Responses to Exogenous Dibutyryl Adenosine 3',5'-Monophosphate of Cardiac Output and Blood Flow in the Renal, Superior Mesenteric and Carotid Arteries in Anesthetized Dogs

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NOZAKI, H. and OKUAKI, A. Responses to Exogenous Dibutyryl Adenosine 3', 5'-Monophosphate of Cardiac Output and Blood Flow in the Renal, Superior Mesenteric and Carotid Arteries in Anesthetized Dogs. Tohoku J. exp. Med., 1975, 115 (2), 145-154 — Changes in the cardiac output and blood flow in the renal, superior mesenteric and carotid arteries in anesthetized dogs were observed, using the non-cannulating electromagnetic flow meter. Dibutyryl cyclic-AMP, 5 mg/kg body weight, was given intravenously and the following results were obtained: 1) Dibutyryl cyclic-AMP increased the stroke volume and the cardiac output, and slightly increased the heart rate. These effects appeared 3 to 5 min after administration of dibutyryl cyclic-AMP. 2) The mean systemic blood pressure as well as the central venous pressure fell slightly. 3) The renal and the superior mesenteric artery blood flow increased markedly, but the carotid artery blood flow did not change. 4) Distribution of the cardiac output to the renal and superior mesenteric arteries did not change but distribution to the carotid artery decreased. 5) Total peripheral resistance, renal artery vascular resistance and superior mesenteric artery vascular resistance decreased, and carotid artery vascular resistance decreased slightly. 6) The cardiac output and blood flow were enhanced by aminophylline (3 mg/kg), and were not blocked by propranolol (0.3 mg/kg). — adenosine 3', 5'-monophosphate; circulatory response; hemodynamic change; peripheral resistance

It is well established that catecholamines cause a rapid increase in cyclic-AMP level in the heart muscle. The increase in cyclic-AMP level precedes the positive inotropic and chronotropic actions. Accordingly, Sutherland and co-workers (1965, 1966, 1968) have established the second messenger theory. But attempts to demonstrate an inotropic effect after addition of cyclic-AMP have been unsuccessful in rabbits (Rail and West 1963) and guinea pigs (De Gubareff and Sleator 1965). On the other hand, Kukovetz (1968) reported that addition of dibutyryl cyclic-AMP in some experiments increased the contractility. More recently, Skelton et al. (1970) reported that dibutyryl cyclic-AMP increased the rate of tension development and the isometric tension of isolated cat papillary muscle.

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It is the purpose of this paper to report the effects of dibutyryl cyclic-AMP in the cardiac output and blood flow in the renal, superior mesenteric and carotid arteries in anesthetized dogs. From these results, distribution of cardiac output to each peripheral vascular bed was calculated in order to suggest the effective site of this compound.

METHODS

Twenty-four adult mongrel dogs of both sexes, weighing 10 to 22 kg, were anesthetized with ketamine chloride (10 mg/kg body weight, i.m.) and sodium pentobarbital (30 mg/kg, i.m.). A cuffed tracheal tube was inserted and each dog was ventilated with air by means of an Aika Respirator (R-60) 20 per min and 10 ml tidal volume per kg. The left chest was opened in the 4th intercostal space and the ascending aorta was exposed to place the flow meter probe around it. The left renal and superior mesenteric arteries were dissected retroperitoneally, and the left carotid artery was exposed. Non-cannulating flow meter probes of adequate size for each artery were placed around the left renal, superior mesenteric and left carotid arteries. Then we measured the ascending aorta blood flow (cardiac output), and blood flow in the renal, superior mesenteric and carotid arteries using an electromagnetic flow meter (Nihon Koden MF-46). The stroke volume was calculated from the flow curve obtained at the ascending aorta. The mean systemic blood pressure and the central venous pressure were measured by electric transducers (Nihon Koden PPU-05 and LPU-05) at the right femoral artery and the intrathoracic vein. ECG (lead II) was connected to the tachometer (Nihon Koden RT-5) and the heart rate recorded.

Vascular resistance for each vascular bed was calculated from the mean systemic blood pressure and the cardiac output, or rate of blood flow, in the renal, superior mesenteric and carotid arteries.

Distribution ratio of each peripheral artery was expressed in percent of the mean rate of blood flow to the cardiac output.

Drugs used were N6-2'-O-dibutyryl adenosine 3', 5'-monophosphate (Dibutyryl cyclic-AMP supplied by Daiichi Seiyaku), propranolol (Sumitomo Kagaku) and aminophylline (Eizai) in this experiment. Dibutyryl cyclic-AMP was dissolved in 4 ml of physiologic saline. Every drug solution was administered intravenously from the left basilic vein. Drug effects were observed for 60 min.

Following abbreviations were used in the present paper: C.A.B.F., carotid artery blood flow; C.A.V.R., carotid artery vascular resistance; C.D.Ra., carotid distribution ratio; C.O., cardiac output; C.V.P., central venous pressure; H.R., heart rate; M.S.B.P., mean systemic blood pressure; R.A.B.F., renal artery blood flow; R.A.V.R., renal artery vascular resistance; R.D.Ra., renal distribution ratio; S.B.P., systemic blood pressure; S.M.A.B.F., superior mesenteric artery blood flow; S.M.A.V.R., superior mesenteric artery vascular resistance; S.M.D.Ra., superior mesenteric distribution ratio; S.V., stroke volume; T.P.R., total peripheral resistance.

RESULTS

Effects of dibutyryl cyclic-AMP on the circulation

After a single injection of dibutyryl cyclic-AMP at the dose of 5 mg/kg to the anesthetized dogs, the cardiac output, the renal and the superior mesenteric artery blood flow increased gradually, but the carotid artery blood flow remained unchanged. The change of blood flow significantly appeared 5 min after addition of dibutyryl cyclic-AMP (Fig. 1). However, the systemic blood pressure did not change after dibutyryl cyclic-AMP injection even though the stroke volume increased at
Changes in the heart rate, mean systemic blood pressure and central venous pressure are illustrated in Fig. 3. The heart rate increased by 8% at 5 min, and maintained this level for 60 min. Both mean systemic blood pressure and central venous pressure decreased slightly.

The results on blood flow are illustrated in Fig. 4. The cardiac output increased by 24% at 30 min and returned to the control value at 60 min. The
stroke volume increased by 14\% at 20 min and returned to the control level at 40 min. The renal artery blood flow increased by 19\% at 20 min and subsided gradually. The superior mesenteric artery blood flow increased by 27\% at 10 min and returned to the control levels at 60 min. The carotid artery blood flow did not vary greatly from the control value.

The results on vascular resistance are illustrated in Fig. 5. The total peripheral resistance as well as vascular resistances of the renal and superior mesenteric arteries decreased about 20\% at 10 min and recovered gradually. The carotid artery vascular resistance decreased slightly.
Fig. 5. Changes in vascular resistance induced by dibutyryl cyclic-AMP, 5 mg/kg, i.v. (mean±S.E.).

Concerning the distribution ratio, the carotid artery distribution ratio decreased about 20% at 10 min and recovered gradually. The renal artery distribution ratio decreased slightly, and the superior mesenteric artery distribution ratio did not vary greatly from the control value (Fig. 6).

**Pretreatment with propranolol**

Fig. 7 shows one of the typical results on the effect of dibutyryl cyclic-AMP
Fig. 7. Circulatory responses to dibutyryl cyclic-AMP, 5 mg/kg, i.v. 5 min after propranolol, 0.3 mg/kg, i.v. (Dog's weight was 16 kg).

Fig. 8. Changes in blood flow induced by dibutyryl cyclic-AMP, 5 mg/kg, i.v. after propranolol treatment. After propranolol (0.3 mg/kg) injection, the systemic blood pressure fell slightly. The cardiac output, the renal, the superior mesenteric and the carotid artery blood flows increased at 5 min.

Changes in blood flow caused by the injection of dibutyryl cyclic-AMP after
Fig. 9. Circulatory responses to dibutyryl cyclic-AMP, 5 mg/kg, i.v. 3 min after aminophylline, 3 mg/kg, i.v. (Dog's weight was 14 kg).

Fig. 10. Changes in blood flow by dibutyryl cyclic-AMP, 5 mg/kg, i.v. after aminophylline, 3 mg/kg (mean ± S.E.).
propranolol treatment are shown in Fig. 8. The cardiac output, the stroke volume and the superior mesenteric artery blood flow increased to the same degree as observed when only dibutyryl cyclic-AMP was injected. The renal artery blood flow increased slightly, and the carotid artery blood flow did not change. These results suggest that the effects of dibutyryl cyclic-AMP were not blocked by propranolol.

Pretreatment with aminophylline

Fig. 9 shows one of the typical results in hemodynamic change induced by the injection of aminophylline (3 mg/kg) and by the injection of dibutyryl cyclic-AMP (5 mg/kg) 3 min after aminophylline treatment. When dibutyryl cyclic-AMP was given 3 min after aminophylline treatment, the systemic blood pressure fell slightly, and the cardiac output, the renal, the superior mesenteric and the carotid artery blood flows increased sooner than when only dibutyryl cyclic-AMP was injected.

Changes in blood flow after aminophylline treatment are shown in Fig. 10. The superior mesenteric artery blood flow, the cardiac output, the renal artery blood flow and the stroke volume increased more markedly than when only dibutyryl cyclic-AMP was injected. From these results, it seems that aminophylline enhanced the effects of dibutyryl cyclic-AMP.

DISCUSSION

Sutherland and co-workers (1965, 1966, 1968) have reported that cyclic-AMP is a “second messenger” mediating various hormonal responses including a positive inotropic effect of catecholamines in the heart. According to this hypothesis, the positive inotropic effect of catecholamines has been postulated to result from an increase in the intracellular level of cyclic-AMP produced by activation of adenyl cyclase. However, attempts to demonstrate an inotropic effect by addition of cyclic-AMP have been unsuccessful because labeled cyclic-AMP cannot penetrate the cell membrane (Rall and West 1963; Robison et al. 1965). But Levine and Vogel (1966) and our previous paper (Nozaki and Okuaki 1974) reported that exogenous cyclic-AMP increased the cardiac output in the dogs. However, in our previous paper, the effects of cyclic-AMP were blocked by propranolol and were not enhanced by aminophylline. These results support the view that cyclic-AMP cannot penetrate the cell membrane, and in our experiments an increase of the cardiac output was not the result of activation of adenyl cyclase. Also it is suspected that the increase of the cardiac output after administration of cyclic-AMP is an unspecific pharmacological effect.

On the other hand, Kukovetz (1968) reported that addition of dibutyryl cyclic-AMP to Langendorff preparations of the hearts of rats, rabbits and guinea pigs increased both contractility and phosphorylase. And recently, Skelton et al. (1970) reported an increase in the rate of tension development and the isometric tension of isolated capillary muscle of cats. Ahren et al. (1971) have reported that
dibutyryl cyclic-AMP could reproduce the inotropic effect in isolated rat heart preparation. More recently, Drummond and Hemmings (1972) have reported that dibutyryl cyclic-AMP produced the positive inotropic and chronotropic effects on perfused rat hearts.

The results of the present study show that the use of dibutyryl cyclic-AMP on dogs increased their heart rates, the stroke volume and their cardiac outputs. These effects appeared 3 to 5 min after injection of dibutyryl cyclic-AMP into the dog's vessel. Moreover, the increase of cardiac output was not depressed by propranolol and slightly enhanced by aminophylline. At the same time, dibutyryl cyclic-AMP increased blood flow in the renal and the superior mesenteric arteries. These results from administration of dibutyryl cyclic-AMP were significantly different from those from cyclic-AMP administration. For example, the heart rate increased after dibutyryl cyclic-AMP administration but decreased after addition of cyclic-AMP. The renal artery blood flow increased after addition of dibutyryl cyclic-AMP, but decreased for the first few minutes and then increased after addition of cyclic-AMP. The effects of cyclic-AMP were blocked by propranolol and were not enhanced by aminophylline. The effects of dibutyryl cyclic-AMP were not blocked by propranolol and were enhanced by aminophylline. The effects of cyclic-AMP appeared within 1 min but the effects of dibutyryl cyclic-AMP were delayed 3 to 5 min after addition of nucleotide. From these results, it is postulated that dibutyryl cyclic-AMP can penetrate the cell membrane and increase the intracellular cyclic-AMP level leading to a positive inotropic effect. In fact, Posternack et al. (1962) and Drummond and Hemmings (1972) have recognized that labeled dibutyryl cyclic-AMP could penetrate the cell membrane.

On the renal blood flow, Gill and Casper (1971) reported that dibutyryl cyclic-AMP did not increase the renal blood flow so much, but that dibutyryl cyclic-AMP had diuretic action in dogs. Our results were opposite to theirs with significantly increased renal blood flow.

On the superior mesenteric artery blood flow, there are no reports except ours which showed an increase in the superior mesenteric artery blood flow. The carotid artery blood flow did not change.

On the distribution ratio of the cardiac output, distribution ratio in the renal and mesenteric arteries did not change after addition of dibutyryl cyclic-AMP. However, carotid artery distribution ratio decreased after addition of dibutyryl cyclic-AMP.

Concerning the vascular resistance, dibutyryl cyclic-AMP decreased total peripheral resistance, renal artery vascular resistance and superior mesenteric artery vascular resistance.

From these results, it is suspected that the increase of blood flow in the renal and superior mesenteric arteries were the results from an increase of the cardiac output as well as from the dilation of each peripheral vascular vessel.
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References


