EFFECTS OF CYCLIC AMP AND DIBUTYRYL CYCLIC AMP ON THE HEART AND CORONARY CIRCULATION

Shoichi IMAI, Takeshi OTORII, Keisuke TAKEDA, Yunmi KATANO and Daijiro HORII*

Department of Pharmacology, Nigata University School of Medicine, Nigata, Japan

Accepted December 10, 1973

Abstract - Using the canine heart-lung preparation supported by a donor dog and the isolated right and left atrial preparations of the guinea-pig and the rat, the cardiac actions of dibutyryl cyclic AMP were studied and compared with those of cyclic AMP. Dibutyryl cyclic AMP produced positive isotropic and chronotropic effects associated with an increase in the coronary flow, while cyclic AMP produced negative isotropic and chronotropic effects, together with an increase in the coronary flow. Propranolol did not alter the effects of dibutyryl cyclic AMP. The negative isotropic and chronotropic effect of cyclic AMP was antagonized by aminophylline, while the positive isotropic and chronotropic effect of dibutyryl cyclic AMP was potentiated by this compound. Whereas the coronary flow increase by dibutyryl cyclic AMP was associated with an increase in the myocardial oxygen consumption, there was no increase in the myocardial oxygen consumption after cyclic AMP. It is concluded that dibutyryl cyclic AMP passed the surface membrane and exerted its effect after having been converted to cyclic AMP within the cell. Cyclic AMP was presumed to remain outside the cell and produce its effect in the same way as adenine nucleotides and adenosine produce their effect.

The positive isotropic effects of catecholamines have been postulated to result from an increase in the intracellular level of cyclic AMP produced by activation of adenyl cyclase. This hypothesis is based on the following observations: (a) myocardial adenyl cyclase in broken cell preparations is activated by catecholamines (1); (b) tissue level of cyclic AMP in the intact heart muscle increases in response to catecholamine stimulation (2-5); and (c) the isotropic response to catecholamines is enhanced by theophylline (6), which has been shown to inhibit phospho-diesterase, the enzyme responsible for metabolic degradation of cyclic AMP to 5'-AMP. One observation at variance with the above hypothesis has been the failure to reproduce the inotropic effects of catecholamines by administration of exogenous cyclic AMP (2, 6-8). Levine and Vogel (9) reported that the injection of large doses of cyclic AMP in unanesthetized humans and dogs resulted in a prompt increase in heart rate and cardiac output, but the possibility that the observed changes could have been reflexly induced cannot be ruled out. However, it has been shown that cyclic AMP does not pass the cell membrane at a rate sufficient to achieve an effective intracellular concentration (2), a finding which may explain the inability of cyclic AMP to alter myocardial contractility. To facilitate passage across cellular membranes, a derivative of cyclic AMP, N6-2'-O-dibutyryl-cyclic 3', 5'-AMP (dibutyryl cyclic

* Present Address: Research Laboratories, Mitsubishi Yuka Pharmaceutical Co., Ltd.
AMP has been synthesized by incorporating lipid-soluble fatty acid residues, a modification that also rendered the substance resistant to enzymatic hydrolysis by phosphodiesterase \(^{10, 11}\). The structural formula of this derivative is shown in Fig. 1, along with that of cyclic AMP. This more lipid-soluble derivative has been studied in several tissues including the heart muscle. It was discovered that the substance could produce qualitatively the same effects on the heart as catecholamines \(^{12-15}\). However, all these data were obtained using the isolated saline-perfused heart or heart preparations immersed in saline, a situation which might seriously affect the permeability of cell membrane to this compound. Such being the case, the present study was undertaken to re-investigate the effects of dibutyryl cyclic AMP using the blood-perfused heart, and compare the results with those of cyclic AMP. Effects of these two substances on the coronary vessels were also studied. Since a definite positive inotropic and chronotropic effect was observed with dibutyryl cyclic AMP, while cyclic AMP produced the opposite effect in the blood-perfused heart, further experiments were performed with isolated atrial preparations of the guinea-pig with a view of determining the underlying mechanism.

**MATERIALS AND METHODS**

*Heart-lung preparations supported by a donor dog*

Twenty-five canine heart-lung preparations (HLP) were set up using mongrel dogs of either sex weighing between 6.5 and 14.0 kg, according to the Krayer-Mendez modification of the original Starling method, the details of which are described in previous papers \(^{16, 17}\). To maintain the coronary blood flow at a constant, low value, the coronary sinus outflow was circulated to another large dog (a donor) via femoral vein and fresh arterial blood was returned by a peristaltic pump (Harvard Model 1203) to the venous reservoir of the HLP from the femoral artery of the donor dog.

*Isolated atrial preparations*

The right and left atrial preparations were prepared, from the freshly excised hearts of stunned guinea pigs (300-600 g) and Wister-Imamichi rats (300-400 g) of either sex. The right atrial preparations, which retained a spontaneous rhythm, were used to assess the chronotropic effect and the left atrial preparations, stimulated at a constant rate.
by a square-wave electronic stimulator (Nihon Kohden MSE-40) via an isolating transformer, were used for the study of the inotropic effect. Only the outer half of the left atrium was used. The preparations were suspended vertically in 20 ml organ bath containing Krebs-Henseleit solution aerated with 95% O\textsubscript{2} + 5% CO\textsubscript{2} and kept at a temperature of 35°±0.3°C. To obtain optimum responses the initial tensions of the preparations were set at about 0.2–0.25 g and the contractile tension was recorded on an ink-writing oscillograph with a force-displacement transducer (Nihon Kohden SB-1T) coupled with a carrier amplifier (Nihon Kohden RP-3). To record the rate of spontaneous beat of the right atrial preparations, the output of the carrier amplifier was fed into a cardiotachometer (Nihon Kohden RT-5) and the output was recorded on another channel of the ink-writing oscillograph. To avoid the initial fluctuations of the preparations, the muscle was left for one hr after mounting.

Na salt of N\textsuperscript{6}-2'-O-dibutyryl-cyclic 3', 5'-adenosine monophosphate (Dibutyryl cyclic AMP) and cyclic 3', 5'-AMP were generously provided by the Dai-ichi Pharmaceutical Co. Ltd. and the Seishin Pharmaceutical Co. Ltd., respectively. Other drugs used were sodium pentobarbital (Mintal, Tanabe) aminophylline (Neophylline, Eisai) propranolol hydrochloride (ICI Japan), adenosine (Nutritional Biochemicals) and AMP (Sigma Chemical).

RESULTS

HLP with donor

In doses above 30 mg, dibutyryl cyclic AMP produced definite positive inotropic and chronotropic effects in HLP with donor. Fig. 2 illustrates the effects of 100 mg of this compound on the cardiac functions. Since the positive inotropic effects can best be seen in the failing heart, 100 mg of pentobarbital was given prior to administration of dibutyryl cyclic AMP. A marked increase in the systemic cardiac output and a marked fall of the right atrial pressure together with an increase in the heart rate can

---

**Fig. 2.** Effects of 100 mg of dibutyryl cyclic AMP (Dib. cyclic AMP) on the canine heart-lung preparation supported by a donor dog.

Dog, 11.0 kg male. Heart wt.: 131 g.

Total blood volume at the beginning of the experiment was 1500 ml.

SOP: systemic cardiac output (Total output of the left ventricle minus the coronary flow), RAP: right atrial pressure, HR: heart rate, P.B.: sodium pentobarbital.
be seen from this figure. There was an increase in the coronary flow (Fig. 3). One characteristic feature of the actions of dibutyryl cyclic AMP in this preparation was the slow onset of action; with 100 mg of this compound about 20 minutes was needed to attain the maximum effect. Propranolol did not alter the effects of dibutyryl cyclic AMP, in doses of 0.3–5 mg, which completely abolished the effects of 0.3–1 mg of isoproterenol (Figs. 4 and 5).

In contrast, cyclic AMP produced only a negative inotropic and negative chronotropic effect, associated with an increase in the coronary flow. Fig. 6 depicts the effects of 20 mg of cyclic AMP on the heart. The effect of this substance on the coronary
vessels is illustrated in Fig. 3, and compared with that of dibutyryl cyclic AMP. In comparison with the effects of dibutyryl cyclic AMP, the onset of action of cyclic AMP was much quicker and the duration, shorter. The effects of AMP and adenosine are also illustrated in these figures. Both of these compounds induced a negative inotropic and chronotropic effect, together with an increase in the coronary flow, although the duration of the action was much shorter.

Although ineffective in itself, a previous administration of 5 mg of aminophylline, a specific inhibitor of the action of adenine nucleotides and adenosine (7, 18), resulted in a marked reduction of all three effects of cyclic AMP on the dog HLP (Figs. 7 and 8) as well as a reduction of the effects of AMP and adenosine, indicating that a similar mechanism is operative in the action of cyclic AMP as in the action of adenine nucleotides and adenosine.
Fig. 7. Inhibitory effect of 5 mg of aminophylline on the cardiac action of cyclic AMP and adenosine (Ads).
HLP 62 donor No. 62 (Dog, 10.0 kg male. Heart wt.: 88 g).
Abbreviations are as in Fig. 2.

Fig. 8. Inhibitory effect of 5 mg of aminophylline on the increase in coronary flow (CF) produced by cyclic AMP and adenosine (Ads).
HLP 62 donor No. 62 (Dog, 10.0 kg, male. Heart wt.: 88 g).

Concomitant with an increase in the coronary flow there was an increase in the myocardial oxygen consumption after administration of dibutyryl cyclic AMP, while the coronary flow increase by cyclic AMP was not related to the increase in the myocardial oxygen consumption. In Fig. 9 the percent increases in the coronary flow are plotted.
**FIG. 9.** Relationship between coronary flow and myocardial oxygen consumption as affected by dibutyryl cyclic AMP (Dib. cyclic AMP), cyclic AMP and adenosine.

*Cor. flow* : increase in coronary flow expressed as percent of the control.

*\( \Delta O_2 \) consumption* : increase in myocardial oxygen consumption expressed as percent of the control.

The regression line designated as Isp represents the 1 to 1 relationship.

**FIG. 10.** Inhibitory effect of cyclic AMP on the coronary flow (CF) increase produced by dibutyryl cyclic AMP (Dib. cyclic AMP).

HLP e donor No. 54 (Dog, 12.0 kg, female. Heart wt.: 130 g).

against the percent increases in the myocardial oxygen consumption. As can be seen from this figure, the regression line for the action of dibutyryl cyclic AMP runs a little steeper than a 1 to 1 relation, indicating that direct dilatatory effect must be taken into
account for this compound (19). The regression line for cyclic AMP corresponds to
the Y-axis, as did the regression lines for AMP and adenosine, thus giving further support
to the hypothesis that the mechanism of the action of cyclic AMP is similar to that of
adenine nucleotides and adenosine.

Cyclic AMP, given in a dose of 30 mg, produced an increase in the coronary flow
associated with a negative inotropic and chronotropic effect. After the subsidence of
these effects, 100 mg of dibutyryl cyclic AMP was given. The effects of the latter com-
ound were greatly reduced. Fig. 10 depicts the inhibitory effect of cyclic AMP on the
coronary flow increase produced by dibutyryl cyclic AMP.

Isolated atrial preparations

As is demonstrated in Fig. 11 the effects of dibutyryl cyclic AMP on atrial prepara-
tions of the guinea pig were qualitatively similar to those found in the dog HLP: a
positive inotropic and chronotropic effect was observed. However, a negative inotropic
effect usually preceded the positive inotropic effect in this preparation, especially with
low concentrations of the compound. The same type of effect was observed in isolated
atrial preparations from the rat (Fig. 11).

As in HLP with donor, pretreatment of the preparations with aminophylline com-
pletely abolished the negative inotropic and chronotropic effects of cyclic AMP on the
atrial preparations (Fig. 12). In contrast, aminophylline produced a potentiation of the
effect of dibutyryl cyclic AMP. Fig. 13 displays the dose-response relation of the effects
of dibutyryl cyclic AMP on isolated atrial preparations from guinea-pig. Dibutyryl
cyclic AMP produced only a negative inotropic and chronotropic effect when the con-
centration was less than $10^{-5}$ M, the positive inotropic and chronotropic effect appearing
at concentrations above $2.4 \times 10^{-4}$ M. After pretreatment of the preparation with $10^{-4}$ M
of aminophylline, there was a shift of the dose-response curve to the left and only the
positive inotropic and chronotropic effect was observed. The effect of aminophylline on
the dose-response curve of the positive inotropic effect of calcium was studied for a comparison. As is demonstrated in Fig. 14, aminophylline $10^{-4}$ M was without effect on the positive inotropic effect of increasing doses of calcium (2.5–9.0 mM), except that there was a slight inhibition of the effect when the highest dose of calcium was administered.

**Fig. 12.** Antagonistic effect of aminophylline on negative inotropic and chronotropic effects of cyclic AMP. HR: spontaneous rate of right atrial preparation of guinea-pig. Tension: contractile tension of left atrial preparations.

**Fig. 13.** Potentiation by aminophylline (AMPH) of positive inotropic and chronotropic effects of dibutyryl cyclic AMP (Dib. cyclic AMP). Contractile tension and atrial rate are expressed as percent of the maximum response obtainable by isoproterenol.

1 atria: left atrial preparation of the guinea-pig.

r atria: right atrial preparation.
DISCUSSION

The present experiments clearly demonstrated that dibutyryl cyclic AMP produced a definite positive inotropic and chronotropic effect irrespective of the animal species. This finding contradicts that of Henion et al. (8) who found that injection of dibutyryl cyclic AMP in a dose of 4 mg/kg, i.v., to anethetized dogs caused only slight and variable changes in the cardiovascular performances and also that of Robison et al. (2) who reported that concentrations of dibutyryl cyclic AMP as high as $6 \times 10^{-4}$ M exerted no effect on the performance of the isolated perfused rat heart. However, the present results are in agreement with the findings of Skelton et al. (13), Kukovetz and Pöch (12), Ahren et al. (14) and Meinertz et al. (15). It is presumed that the results of the earlier studies were inconsistent because too small a dose of dibutyryl cyclic AMP was administered.

Since the beta-receptor blocking agent, propranolol, had no effect on the action of dibutyryl cyclic AMP, it is concluded that direct stimulation of the beta-receptor or the release of endogenous noradrenaline is not involved in the stimulant action of this compound.

Although there is some possibility that the positive inotropic and chronotropic effects of dibutyryl cyclic AMP occurred via a mechanism independent of its structural relation to cyclic AMP, it seems more likely that the differences observed between inotropic and chronotropic effects of these two compounds are related to differences in their capability to penetrate the myocardial cell membrane. Kaukel and Hilz (20) provided direct evidence that dibutyryl cyclic AMP penetrates the HeLa cell membrane more rapidly than cyclic AMP. It is, therefore, probable that after passing the cell membrane this compound undergoes deacylation to cyclic AMP, the accumulation within the cell of which results in positive inotropic and chronotropic effects. The following three facts support this idea. First, it was shown by Posternak et al. (10) with extracts
of the liver and the heart that the biological activities of dibutyryl cyclic AMP evaluated according to the results on the activation of phosphorylase were negligible, as compared with the effects of cyclic AMP. Secondly, Blecher and Hunt (21) have shown that the heart muscle of the rat contains enzymes which deacylated dibutyryl cyclic AMP to cyclic AMP and the rates of deacylation were of a magnitude sufficient to produce biologically effective amounts of cyclic AMP. Finally and most conclusively there was a potentiation of the effects of dibutyryl cyclic AMP by aminophylline, an inhibitor of phosphodiesterase. Since N\textsuperscript{6}-substituted cyclic AMP's are found to be resistant to phosphodiesterase degradation (10), inhibition of phosphodiesterase by aminophylline would not enhance the effects of dibutyryl cyclic AMP, unless the compound was deacylated to cyclic AMP before exerting its effects. Aminophylline potentiation of the effects of dibutyryl cyclic AMP would also exclude the hypothesis of Heersche et al. (22) that dibutyryl cyclic AMP acted primarily as inhibitor of phosphodiesterase.

In contrast, cyclic AMP, incapable of penetrating the cell membrane, remains outside the cell and exerts its effect on the surface membrane and produces the negative inotropic and chronotropic effect with an increase in the coronary flow just as is seen with adenine nucleotides and adenosine. The fact that aminophylline blocked all these three effects of cyclic AMP is compatible with this interpretation. It is possible that cyclic AMP produced its effect either as AMP or as adenosine, since the rapid degradation of this substance to purine nucleotides and adenosine was demonstrated in HeLa cells by Kaukel and Hilz (20).

The mechanism by which an increase in the intracellular cyclic AMP produces a positive inotropic effect is not entirely clear. Entman et al. (23) have shown that the calcium accumulation within a microsomal fraction of canine myocardium, generally admitted to represent sarcoplasmic reticulum, is increased by noradrenaline and cyclic AMP. Presumably activation of adenyl cyclase by catecholamine brings about an increase in cyclic AMP within the cells, which in turn modulates the strength of myocardial contraction by increasing the amount of sarcotubular calcium released following membrane depolarization.

In conclusion, the demonstration that dibutyryl cyclic AMP causes a marked positive inotropic effect offers further support to the idea that the positive inotropic and chronotropic response to catecholamines is mediated by an increase in the intracellular level of cyclic AMP. Although Shanfeld et al. (24) have shown in the perfused rat heart that N-isopropylmethoxamine can block the increase of cyclic AMP produced by noradrenaline without preventing the inotropic effect of the substance, too much stress should not be laid upon their findings, since even a minute increase in cyclic AMP which cannot be detected by the presently available method would be sufficient to produce a positive inotropic effect.

**Acknowledgements:** The authors wish to express their thanks to Mr. I. Matsubara for his excellent technical assistance and to Miss S. Tase for help in preparing the manuscript.
REFERENCES

1) Murad, F., Chi, Y.M., Rall, T.W. and Sutherland, E.W. : J. biol. Chem. 237, 1233 (1962)
2) Robison, G.A., Butcher, R.W., Fyl, L., Morgan, H.E. and Sutherland, E.W. : Mol. Pharmacol. 1, 168 (1965)
3) Cheung, W.Y. and Williamson, J.R. : Nature 207, 979 (1965)
4) Hammermeister, K.E., Yunis, A.A. and Krebs, E.G. : J. biol. Chem. 240, 986 (1965)
5) Drummond, G.L., Duncan, L. and Hertzman, E. : J. biol. Chem. 241, 5899 (1966)
6) Rall, T.W. and West, T.C. : J. Pharmacol. exp. Ther. 139, 269 (1963)
7) De Gubareff, T. and Sleator, W. : J. Pharmacol. exp. Ther. 148, 202 (1965)
8) Henion, W.F., Sutherland, E.W. and Posternak, Th. : Biochim. biophys. Acta 148, 106 (1967)
9) Levine, R.A. and Vogel, J.A. : Nature 207, 987 (1965)
10) Posternak, Th., Sutherland, E.W. and Henion, W.F. : Biochim. biophys. Acta 65, 558 (1962)
11) Falbriard, J.G., Posternak, Th. and Henion, W.F. : Biochim. biophys. Acta 148, 99 (1967)
12) Kukovetz, W.R. and Pöch, G. : Arch. Pharmacol. 266, 236 (1970)
13) Skelton, C.L., Levey, G.S. and Epstein, S.E. : Circulation Res. 26, 35 (1970)
14) Ahren, K., Hjalmarson, A. and Isaksson, O. : Acta physiol. scand. 82, 79 (1971)
15) Meinertz, T., Nawrath, H. and Scholz, H. : Pflügers Arch. 333, 197 (1972)
16) Imai, S., Shigei, T. and Hashimoto, K. : Circulation Res. 9, 552 (1961)
17) Katori, M., Takeda, K. and Imai, S. : Tohoku J. exp. Med. 101, 67 (1970)
18) Afonso, S. : Circulation Res. 26, 743 (1970)
19) Imai, S., Takeda, K. and Katano, Y. : J. Japan. College of Angiology 13, 351 (1973) (in Japanese)
20) Kauki, E. and Hilz, H. : Biochem. biophys. Res. Commun. 46, 1011 (1972)
21) Blecher, M. and Hunt, N.H. : J. biol. Chem. 247, 7479 (1972)
22) Heersche, J.N.M., Fedak, S.A. and Aurbach, G.D. : J. biol. Chem. 246, 6770 (1971)
23) Entman, M.L., Levey, G.S. and Epstein, S.E. : Circulation Res. 25, 429 (1969)
24) Shanfield, J., Frazer, A. and Hess, M.E. : J. Pharmacol. exp. Ther. 169, 315 (1969)