Cardiovascular Effects of Dibutyryl Cyclic AMP in the Intact Dog Heart and the Isolated Cross-Perfused Right Atrium

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Summary

This study used an isolated right atrial preparation, cross-perfused with arterial blood from a support dog. We investigated the effects of dibutyryl cyclic AMP (DBcAMP) on heart rate and arterial blood pressure of the support dog and on the sinus rate and atrial contractile force of the isolated perfused atrium. DBcAMP was injected into the external jugular vein of the support dog or into the sinus node artery of the isolated atrium. DBcAMP to the support dog induced a small increase and/or decrease in arterial blood pressure at a dose of 3 or 10 mg/Kg i.v. It produced a decrease, with or without an increase in blood pressure, at a dose of 30 mg/Kg i.v., and a dose-dependent increase in heart rate in the support dog. In the isolated atrium, positive chronotropic and inotropic effects were observed. Direct injection of DBcAMP (3–30 mg) into the sinus node artery of the isolated atrium induced positive chronotropic and inotropic effects, after initial brief negative effects, in a dose-dependent manner. DBcAMP did not change norepinephrine-induced positive chronotropic and inotropic effects in the isolated atrium. These results demonstrate that DBcAMP induces a decrease in systemic arterial blood pressure with increases in sinus rate and atrial contractile force, and acts additively with the norepinephrine-induced positive cardiac effects in the dog heart.

Additional Indexing Words:
Arterial blood pressure Contractility Dibutyryl cAMP Intact dog heart Isolated atrium Heart rate Phosphodiesterase

The hypothesis that cyclic AMP (cAMP) acts as a second messenger for catecholamines is widely accepted, although it does not explain all catecholamine effects.13–9 However, cAMP does not produce positive cardiac effects like catecholamines when applied exogenously due to poor penetration
of the cell membrane. On the other hand, the N6-2'-O-dibutyryl derivative of cAMP (DBcAMP) readily crosses cellular membranes and accumulates as cAMP and monobutyryl cAMP in the cell. After Kukovetz reported positive inotropic effects of DBcAMP in perfused mammalian hearts, positive chronotropic and inotropic effects of DBcAMP were demonstrated in isolated hearts. An increase in coronary flow was also reported, together emphasizing the similarity of cardiac effects of DBcAMP and catecholamines. In addition, it was reported that derivatives of cAMP, including DBcAMP, can inhibit cAMP phosphodiesterase (PDE) in cardiac tissues of the cat and that DBcAMP decreases peripheral vascular resistance in anesthetized dogs. Recently, beneficial vasodilating effects of DBcAMP were reported in patients with congestive heart failure by Hashimoto et al and Matsui et al. In the present study, we compared the effects of DBcAMP on heart rate and arterial blood pressure of an anesthetized support dog with the effects of DBcAMP on sinus rate and atrial contractility of an isolated right atrium perfused with arterial blood from the support dog. We also examined the effects of DBcAMP on catecholamine-induced cardiac effects in the same system.

**Methods**

Isolated atria were obtained from 20 mongrel dogs. Each preparation was perfused with arterial blood from an anesthetized support dog. The details of this preparation have been described previously.

The support dogs, which weighed 10 to 25 Kg, were anesthetized with sodium pentobarbital (30 mg/Kg, i.v.) and ventilated by means of a Harvard respirator. Sodium heparin (500 units/Kg) was administered intravenously to each dog at the beginning of the perfusion and 200 units/Kg were added each hour thereafter. The recipient dogs, weighing 7 to 18 Kg, were also anesthetized with sodium pentobarbital. Heparin (200 units/Kg) was given intravenously and the right atria were excised and immersed in cold Tyrode's solution. The wet weight of the isolated preparations varied from 6 to 14 g. In each preparation, the sinus node artery was cannulated via the right coronary artery and was perfused with blood conducted from the carotid artery of the support dog with the aid of a peristaltic pump (Harvard Apparatus model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained at a constant level of 100 mmHg. The blood flow rate through the isolated atrium was 4 to 10 ml/min. The venous effluent from the preparation was led to a collecting funnel, from which it was returned continuously to the sup-
Port dog via an external jugular vein.

The ventricular margin of the atrium was attached to a rigid stainless steel bar and the preparation was placed in a glass container. The superior part of the atrium was connected to a force transducer (Nihon Kohden AP-620) by a silk thread. The atrial muscle was usually stretched to a resting tension of 2 g. The isometric tension was recorded on a thermal writing rectigraph (Nihon Kohden). A pair of electrodes, placed on the atrial free wall, was used to record the electrogram. The sinus rate was derived from the atrial electrogram (Nihon Kohden AT600G). The heart rate derived from the ECG (lead II) and the femoral arterial blood pressure of the support dog were also recorded on the rectigraph.

Drugs used in the present study were dibutyryl cyclic AMP (sodium N-2'-O-dibutyryl adenosine 3', 5' cyclic phosphate; DBcAMP, Daiichi Seiyaku), d,l-norepinephrine hydrochloride (Sankyo), l-isoproterenol hydrochloride (Nikken Kagaku) and propranolol hydrochloride (Sigma). DBcAMP was dissolved in distilled water and other drugs were dissolved in physiological saline. The drug was injected into the external jugular vein of the support dog or into the sinus node artery of the isolated atrium. Data reflect the maximum change in each response. The data were analyzed by Student's t-test for paired values.

**Results**

*Effects of DBcAMP, injected intravenously into the support dog, on heart rate and systemic arterial blood pressure of support dogs and on sinus rate and atrial contractile force of isolated, blood-perfused atria:*

Figure 1 shows the effects of intravenous DBcAMP (30 mg/Kg) and isoproterenol (0.1 µg/Kg) on the heart rate and femoral arterial blood pressure of a support dog and on the sinus rate and atrial contractile force of an isolated right atrial preparation, perfused with arterial blood from the support dog. DBcAMP induced an increase in heart rate and triphasic changes, (a decrease with a transient increase followed by a long-lasting increase) in arterial blood pressure. In the isolated atrial preparation, about 3 min after the drug injection, the sinus rate increased markedly but the atrial contractile force increased only slightly (only 15%). On the other hand, isoproterenol (0.1 µg/Kg, i.v.) increased the heart rate and decreased the arterial blood pressure of the support dog, while increasing pacemaker activity and atrial contractility of the isolated atrium. These effects of isoproterenol were of greater amplitude and shorter duration than those of DBcAMP. The effects of DBcAMP on the cardiovascular responses are summarized in Fig.
Fig. 1. Effects of dibutyryl cyclic AMP (DBcAMP, 30 mg/Kg i.v.) and isoproterenol (0.1 µg/Kg i.v.) on the heart rate and femoral arterial blood pressure of a support dog. The effects on the sinus rate and atrial contractile force of an isolated right atrial preparation perfused with arterial blood from the support dog are also displayed.

Fig. 2. Dose-response curves of the effects of dibutyryl cyclic AMP (DBcAMP) injected into the jugular vein on maximum changes in heart rate and systolic and diastolic femoral arterial blood pressure of the support dog. The maximum changes in sinus rate and atrial contractile force of the isolated, cross-perfused atrial preparation are also shown for 6 experiments. The control levels of heart rate and systolic blood pressure (SBP) and diastolic blood pressure (DBP) in 6 support dogs were 151±13.5 (mean±SEM) beats/min and 146±16.4 and 80±7.6 mm Hg, respectively, and the control levels for sinus rate and atrial contractile force in 6 isolated atria were 105±3.5 beats/min and 2.0±0.4 g, respectively. Solid and dotted lines (left lower panel) present an increase and a decrease in arterial blood pressure, respectively. Vertical bars show SEM. *p<0.05 and **p<0.01.
2. DBcAMP, at a dose of 1 or 3 mg/Kg i.v., did not produce clear cardiac effects in either the support dog or the isolated, perfused atrium. DBcAMP, at a dose of 10 mg/Kg i.v., induced small increases in systolic and diastolic arterial blood pressure and heart rate of the support dog. In 2 out of 6 experiments, an increase followed by a decrease in the blood pressure response was observed. In the isolated, cross-perfused atrial preparation, a small but significant increase in sinus rate, but not atrial contractile force, was produced. However, DBcAMP at a dose of 30 mg/Kg i.v. induced a long-lasting increase in heart rate (p<0.05). It also produced either biphasic (a transient increase followed by a long-lasting decrease in arterial blood pressure in 3 out of 6 experiments) or triphasic hemodynamic responses (a decrease with transient increase followed by an increase in blood pressure). Although variable changes in blood pressure responses were observed in the support dog, positive chronotropic and inotropic effects were observed in the isolated, perfused atrial preparation (p<0.05) after DBcAMP administration.

**Effects of DBcAMP on NE-induced positive chronotropic and inotropic responses of the isolated, perfused atrium**

When DBcAMP was injected at a dose of 3 to 30 mg into the sinus node artery of the isolated, blood-perfused atrium, dose-dependent positive chronotropic and inotropic effects were observed after initial transient negative effects (Fig. 3). The positive cardiac effects of DBcAMP were observed after
Fig. 4. Effects of dibutylryl cyclic AMP on norepinephrine-induced increases in atrial rate and contractile force in 5 isolated, blood-perfused dog atria. The number in parentheses shows the number of experiments at each dose. Vertical bars represent SEM.

treatment with propranolol as previously reported. The NE-induced positive cardiac effects were not affected by treatment with 10 mg of DBcAMP in 5 experiments (Fig. 4) or with 30 mg of DBcAMP in 2 experiments.

**DISCUSSION**

We have demonstrated two basic effects of DBcAMP in cross-perfused atrial preparations. First, DBcAMP at doses of 10–30 mg/Kg induced either a decrease or an increase in systemic arterial blood pressure of the support dog, accompanied by increases in sinus rate and contractile force in the isolated, cross-perfused right atrial preparation. Second, DBcAMP had an additive effect on the positive chronotropic and inotropic actions of NE in the isolated atrium.

It has been reported that DBcAMP induces positive cardiac effects, with increases in both the sinus rate and contractile force in isolated mammalian hearts. The DBcAMP-induced cardiotonic effects are not blocked by propranolol, a β-adrenergic receptor antagonist, and are enhanced by amino-
phylline, a phosphodiesterase inhibitor. After DBcAMP penetrates the cell membrane, it is converted to cAMP and monobutyryl cAMP. Thus, it is likely that cardiotonic effects of DBcAMP are direct effects of cAMP in cardiac cells. Coronary flow is also increased by treatment with DBcAMP in perfused mammalian heart preparations and dog heart-lung preparations. In the electrophysiological investigations, DBcAMP has increased the slow inward current in mammalian ventricular muscle fibers and the slope of diastolic depolarization in sinoatrial nodal cells of the rabbit. We have confirmed the presence of persistent positive chronotropic and inotropic responses to DBcAMP after transient negative responses in isolated, perfused atrial preparations. The transient negative chronotropic and inotropic effects of DBcAMP may be mediated through adenosine receptors, because the DBcAMP-induced negative effects were blocked by aminophylline.

Effects in our study were only observed at a dose of 10-30 mg/Kg, i.v., though Nozaki and Okuaki reported effects of DBcAMP at a dose of 5 mg/Kg i.v. on the cardiovascular system in the anesthetized dog. They also observed decreases in renal and superior mesenteric arterial vascular resistance, a small decrease in systemic blood pressure, and increases in stroke volume, cardiac output and heart rate. In the present study, DBcAMP at a dose of 10 mg/Kg i.v., which is greater than a clinical dosage (0.025-0.3 mg/Kg/min), induced hemodynamic changes in the support dog but only small responses in the isolated atrium. In addition, DBcAMP at a dose of 30 mg/Kg i.v. evoked both a decrease with or without a prior increase, in systemic blood pressure and an increase in heart rate of the support dog. These changes were accompanied by consistent increases in sinus rate and atrial contractile force in the isolated, cross-perfused atrial preparation. Therefore, these results suggest that the vasodilating effect of DBcAMP is more prominent than the cardiotonic effects in the cardiovascular system of the dog.

Cyclic AMP plays an important role in the relaxation of vascular smooth muscle as reviewed by Kuriyama et al. However, the effects of DBcAMP on arterial blood pressure were variable between experiments. Although the effects of DBcAMP on the cardiovascular system were similar to those of isoproterenol, the effects of isoproterenol were more consistent. Isoproterenol-induced responses were definitely influenced by the density of ß-adrenoceptors in the tissues. However, DBcAMP-induced responses were not modified by ß-adrenoceptors, indicating different hemodynamic actions of these substances.

Several new phosphodiesterase inhibitors have been developed recently to improve cardiac contractility for pharmacological treatment of congestive heart failure. Harris et al. studied the effect of cyclic nucleotides on the
activity of cAMP phosphodiesterase in cat hearts and rat brains. In their experiments, DBcAMP and other cyclic nucleotides inhibited cat heart phosphodiesterase. This result raises the possibility that DBcAMP potentiates the effects of a β-adrenergic receptor agonist on the pacemaker activity and contractility in the heart, because an increase in cAMP is observed after treatment with catecholamines in cardiac tissues.1)-3), 21) However, in the isolated, perfused dog atrium, DBcAMP did not alter the NE-induced positive chronotropic and inotropic effects. These results suggest that DBcAMP has no significant inhibition of phosphodiesterase activity in perfused preparations.

References


