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Dibutyryl cyclic AMP increases the contractility of fatigued diaphragm in dogs

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Abstract: The effects of dibutyryl cyclic AMP (DBcAMP) on the contractility of nonfatigued and fatigued diaphragms were studied in 36 anesthetized and mechanically ventilated dogs. The animals were divided into four groups. In group C_1 (n = 8), dogs without fatigue received only Ringer's lactate solution. In group D_1 (n = 8), dogs without fatigue were given a continuous infusion of DBcAMP 0.2 mg·kg^{-1}·min^{-1}. In groups C_2 and D_2 (n = 10 each), diaphragmatic fatigue was induced by intermittent supramaximal bilateral electrophrenic stimulation at a frequency of 20 Hz applied for 30 min. In group D_2, after producing fatigue, DBcAMP 0.2 mg·kg^{-1}·min^{-1} was administered. In groups C_2, only Ringer's solution was administered during this period. Diaphragmatic contractility was assessed by measuring the transdiaphragmatic pressure (P_{di}, cmH_2O). No difference in P_{di} was observed in groups C_1 and D_1. After diaphragmatic fatigue in groups C_2 and D_2, P_{di} at low-frequency (20-Hz) stimulation decreased significantly compared with the prefatigue values (group C_2; 9.3 ± 1.9 *vs* 12.5 ± 2.4, group D_2; 9.3 ± 2.1 *vs* 12.5 ± 2.6; mean ± SD; $P < 0.05$), whereas no change in P_{di} was observed at high-frequency (100-Hz) stimulation. In group D_2, P_{di} at both stimuli increased significantly with an infusion of DBcAMP compared with the fatigue values (20 Hz; 13.3 ± 3.3 *vs* 9.3 ± 2.1, 100 Hz; 23.4 ± 3.6 *vs* 21.3 ± 3.2; $P < 0.05$). In group C_2, the speed of recovery from fatigue was relatively slower at 20-Hz stimulation than at 100-Hz stimulation. It is concluded that DBcAMP increases the contractility of fatigued diaphragm, but that this agent does not affect the contractility of nonfatigued diaphragm.

Key words: Diaphragm, Fatigue, Dibutyryl cyclic AMP

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Introduction

Several investigators have demonstrated that aminophylline, β_2 agonists, digoxin, and dopamine have positive inotropic effects on fatigued diaphragm [1–4]. Recently, we have also shown that dobutamine and amrinone increase the contractility of fatigued diaphragm [5,6]. Thus, these pharmacological agents which have been shown to augment myocardial contraction may increase the contractility of fatigued diaphragm. It is known that dibutyryl cyclic AMP (DBcAMP) increases myocardial contraction [7]. However, to our knowledge, the effects of DBcAMP on the contractility of fatigued diaphragm have not been reported. Therefore, the present study was designed to assess the changes in contractility of fatigued diaphragm after administration of dibutyryl cyclic AMP (DBcAMP).

Methods

Animal preparation

Institutional approval for the experiment was obtained from the Animal Care and Use Committee of Tokyo Medical and Dental University School of Medicine. Thirty-six healthy mongrel dogs weighing between 10 and 15 kg were anesthetized with ketamine 20 mg·kg^{-1} im and with supplemental doses of pentobarbital sodium 2 mg·kg^{-1}·hr^{-1} iv to abolish spontaneous movement. No muscle relaxants were used. The animals were placed in the supine position, their tracheas were intubated with a cuffed tracheal tube, and the lungs were mechanically ventilated with a mixture of oxygen and air (FiO_2 = 0.4) to maintain over 100 torr of Pao_2, 35–40 torr of $Paco_2$, and a pH of 7.35–7.45. The right femoral artery was cannulated to monitor arterial blood pressure and to draw blood samples for measurement of

arterial blood gas tensions. The right femoral vein was cannulated to administer $10\,ml \cdot kg^{-1} \cdot hr^{-1}$ of Ringer's lactate solution, pentobarbital sodium, and bicarbonate to correct metabolic acidosis. The left femoral vein was also cannulated for administering DBcAMP. A pulmonary artery catheter was advanced via the right external jugular vein into the pulmonary artery for cardiac output measurement by the thermodilution technique. Rectal temperature was monitored continuously and maintained at 37 ± 1°C.

The phrenic nerves were exposed bilaterally in the neck and the stimulating electrodes were placed around them under mineral oil. Transdiaphragmatic pressure (P_{di}) was measured by means of two thin-walled latex balloons, one positioned in the stomach, the other in the middle third of esophagus. Balloons were connected to a differential pressure transducer (Pressure Head, Tokyo Keiki, Tokyo, Japan) and an amplifier (Type 1212, Nihondenki San-ei, Tokyo, Japan). Supramaximal electrical stimuli (10–15 volts) of 0.1-ms duration lasting 2 s were applied at low frequency (20 Hz) and high frequency (100 Hz) with an electrical stimulator (Electronic Stimulator 3F37, Nihondenki San-ei). Diaphragmatic contractility was evaluated by measuring the maximal P_{di} after airway occlusion at functional residual capacity (FRC), so that the initial length of diaphragm was maintained at the same level. Transpulmonary pressure (P_{tp}), the difference between airway and esophageal pressures, was kept constant by maintaining the same lung volume before each phrenic stimulation. End-expiratory diaphragmatic geometry and muscle fibers were kept constant by placing a close-fitting plaster cast around the abdomen and lower one-third of the rib cage. The electrical activity of the diaphragm was measured with needle electrodes inserted percutaneously into the diaphragm from the upper abdominal area, and was rectified and integrated with a permeable integrator (Type 1310, Nihondenki San-ei) with a time constant of 0.1 s. This was regarded as the integrated diaphragmatic electrical activity (E_{di}).

Experimental protocol

The dogs were randomly divided into four groups: groups C_1 ($n = 8$) and D_1 ($n = 8$) in the nonfatigued model, and groups C_2 ($n = 10$) and D_2 ($n = 10$) in the fatigued model. After pre-DBcAMP (baseline) measurements of P_{di}, E_{di}, and hemodynamic variables including heart rate (HR), mean arterial pressure (MAP), right atrial pressure (RAP), mean pulmonary arterial pressure (MPAP), pulmonary wedge pressure (PCWP), and cardiac output ($\dot{Q}t$), the dogs in group D_1 were given a continuous administration of DBcAMP $0.2\,mg \cdot kg^{-1} \cdot min^{-1}$ iv clinically with an electrical infusion pump (Terumo, Tokyo, Japan) for 30 min. Thirty min after the onset of DBcAMP infusion and 60 min after the end of DBcAMP infusion, P_{di}, E_{di}, and hemodynamic variables were measured, and $\dot{Q}t$ was evaluated by the thermodilution technique. In group C_1, only Ringer's lactate solution was administered and these measurements were made at 30 and 90 min to verify the stability of this preparation.

After measuring the prefatigue (baseline) values of P_{di}, E_{di}, and hemodynamic variables including HR, MAP, PAP, PCWP, and $\dot{Q}t$ in groups C_2 and D_2, diaphragmatic fatigue was induced by intermittent supramaximal bilateral electrophrenic stimulation applied for 30 min at a frequency of 20 Hz, with an entire cycle of 4 s and a duty cycle of 0.5 s (low-frequency fatigue) [8]. In group D_2, DBcAMP $0.2\,mg \cdot kg^{-1} \cdot min^{-1}$ iv was administered continuously with an infusion pump for 30 min after producing fatigue. At 30 min after the start of DBcAMP infusion and 60 min after the cessation of DBcAMP infusion, P_{di}, E_{di}, and hemodynamic variables were measured. In group C_2, only Ringer's lactate solution was administered and these measurements were made at 30 and 90 min after the fatigue-producing period (recovery period).

Data analysis

All values are expressed as mean ± standard deviation (SD). Statistical analysis was performed using analysis of variance (ANOVA) for repeated mmeasurements, and a multiple comparison test (Duncan) was used for determining different mean values. A $P < 0.05$ was regarded as statistically significant.

Results

Effects of DBcAMP on hemodynamics, P_{di}, and E_{di} in nonfatigued diaphragm

No differences were observed in the baseline hemodynamic variables between groups C_1 and D_1 (Table 1). In group D_1, with an infusion of DBcAMP, HR and $\dot{Q}t$ increased ($P < 0.05$) and MAP, MPAP, and PCWP decreased ($P < 0.05$) compared with the baseline values. After the end of administration, these values returned to the baseline. In group C_1, no changes in hemodynamic variables were observed.

The P_{di} values in groups C_1 and D_1, obtained at each frequency stimulation, are shown in Table 2. P_{di} was not affected by infusion of DBcAMP in group D_1. No change in E_{di} was observed in groups C_1 and D_1 throughout the experiment.

Table 1. Hemodynamic changes in the nonfatigue model

			Recovery 30 min 90 min (Group C_1)	
Variable	Group	Pre-DBcAMP	DBcAMP	Post-DBcAMP (Group D_1)
HR	C_1	144 ± 13	145 ± 12	144 ± 10
(bpm)	D_1	145 ± 15	156 ± 14[a,b]	143 ± 14
MAP	C_1	106 ± 11	105 ± 13	105 ± 11
(mmHg)	D_1	105 ± 10	94 ± 9[a,b]	103 ± 13
RAP	C_1	5 ± 2	5 ± 1	5 ± 1
(mmHg)	D_1	5 ± 1	5 ± 1	5 ± 1
MPAP	C_1	14 ± 3	14 ± 2	14 ± 3
(mmHg)	D_1	14 ± 2	12 ± 2[a,b]	14 ± 2
PCWP	C_1	9 ± 2	9 ± 2	9 ± 2
(mmHg)	D_1	9 ± 2	7 ± 1[a,b]	9 ± 2
$\dot{Q}t$	C_1	2.1 ± 0.2	2.2 ± 0.4	2.1 ± 0.3
($l \cdot min^{-1}$)	D_1	2.2 ± 0.3	2.8 ± 0.3[a,b]	2.2 ± 0.5

DBcAMP, dibutyryl cyclic AMP; HR, heart rate; MAP, mean arterial pressure; RAP, right atrial pressure; MPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; $\dot{Q}t$ = cardiac output.
All values are expressed as mean ± SD.
[a] $P < 0.05$ vs Pre-DBcAMP.
[b] $P < 0.05$ vs group C_1.

Table 2. Changes in $P_{di}(cmH_2O)$ in nonfatigued diaphragm

			Recovery 30 min 90 min (Group C_1)	
Frequency	Group	Pre-DBcMAP	DBcAMP	Post-DBcAMP (Group D_1)
20 Hz	C_1	12.5 ± 2.5	12.4 ± 2.8	12.5 ± 2.7
	D_1	12.6 ± 2.4	12.9 ± 3.0	12.4 ± 3.5
100 Hz	C_1	21.4 ± 3.3	21.3 ± 3.0	21.2 ± 2.8
	D_1	21.3 ± 3.0	21.4 ± 2.9	21.1 ± 3.1

P_{di}, transdiaphragmatic pressure.
All values are expressed as mean ± SD.

Table 3. Hemodynamic changes in fatigued model

				Recovery 30 min 90 min (Group C_2)	
Variable	Group	Prefatigue	Fatigue	DBcAMP	Post-DBcAMP (Group D_2)
HR	C_2	143 ± 11	145 ± 14	145 ± 10	141 ± 13
(bpm)	D_2	143 ± 15	141 ± 7	158 ± 17[a,b,c]	141 ± 18
MAP	C_2	104 ± 12	103 ± 12	103 ± 11	103 ± 13
(mmHg)	D_2	102 ± 13	102 ± 11	91 ± 10[a,b,c]	101 ± 9
RAP	C_2	5 ± 1	5 ± 1	5 ± 1	5 ± 2
(mmHg)	D_2	5 ± 2	5 ± 1	5 ± 1	5 ± 1
MPAP	C_2	14 ± 3	14 ± 2	14 ± 2	14 ± 3
(mmHg)	D_2	14 ± 2	14 ± 2	12 ± 2[a,b,c]	14 ± 2
PCWP	C_2	9 ± 2	9 ± 3	9 ± 2	9 ± 2
(mmHg)	D_2	9 ± 2	9 ± 2	7 ± 2[a,b,c]	9 ± 2
$\dot{Q}t$	C_2	2.1 ± 0.3	2.1 ± 0.4	2.2 ± 0.4	2.1 ± 0.3
($l \cdot min^{-1}$)	D_2	2.2 ± 0.6	2.2 ± 0.5	2.9 ± 0.7[a,b,c]	2.2 ± 0.5

HR, heart rate; MAP, mean arterial pressure; RAP, right atrial pressure; MPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; $\dot{Q}t$ = cardiac output.
All values are expressed as mean ± SD.
[a] $P < 0.05$ vs Prefatigue.
[b] $P < 0.05$ vs Fatigue.
[c] $P < 0.05$ vs group C_2.

Effects of DBcAMP on hemodynamics, P_{di}, and E_{di} in fatigued diaphragm

The hemodynamic results in groups C_2 and D_2 are summarized in Table 3. There were no differences between the groups in hemodynamic variables during the prefatigue period. In group D_2, with an infusion of DBcAMP, increases in HR and $\dot{Q}t$ ($P < 0.05$) and decreases in MAP, MPAP, and PCWP ($P < 0.05$) were observed compared with the prefatigue values. After the cessation of administration, these values returned to the prefatigue levels. In group C_2, there were no hemodynamic changes.

The P_{di} values in both groups are shown in Table 4. After the fatigue-producing period, P_{di} at low-frequency (20-Hz) stimulation decreased from the prefatigue values ($P < 0.05$), whereas P_{di} at high-frequency (100-Hz) stimulation did not change. In group D_2, P_{di} at both stimuli increased compared with the fatigue values ($P < 0.05$) during DBcAMP administration. After the end of infusion, P_{di} returned to the fatigue values. In group C_2, the speed of recovery from fatigue was relatively slower at 20-Hz stimulation than at 100-Hz stimulation. There was no change in E_{di} in groups C_2 and D_2 throughout the experiment.

Discussion

The major findings of the present study were: (a) P_{di} of nonfatigued diaphragm was not affected by infusion of DBcAMP and (b) DBcAMP increased the P_{di} of fatigued diaphragm.

The pressure generated by the diaphragm after a given electrical stimulation depends on its length and geometry [9]. The change in P_{di} might conceivably be secondary to changes in end-expiratory lung volume. However, in the present study, the airway was concluded at the end-expiratory lung volume during the measurements and its constancy was monitored by measuring the end-expiratory P_{tp}. Therefore, changes in lung volume during the experimental procedures can reasonably be excluded. Furthermore, the plaster cast around the lower one-third of the thorax and the abdomen was also placed to prevent deformation of the thoracoabdominal structure.

Hypoxemia, hypercapnia, and metabolic acidosis decrease the contractility of fatigued diaphragm [10,11]. However, in the present study, Pao_2, $Paco_2$, and serum bicarbonate were controlled within normal ranges. Therefore, major factors which may affect diaphragmatic contractility were excluded.

As the dogs were basically anesthetized with pentobarbital, we may have to consider the combined effects of DBcAMP and pentobarbital. However, it has been previously reported that pentobarbital, at the dose used in the present study, does not affect the contractility of nonfatigued diaphragm [6]. This was also in accordance with constant P_{di} observed in group C_1 throughout the experiment.

Low-frequency fatigue is of particular clinical importance because the spontaneous, natural rate of phrenic nerve discharge is mainly in the low-frequency range (5–30 Hz) [12]. Therefore, in the present study, the effect of DBcAMP on contractility was examined in fatigued diaphragm induced by 20-Hz stimulation (low-frequency fatigue).

The results of group C_2, in which P_{di} was observed without an administration of DBcAMP in fatigued diaphragm, showed that P_{di} at 20-Hz stimulation had a tendency to recover more slowly than that of P_{di} at 100-Hz stimulation and that of E_{di} at either frequency stimulation. This was in agreement with our previous studies [5,6].

The present study demonstrated that DBcAMP did not affect P_{di} in nonfatigued diaphragm (group D_1), but that P_{di} of fatigued diaphragm was increased with an infusion of DBcAMP (group D_2). The mechanism by which DBcAMP enhances P_{di} only in fatigued diaphragm is not entirely clear. However, it has been postulated that DBcAMP may inhibit phosphodiesterase, eventually increasing the intracellular cyclic AMP level,

Table 4. Changes in $P_{di}(cmH_2O)$ in fatigued diaphragm

Frequency	Group	Prefatigue	Fatigue	Recovery 30 min 90 min (Group C_2)	
				DBcAMP	Post-DBcAMP (Group D_2)
20 Hz	C_2	12.5 ± 2.4	9.3 ± 1.9[a]	9.4 ± 2.2[a]	9.4 ± 2.4[a]
	D_2	12.5 ± 2.6	9.3 ± 2.1[a]	13.3 ± 3.3[b,c]	9.5 ± 2.0[a]
100 Hz	C_2	21.4 ± 2.3	21.1 ± 2.3	21.1 ± 2.3	21.2 ± 2.2
	D_2	21.6 ± 3.1	21.3 ± 3.2	23.4 ± 3.6[b,c]	21.4 ± 3.2

Pdi, transdiaphragmatic pressure.
All values are expressed as mean ± SD.
[a] $P < 0.05$ vs Prefatigue.
[b] $P < 0.05$ vs Fatigue.
[c] $P < 0.05$ vs group C_2.

and that the resulting increases in intracellular cyclic AMP may promote the activation of Ca^{2+} transport from the sarcoplasmic reticulum [7]. Low-frequency fatigue is closely related to the impairment of excitation-contraction coupling [13], and this impairment is suggested to be the result of the impediment of mobility of Ca^{2+} from the sarcoplasmic reticulum [14]. Therefore, it is suggested that DBcAMP increases the contractility of fatigued diaphragm with the reversal of this impaired Ca^{2+} release from the sarcoplasmic reticulum.

Diaphragmatic contractility depends on the energy supplies of the diaphragm, which are related to its blood supply [8], and $\dot{Q}t$ is one of the major factors determining blood flow to the diaphragm [15]. Diaphragmatic fatigue occurs when the muscular energy demands exceed the capacities of the energy supply associated with blood flow [16,17]. Therefore, an increase in $\dot{Q}t$, observed in the present study with an infusion of DBcAMP, may also have led to an increase in blood flow to the diaphragm, and thereby increased the contractility of fatigued diaphragm. However, on the basis of our findings that DBcAMP did not increase P_{di} of nonfatigued diaphragm (group D_1), an increase in diaphragmatic blood flow induced by DBcAMP may be a relatively small factor in augmenting the contractility of fatigued diaphragm.

In conclusion, the present study suggests that DBcAMP increases the contractility of fatigued diaphragm, and that this effect may be caused by the reversal of impaired Ca^{2+} release in fatigued diaphragm.

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