreased from baseline (all, $P = 0.001$) in the presence of hypercapnia. As we have stated in the past, the mechanism by which hypercapnia decreases diaphragmatic

<table>
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<th>Variables</th>
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<th>Treatment</th>
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<td>155 (3)*††‡</td>
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HR = heart rate; group 1 = DBcAMP 0.05 mg/kg/min; group 2 = DBcAMP 0.2 mg/kg/min; group 3 = placebo (saline); MAP = mean arterial pressure; pHa = partial pressure of hydrogen in arterial blood; PaCO₂ = partial pressure of carbon dioxide in arterial blood; PaO₂ = partial pressure of oxygen in arterial blood.

* $P = 0.001$ versus baseline.
† $P = 0.001$ versus hypercapnia.
‡ $P = 0.001$ versus control (group 3).
§ $P = 0.002$ versus group 1.
‖ $P = 0.025$ versus group 1.
contractility is unknown, but there is a possibility that an alteration of muscle pH may be related to a decrease in the affinity of troponin for calcium, an increase in the binding of calcium by the sarcoplasmic reticulum, or a decrease in the rate of glycolysis and, therefore, adenosine triphosphate resynthesis.15–17

In the present study, we found that Pdi at 20- and 100-Hz stimulation significantly increased from hypercapnia-induced values with an infusion of DBcAMP (0.05 or 0.2 mg/kg/min). The exact mechanism by which DBcAMP improves diaphragm...
matic contractility during hypercapnia is not known. However, it has been suggested that DBcAMP may have a direct positive effect on diaphragmatic contractility. DBcAMP was associated with increased intracellular cAMP, which promotes the activation of calcium ion (Ca$^{2+}$) transport from the sarcoplasmic reticulum. Therefore, DBcAMP may increase the diaphragmatic contractility by influencing Ca$^{2+}$ transport across the cell membrane.

No significant changes in %Edi-cru and %Edi-cost were observed in any group. This suggests that hypercapnia and DBcAMP (0.05 or 0.2 mg/kg/min) administration do not affect the electrical activity of the diaphragm.

Signs of CO$_2$ retention are tachycardia and hypertension. When hypercapnia (80–90 mm Hg) was established in this study, HR and MAP significantly increased from baseline. With an infusion of DBcAMP, HR increased significantly and MAP decreased significantly from that obtained during hypercapnia in groups 1 and 2. These hemodynamic changes during DBcAMP administration are consistent with those we found previously.

The data described from this experiment provides information on the improvement in hypercapnic depression of diaphragmatic contractility associated with DBcAMP administration. However, this must be considered within the context of the study’s limitations. First, the effective dose of DBcAMP for diaphragm muscle function is unknown. The recommended dose of DBcAMP for the improvement of myocardial performance is between 0.05 and 0.2 mg/kg/min. Previously, we examined the diaphragm muscle function during fatigue by administering DBcAMP 0.2 mg/kg/min in dogs. Second, the exact mechanism by which DBcAMP affected hypercapnic depression of diaphragmatic contractility was not studied. However, there is a possibility that DBcAMP may increase the diaphragmatic contractility by influencing Ca$^{2+}$ transport across the cell membrane. Regardless of the unknown mechanism of action, our results have therapeutic implications for diaphragm muscle dysfunction associated with hypercapnia. Third, our sample size was small. However, when a power analysis was performed, a total of 7 dogs in each group were found to be sufficient to detect a significant difference with $\alpha$ of 0.05 and a power of 80%. Lastly, the study was not blinded. Further studies should consider these limitations.

**CONCLUSION**

DBcAMP, in a dose-dependent manner, was associated with improved hypercapnic depression of diaphragmatic contractility in pentobarbital-anesthetized dogs.

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