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 C1 (n = 8), dogs without fatigue received only Ringer's lactate solution. In group D1 (n = 8), dogs without fatigue were given a continuous infusion of DBcAMP 0.2mg.kg⁻¹.min⁻¹. In groups C2 and D2 (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 D2, after producing fatigue, DBcAMP 0.2mg.kg⁻¹.min⁻¹ was administered. In groups C2, only Ringer's solution was administered during this period. Diaphragmatic contractility was assessed by measuring the transdiaphragmatic pressure (Pd, cmH₂O). No difference in Pd was observed in groups C1 and D1. After diaphragmatic fatigue in groups C2 and D2, Pd at low-frequency (20 Hz) stimulation decreased significantly compared with the prefatigue values (group C2; 9.3 ± 1.9 vs 12.5 ± 2.4, group D2; 9.3 ± 2.1 vs 12.5 ± 2.6; mean ± SD; P < 0.05), whereas no change in Pd was observed at high-frequency (100 Hz) stimulation. In group D2, Pd at both stimuli increased significantly with an infusion of DBcAMP compared with the fatigue values (20 Hz; 13.5 ± 3.3 vs 9.3 ± 2.1, 100 Hz; 23.4 ± 3.6 vs 21.3 ± 3.2; P < 0.05). In group C2, the speed of recovery from fatigue was relatively slower at 20-Hz stimulation than at 100-Hz stimulation. It is concluded that dibutyryl cyclic AMP (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

Introduction

Several investigators have demonstrated that aminophylline, β₂ 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 (FiO₂ = 0.4) to maintain over 100 torr of Pao₂, 35-40 torr of Paco₂ 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 kg\(^{-1}\) 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 2s 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 \(P_a\) 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 \(P_a\), \(E_{di}\), 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 (Qt), the dogs in group \(D_1\) were given a continuous administration of DBCAMP 0.2 mg kg\(^{-1}\) 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, \(P_a\), \(E_{di}\), and hemodynamic variables were measured, and \(Qt\) 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 \(P_a\), \(E_{di}\), and hemodynamic variables including HR, MAP, PAP, PCWP, and \(Qt\) 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 kg\(^{-1}\) 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, \(P_a\), \(E_{di}\), 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 measurements, 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, \(P_a\), 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 \(Qt\) 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 \(P_a\) values in groups \(C_1\) and \(D_1\) obtained at each frequency stimulation, are shown in Table 2. \(P_a\) 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.