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EFFECTS OF VARIOUS DRUGS ON MYOCARDIAL CYCLIC AMP

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ABSTRACT

Effects of norepinephrine, β-adrenergic blocking agents, theophylline ethylenediamine (TEDA) and digoxin on myocardial cyclic AMP concentration and those of various vasodilators both on myocardial cyclic AMP *in vivo* and phosphodiesterase (PDE) activity *in vitro* were investigated by radioimmunoassay of cyclic AMP.

The mean concentration of myocardial cyclic AMP was 0.318 p mole/mg in the left ventricle, 0.204 p mole/mg in the right ventricle, 0.131 p mole/mg in the left atrium and 0.094 p mole/mg in the right atrium. Norepinephrine increased the concentration of cyclic AMP in the left ventricle significantly, while digoxin increased it only slightly, β-adrenergic blocking agents did not decrease it, and TEDA elevated the concentration of left ventricular cyclic AMP. The myocardial cyclic AMP concentration of the ventricular fibrillation group increased significantly as compared with the non-ventricular fibrillation group. Dipyridamole, TEDA and prenylamine were determined as competitive inhibitors of purified heart PDE *in vitro*.

These findings suggest that norepinephrine, TEDA and propranolol affect myocardial contractility through the cyclic AMP-PDE system and that TEDA, dipyridamole and prenylamine cause increase of myocardial cyclic AMP through the inhibition of PDE. It also suggests that there is a relationship between high myocardial concentration of cyclic AMP and ventricular fibrillation induced by PDE.

INTRODUCTION

Cyclic AMP was discovered by Sutherland and Rall[1] in the course of investigation of the effects of adrenergic agents on carbohydrate metabolism. Its metabolic effects were investigated on the liver, adipose tissue and nervous system. It was demonstrated that phosphorylase and lipase were activated by cyclic AMP, resulting in accelerated glycogenolysis and lipolysis.

The first study on the cardiac effect of cyclic AMP reported by Murad et al.[2] in the broken cell preparation from the dog heart stated that the relative potencies of catecholamines in stimulating adenyl cyclase were similar to their relative potencies as inotropic agents *in vivo* (isoproterenol > epinephrine > norepinephrine). Later, studies in rat hearts revealed that the activation of adenyl cyclase was an extremely rapid process, preceeding both the inotropic response and the activation of phosphorylase.[3] There are many reports[3~9] that the inotropic action of myocardium is related with the myocardial cyclic AMP, but these series of studies were not directly measured on the myocardial cyclic AMP. In 1969, a sensitive and specific radioimmunoassay for the cyclic AMP was developed by Steiner,[10] which could measure a low level of cyclic AMP accurately. In order to investigate the biochemical and physiological roles of the myocardial cyclic

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AMP, the concentration of cyclic AMP with radioimmunoassay in the heart muscles of normal dogs was determined, and the effects of various drugs on the myocardial cyclic AMP, the heart rate, the left ventricular pressure and the max dp/dt were investigated.

Recently, it has been shown that the cyclic AMP and phosphodiesterase were related to the mechanism of smooth muscle relaxation and vasodilatation.[11] However, there has been no report on how the coronary vasodilators affect the myocardial cyclic AMP. Therefore, we investigated the effects of various coronary vasodilators on myocardial cyclic AMP and their inhibitory effects on phosphodiesterase, using purified phosphodiesterase of the bovine heart.

MATERIALS AND METHOD

1. Materials

Eighty-four normal mongrel dogs, weighing 7 – 15 kg, were divided into the following eleven groups, namely, control group, norepinerphrine group, digoxin group, propranolol group, practolol group, pindolol group, theophylline ethylenediamine group, dipyridamole group, prenylamine group, carbochromen group and nitroglycerin group. Dogs were anesthetized with hexobarbital sodium and thoracotomized under artificial respiration. In each experimental group, the individual drug was injected from the right femoral vein. The heart muscles of each chambers were removed with stainless steel tongs, precooled in liquid nitrogen and used for cyclic AMP assay, when the dp/dt reached the maximum after the injection of 100 μg/kg of norepinephrine and of 30 mg/kg theophylline ethylenediamine, thirty minutes after the intravenous administration of 60 μg/kg of digoxin and five minutes after the administration of 1 mg/kg of propranolol, of 2.5 mg/kg of practolol and of 0.1 mg/kg of pindolol, respectively. The left ventricular myocardium was also removed five minutes after the injection of 0.5 mg/kg of dipyridamole, 1.0 mg/kg of prenylamine and 2.0 mg/kg of carbochromen and one minute after the injection of 0.02 mg/kg of nitroglycerin.

Adenosin-H^3(G) 3′, 5′ - cyclic phosphate, ammonium salt was obtained from New England Nuclear, and Adenosine 3′, 5′ - cyclic AMP and purified phosphodiesterase (beef heart) from Boehringer Mannheim. Millipore membrane filter (H. A. poresize 0.45 μ) was from Millipore Corporation. Radioactivity was determined with a liquid scintillation counter (Aloka 100).

2. Determination of tissue cyclic AMP

Cyclic AMP concentration was measured with radioimmunoassay of Steiner[10] and Okabayashi.[12] The heart muscle, weighing 200–300 mg, was boiled at 100°C for 10 minutes and then homogenized with 1 ml of 0.05 M sodium acetate buffer (pH 6.2). After centrifugation at 3,000 r.p.m. for 10 minutes, 50 μl of the supernatant solution, 10 μl of 0.05 M sodium acetate buffer (pH 6.2), 10 μl of 0.5 % bovine albumine, 10 μl of 0.2 p mole H^3-cyclic AMP and 10 μl of cyclic AMP antibody (kindly donated by Dr. Okabayashi, Shionogi Research Laboratory) were mixed, and then made to react in ice water for 1 hour. Fifty μl of the reaction product filtrated through a millipore membrane filter placed on a Pyrex microanalysis filter holder and followed with 5 ml of 0.05 M sodium acetate buffer for three times. The millipore membrane filter on which H^3-cyclic AMP bounded antibody absorbed, was added to 10 ml of Bray's solution containing 0.5 ml of H_2O in a counting vial and the radioactivities were counted with a liquid scintilla-

tion counter for 5 minutes. Figure 1 shows the standard radioimmunoassay curve. This curve indicated that the lowest amount of cyclic AMP measurable by this method was 0.5 p mole. In tissues weighing 100–300 mg, the levels of cyclic AMP had a linear correlation with the weight.

3. Determination of phosphodiesterase activity in vitro

The activity of purified phosphodiesterase (PDE) of the beef heart was measured with a modification[13] of the method of Krishna *et al.* The incubation medium contained 0.3 ml of 4×10^{-2} M Tris HCl (pH 7.4), 0.1 ml of 2×10^{-3} M $MgCl_2$, 0.1 ml of 2×10^{-4} - 1.5×10^{-3} M cyclic AMP, 0.1 ml of 2 μg purified PDE (beef heart) and 0.1 ml of 1×10^{-4} M various vasodilators. The reaction was carried out with 0.7 ml reaction mixture at 37°C for 30 minutes and ended by the addition of 1 ml of 2 % $ZnSO_4$ and 1 ml of 1.8 % $Ba(OH)_2$. After centrifugation at 3,000 r.p.m. for 15 minutes, the supernatant solution was diluted from 20 times to 1,500 times with H_2O, to determine the concentration of cyclic AMP. Cyclic AMP of the diluted solutions was measured by radioimmunoassay. The product of the PDE reaction, 5'-AMP, was calculated from the results obtained by radioimmunoassay of cyclic AMP.

4. Method of measurement of the left ventricular pressure and the dp/dt

Normal mongrel dogs were anesthetized and thoracotomized under artificial respiration. The heart was exposed, and a Cournand catheter was inserted through the left femoral artery into the left ventricular cavity and the changes of the left ventricular pressure and the dp/dt were examined with an electronic manometer (Nihon Koden, MP-3A).

Results were analyzed for statistical significance by Student's "t" test.

RESULTS

1. The level of cyclic AMP in the heart of normal dogs

Samples of each chambers of the heart were removed from 8 anesthetized dogs. Table 1 shows that the mean concentrations of the cyclic AMP were 0.318 ± 0.022 (S.E.M.) p mole/mg in the left ventricle, 0.204 ± 0.024 p mole/mg in the right ventricle, 0.131 ± 0.031 p mole/mg in the left atrium and 0.094 ± 0.025 p mole/mg in the right atrium.

Table 1. Cyclic AMP concentration in dog heart

Heart	No. of Experiments	Cyclic AMP p mole/mg
Left Ventricle	8	0.318 ± 0.022
Right Ventricle	8	0.204 ± 0.024
Left Atrium	8	0.131 ± 0.031
Right Atrium	8	0.094 ± 0.025

2. The effects of various drugs on myocardial cyclic AMP and hemodynamics of normal dogs

Table 2 shows changes of the heart rate, the left ventricular pressure, the max dp/dt and the level of cyclic AMP of the left and the right ventricles of six normal dogs.

1) The effects of norepinephrine

The heart rate increased from 168.8 ± 10.9/min to 224.7 ± 26.8/min after the admini-

Table 2. The effects of various drugs on hemodynamics and myocardial cyclic AMP concentration

	No. of Experiments	Heart Rate (beats/min) before	Heart Rate (beats/min) after	Pressure of L.V. (mmHg) before	Pressure of L.V. (mmHg) after	% change of max dp/dt	Cyclic AMP L. V. p mole/mg	Cyclic AMP R.V. p mole/mg
control	6						0.315 ± 0.047	0.249 ± 0.024
norepinephrine (100 μg/kg)	6	168.8 ± 10.9	224.7 ± 26.8	89.0 ± 26.8	190.6 ± 17.7**	240.3 ± 27.8**	0.596 ± 0.095*	0.379 ± 0.084
digoxin (60 μg/kg)	6	153.2 ± 8.5	166.0 ± 8.3	90.5 ± 8.2	125.0 ± 5.6**	150.1 ± 23.8	0.332 ± 0.067	0.200 ± 0.057
propranolol (1 mgTkg)	6	165.3 ± 5.1	140.0 ± 6.4*	69.3 ± 7.4	66.3 ± 3.9	75.5 ± 9.7	0.231 ± 0.053	0.197 ± 0.038
Practolol (2.5 mgTkg)	6	156.6 ± 5.9	144.5 ± 4.8	122.7 ± 9.2	123.7 ± 8.0	87.1 ± 2.5	0.226 ± 0.037	0.202 ± 0.032
pindolol (0.1 mg/kg)	6	152.7 ± 10.7	137.0 ± 6.6	120.7 ± 13.1	112.2 ± 8.7	85.4 ± 11.4	0.230 ± 0.049	0.155 ± 0.020*

mean ± s.e. L.V. : left ventricle R.V. : right ventricle * $P < 0.05$ ** $P < 0.01$

Table 3. The effects of theophylline ethylenediamine on hemodynamics and myocardial cyclic AMP concentration

			No. of Experiments	Heart Rate (beats/min) before	Heart Rate (beats/min) after	Pressure of L.V. (mmHg) before	Pressure of L.V. (mmHg) after	% change of max dp/dt	Cyclic AMP L.V. p mole/mg	Cyclic AMP R.V. p mole/mg
control			6						0.315 ± 0.047	0.249 ± 0.024
theophylline ethylenediamine 30 mgTkg	occurrence of V.F.	–	6	164.0 ± 15.3	201.1 ± 17.0	103.2 ± 8.0	114.2 ± 1.9	160.3 ± 17.8*	0.496 ± 0.039*](**)	0.428 ± 0.027**](*)
		+	3	determination was incapable for ventricular fibrillation					0.835 ± 0.035	0.591 ± 0.051

mean ± s.e. V.F. : ventricular fibrillation L.V. : left ventricle R.V. : right ventricle * $P < 0.05$ ** $P < 0.01$

(*) t test was made between non-occurrence group and occurrence group of ventricular fibrillation after the injection of theophylline ethylenediamine.

stration of norepinephrine, whereas, the left ventricular pressure increased significantly from 89.0 ± 16.9 mmHg to 190.6 ± 17.7 mmHg. The left ventricular max dp/dt was significantly increased by 240.3 ± 27.8 % compared with control group ($P < 0.01$). The level of cyclic AMP of the heart muscle was measured at the time, when the dp/dt reached the maximum after the intravenous injection of 100 μg/kg of norepinephrine. The mean concentration of the left ventricular cyclic AMP was 0.596 ± 0.095 p mole/mg in norepinephrine group and significantly increased as compared with that of control group ($P < 0.05$). However, that of the right ventricle did not significantly increase as compared with control group.

2) The effects of digoxin

The heart rate was not significantly changed after the injection of digoxin. The mean pressure of the left ventricle significantly increased after digoxin ($P < 0.01$). The mean max dp/dt increased by 150.1 ± 23.8 % after the injection, but the difference was not significant. The mean concentrations of cyclic AMP of the left and right ventricles, measured thirty minutes after the intravenous injection of 60 μg/kg of digoxin, were 0.332 ± 0.067 p mole/mg and 0.200 ± 0.057 p mole/mg, respectively. There was no significant difference as compared with the control group.

3) The effects of β-adrenergic blocking agents

The mean heart rates after the injection of β-adrenergic blocking agents decreased, however, the decrease of propranolol group was statistically significant ($P < 0.05$). The left ventricular pressure in all three groups did not show a significant change. The mean max dp/dt decreased by 75.5 ± 9.7 % in the propranolol group, by 87.1 ± 2.5 % in the practolol group and by 85.4 ± 11.4 % in the pindolol group, as compared with the control group. The mean cyclic AMP concentrations of the left ventricle decreased from 0.315 ± 0.047 p mole/mg in the control group to 0.231 ± 0.053 p mole/mg in the propranolol group, to 0.226 ± 0.037 p mole/mg in the practolol group and to 0.230 ± 0.049 p mole/mg in the pindolol group, but these were not statistically significant. The mean cyclic AMP concentrations of the right ventricle of the three groups decreased, but the decrease from 0.249 ± 0.024 p mole/mg to 0.155 ± 0.020 p mole/mg in the pindolol group was significant ($P < 0.05$).

3. The effects of various vasodilators on the myocardial cyclic AMP

The hemodynamics of theophylline ethylenediamine were investigated.

1) The effects of theophylline ethylenediamine.

Table 3 shows the effects of theophylline ethylenediamine on the hemodynamics and concentration of cyclic AMP of the heart. Ventricular fibrillation was spontaneously induced in three dogs soon after the injection of theophylline ethylenediamine (ventricular fibrillation group), but six other dogs produced no arrhythmia (non-ventricular fibrillation group).

i) Non-ventricular fibrillation group

The mean heart rate of 164.0 ± 15.3/min before injection increased to 202.1 ± 17.0/min after injection, but was not significant. The mean max dp/dt significantly increased by 160.3 ± 17.8 % ($P < 0.05$). When the dp/dt reached a maximum after the injection of 30 mg/kg of theophylline ethylenediamine, the mean cyclic AMP concentration of the left ventricle became 0.496 ± 0.039 p mole/mg in the theophylline ethylenediamine group and was significantly increased as compared with the control group ($P < 0.05$). The cyclic AMP concentration of the right ventricule was significantly increased ($P < 0.01$).

(ii) Ventricular fibrillation group

The mean cyclic AMP concentration of the left ventricle of the ventricular fibrillation group was 0.835 ± 0.035 p mole/mg. This was a most significant increase of cyclic AMP as compared with the non-ventricular fibrillation group of 0.496 ± 0.039 p mole/mg ($P < 0.01$). The mean right ventricular cyclic AMP concentration of the ventricular fibrillation group also significantly increased when compared with that of the non-ventricular fibrillation group ($P < 0.05$).

2) The effects of other vasodilators

Table 4 shows the effects of various coronary vasodilators on myocardial cyclic AMP. The mean cyclic AMP concentration of the left ventricle increased after the injection of dipyridamole, prenylamine, carbochromen and nitroglycerin as compared with the control group.

Table 4. The effects of various coronary vasodilators on myocardial cyclic AMP

	Dose	No. of Experiments	Cyclic AMP concentration of left ventricle p mole/mg
control		6	0.214 ± 0.018
dipyridamole	0.5 mg/kg	6	0.378 ± 0.118
prenylamine	1.0 mg/kg	6	0.452 ± 0.100
carbochromen	2.0 mg/kg	6	0.340 ± 0.057
nitroglycerin	0.02 mg/kg	7	0.469 ± 0.098

mean ± s.e.

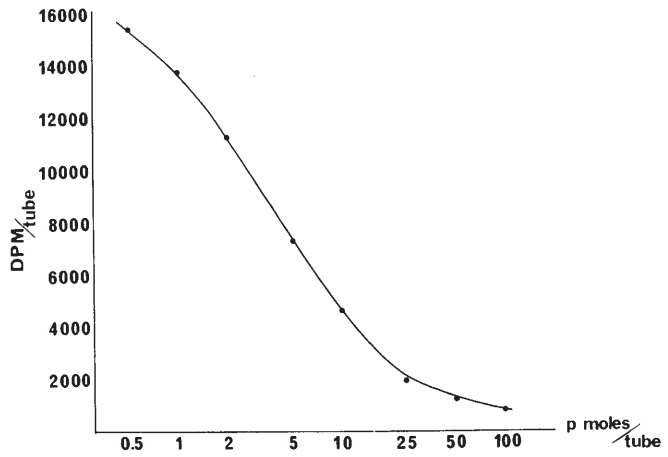

Fig. 1. Standard immunoassay curve for cyclic AMP.

4. Correlation between the left ventricular cyclic AMP concentration and the max dp/dt

Figure 2(A) shows that there was a statistically significant correlation between the left ventricular cyclic AMP concentration and the max dp/dt after the injection of 100 μg/kg of norepinephrine, 1 mg/kg of propranolol and 30 mg/kg of theophylline ethylenediamine ($r = 0.656$ $P < 0.005$), but if the cyclic AMP concentration of the digoxin group (Figure B) was added to Figure A, there was seen no correlation between the cyclic AMP concentration and the max dp/dt ($r = 0.320$ $P < 0.2$). Figure 2(B) shows that there was no correlation

between the left ventricular cyclic AMP concentration and the max dp/dt after the injection of 60 μg/kg of digoxin (r = −0.223).

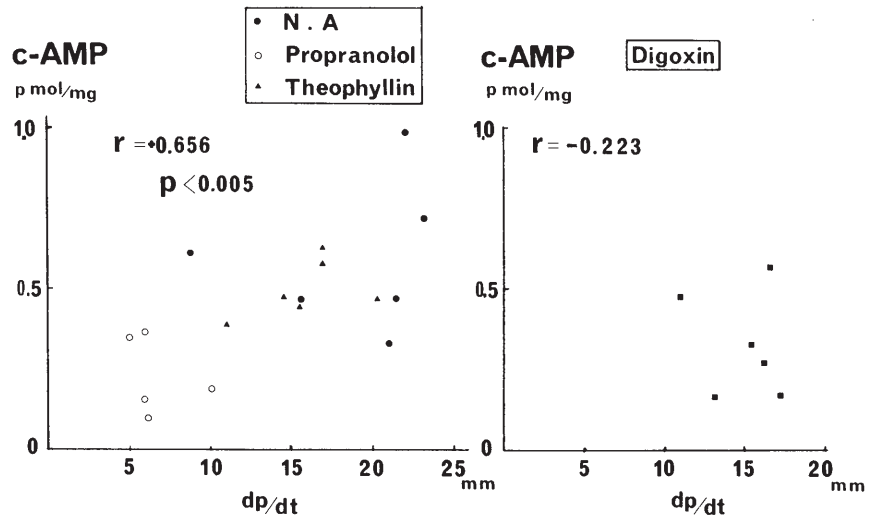

Fig. 2A. Fig. 2B.

Fig. 2. Correlation between left ventricular cyclic AMP concentration and max dp/dt after the injection of norepinephrine, propranolol and theophylline ethylenediamine (Fig. 2A) and digoxin (Fig. 2B).

5. The effects of various vasodilators on phosphodiesterase (PDE) activity in vitro

Figure 3 shows the Lineweaver and Burk plots in the presence and the absence of various vasodilators as PDE inhibitor. In the panel, vertical line indicates the plots of the reciprocal of the reaction velocity, and horizontal line shows the plots of that of the molar concentration of substrates. These plots yielded identical V max in the dipyridamole, theophylline ethylenediamine, prenylamine, carbochromen, nitroglycerin and control (non-inhibitor) groups, and were 4.3×10^{-5} M/min. These plots showed that Km was 1.17×10^{-3} M in the non-inhibitor, that Kp was 2.50×10^{-3} M in the dipyridamole, 1.69×10^{-3} M in the theophylline ethylenediamine, 1.43×10^{-3} M in the prenylamine, and 1.33×10^{-3} M in carbochromen and nitroglycerin.

Table 5 shows that inhibitions of PDE activity by dipyridamole, theophylline ethylenediamine, prenylamine, carbochromen, nitroglycerin in 6×10^{-4} M of cyclic AMP were 44.1, 32.5, 13.3, 12.0 and 9.8 % respectively, and the inhibitor constants (Ki-value) of dipyridamole, theophylline ethylenediamine, prenylamine, carbochromen and nitroglycerin were 87, 227, 455, 714 and $714 \times \mu$M respectively.

DISCUSSION

There have been many reports on the cyclic AMP concentrations of normal animals which indicated that these differ with the animals used and the methods of measurement of cyclic AMP. A sensitive and specific radioimmunoassay for cyclic AMP has been developed which enables the measurement of nucleotide in extracts of small quantity of tissue. Radioimmunoassay is relatively easy and sufficiently specific for cyclic AMP and

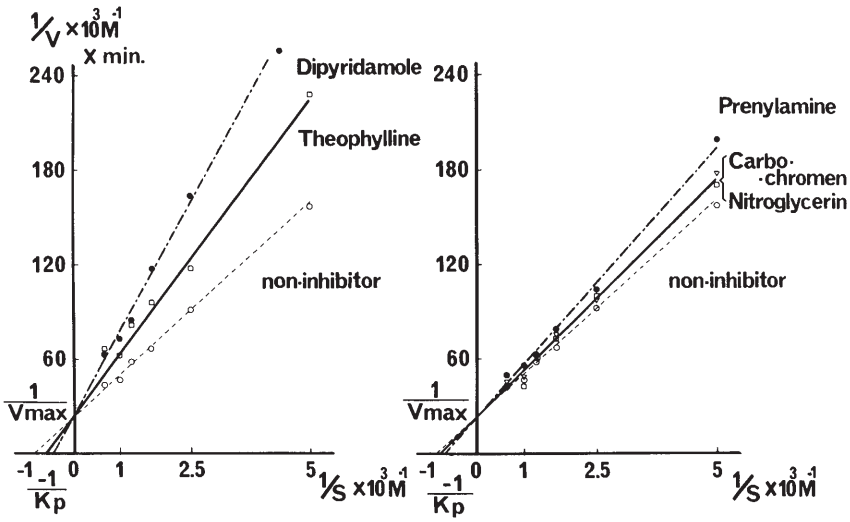

Fig. 3. Inhibition of phosphodiesterase activity. Plots of reciprocal of initial velocity (V) vs. reciprocal of molar concentration of cyclic AMP in the presence and absence of dipyridamole, theophylline ethylenediamine, prenylamine, carbochromen and nitroglycerin.

Table 5. The effects of various coronary vasodilators on myocardial phosphodiesterase activity

	Molar Concentration	No. of Experiments	% Inhibition at 6×10^{-4} mole	Ki Value (μM)
dipyridamole	1×10^{-4} mole	4	44.1 %	87
theophylline	1×10^{-4} mole	4	32.5 %	227
prenylamine	1×10^{-4} mole	4	13.3 %	455
carbochromen	1×10^{-4} mole	4	12.0 %	714
nitroglycerin	1×10^{-4} mole	4	9.8 %	714

Ki values were determined from Lineweaver-Burk plots

i : concentration of inhibitor

Km : the Michaelis constant

Kp : apparent value of Km

$$Ki = \frac{i}{\frac{Kp}{Km} - 1}$$

eliminates the need for prior chromatographic separation of the cyclic nucleotide from other tissue nucleotides. In the present study, the concentration of cyclic AMP in heart muscles and the effects of various drugs on myocardial cyclic AMP levels were measured by this radioimmunoassay.

It has been reported that the catecholamine concentration is highest in the right atrium, next in the left atrium, the right ventricle and lowest in the left ventricle.[14),15)] It was reported by Kuo[16)] that the ventricle was richer in cyclic AMP than the atrium. These experiments indicated the distribution of the cyclic AMP levels of the heart to be the highest in the left ventricle, followed by those in the right ventricle, the left atrium and lowest in the right atrium. It is interesting that there is a negative correlation between the concentration of cyclic AMP and catecholamine, but a positive correlation to exist between the inotropic action of the heart muslce and the concentration of cyclic AMP.

There is considerable evidence to suggest that the positive inotropic effect of catecholamines is mediated by cyclic AMP.[3~9] After the administration of catecholamines, the myocardial cyclic AMP concentrations rises before or at least simultaneously with the positive inotropic response.[3),6),17)] Effects on phosphorylase transformation and glycogenolysis occur after the changes in contractility are evident.[18),19)] Catecholamine agonists exhibit the same order of potency in stimulating adenyl cyclase *in vitro* and increasing contractility of the intact heart.[20)] Phosphodiesterase inhibition appears to potentiate the positive inotropic effects of catecholamines.[21)]

In these experiments, after the injection of norepinephrine, the cyclic AMP concentration of the left ventricle increased with the enhancement of the left ventricular pressure and the dp/dt, and suggested that the cyclic AMP increased by activated adenyl cyclase mediated by β-adrenergic receptor of norepinephrine and enhanced the left ventricular pressure and the dp/dt.

From the studies of β-adrenergic blocking agents, it was initially shown by Murad[2)] *et al.* that the effect of epinephrine on cyclic AMP in the liver preparation of dog could be competitively antagonized by DCI. Subsequently by Robison,[3)] Levey[22)] and Drummond,[23)] it was shown that the order of potency of the blocking agents seemed to be the same in both preventing the formation of cyclic AMP and in opposing the inotropic response. Recently it was reported by Mayer[24)] in isolated perfused rat hearts that DCI and pronethalol abolished the epinephrine-induced increase in cyclic AMP concentration, but these β-blocking agents did not decrease the cyclic AMP concentration so much.

In the present study with whole animals, it was shown that the myocardial cyclic AMP concentration of the left ventricle was not so much decreased by propranolol, practolol and pindolol, and only the right ventricular cyclic AMP decreased significantly by pindolol. It suggested that the β-blocking agent itself did not decrease the myocardial cyclic AMP concentration significantly. On comparing these β-adrenergic blocking agents, there was seen a same tendency as to the left ventricular pressure, the max dp/dt and the left ventricular cyclic AMP concentration, but no significant difference was seen between these drugs.

The evidence was first presented by Butcher and Sutherland[25)] that methylxanthine had an ability of inhibiting phosphodiesterase (PDE) from the heart muscle, and by Rall and West[26)] that it was capable of enhancing the positive inotropic response to catecholamine. The ability of theophylline to increase the cyclic AMP levels in the particulate cardiac preparation was well recognized and led to its use in the adenyl cyclase assay system by Krishna.[21)] However, there is no report concerning the effect of theophylline on the cyclic AMP levels in intact myocardial tissue. In the present study with whole animals, the left ventricular cyclic AMP levels and the max dp/dt increased after the injection of theophylline ethylenediamine. It was interesting that the ventricular fibrillations were induced soon after the injection of theophylline ethylenediamine, and the myocardial cyclic AMP concentration of the ventricular fibrillation group became significantly higher than that of the non-fibrillation group. Based on the above findings, the treatment of acute myocardial infarction with theophylline ethylenediamine should be made with caution.

As illustrated in Figure 2, there was a positive correlation between the max dp/dt and the left ventricular cyclic AMP after the injection of norepinephrine, theophylline ethylenediamine and propranolol. On the other hand, when the results of digoxin were added, there was seen no correlation between the max dp/dt and the left ventricular cyclic AMP. It may be suggested that from these findings that the alteration of myocardial contractility

by norepinephrine, theophylline ethylenediamine and propranolol is mediated through myocardial cyclic AMP, but the enhanced contractility by digoxin is not mediated through cyclic AMP. Furthermore, it was shown that digoxin enhanced the max dp/dt without the increase of cyclic AMP.

There have been many reports on the mechanism of coronary vasodilators, but little work to study the effect of these drugs on myocardial cyclic AMP and phosphodiesterase. Kukovetz *et al.*[11] stated that papaverine and certain other smooth muscle relaxants were very potent inhibitors of phosphodiesterase (PDE), particularly in coronary arterial tissue. The effect of papaverine on PDE was dose dependent and preceded the onset of mechanical relaxation in the circular strips of the coronary arteries, suggesting that the relaxant effect of papaverine like drugs on the smooth muscle was due to their inhibitory action on PDE, and mediated by accumulated cyclic AMP. Recent experiments[27] with an isovolumic perfused heart preparation, when the heart rate was maintained constant, have shown that papaverine leads to a marked increase in myocardial cyclic AMP, as well as transformation of phosphorylase b to phosphorylase a, but no change in the myocardial contractility. In this experiment the left ventricular pressure and the max dp/dt were not determined.

In the present study, dipyridamole exhibited the strongest inhibition of PDE activity. This result showed the same tendency as the report by Kukovetz *et al.*[11] of the coronary artery. But a marked increase in the left ventricular cyclic AMP concentration *in vivo* could not be elucidated. This may be caused by the difference in experimental condition between the purified beef heart PDE *in vitro* and the dog heart muscle *in vivo*. Theophylline ethylenediamine inhibited the PDE activity *in vitro*, and as stated in above, increased the left ventricular cyclic AMP. Prenylamine inhibited the PDE activity and increased the left ventricular cyclic AMP, as well as theophylline ethylenediamine. It suggested that the intracellular cyclic AMP increased by the inhibition of PDE activity of theophylline ethylenediamine and prenylamine. It was reported by Nitz[28] that carbochromen inhibited the decomposition of cyclic AMP by PDE. This effect was proved on both an enzyme from snake venom and on a raw enzyme preparation from rat hearts. Moreover, carbochromen was able to compensate the activation of PDE by imidazole. In the present experiments, carbochromen did not inhibit the PDE activity, but increased the left ventricular cyclic AMP. Anderson[29] reported that the rabbit colon muscle is relaxed by papaverine and nitroglycerin, and was preceded by an increase of the cyclic AMP. Carbochromen and nitroglycerin increased the left ventricular cyclic AMP concentration but did not inhibit the PDE activity. It was suggested that intracellular cyclic AMP do not increase by the inhibition of PDE activity of carbochromen and nitroglycerin.

SUMMARY

The concentration of cyclic AMP in the heart muscles of normal dogs and the effects of various drugs on myocardial cyclic AMP were investigated by radioimmunoassay. Further, studies were made on the effects of various coronary vasodilators on myocardial cyclic AMP in whole animal and phosphodiesterase (PDE) activity *in vitro*.

1) The concentration of myocardial cyclic AMP was highest in the left ventricle (0.318 ± 0.022 p mole/mg), in the right ventricle (0.204 ± 0.024 p mole/mg), in the left atrium (0.131 ± 0.031 p mole/mg) and lowest in the right atrium (0.094 ± 0.025 p mole/mg).

2) Norepinephrine significantly increased the left ventricular cyclic AMP concentration, the left ventricular pressure and the max dp/dt.

3) Digoxin enhanced the left ventricular pressure and the max dp/dt, but increased the myocardial cyclic AMP without significance.
4) β-adrenergic blocking agents did not significantly decrease the myocardial cyclic AMP concentration and did not strongly reduce the left ventricular pressure and the max dp/dt. With propranolol, practolol and pindolol, there was seen the same tendency as regards the left ventricular cyclic AMP, the left ventricular pressure and the max dp/dt but there was seen no significant difference in the effects between these three drugs.
5) Theophylline ethylenediamine increased the left ventricular cyclic AMP and the max dp/dt. Out of 9 dogs, in 3 dogs ventricular fibrillation was induced immediately after the injection of theophylline ethylenediamine. The myocardial cyclic AMP concentration of the ventricular fibrillation group was significantly increased when compared with the non-ventricular fibrillation group.
6) Dipyridamole, prenylamine, carbochromen and nitroglycerin increased the myocardial cyclic AMP concentration.
7) Dipyridamole, theophylline ethylenediamine and prenylamine were competitive inhibitors of purified beef heart PDE *in vitro*.

From these findings it may be concluded that norepinephrine, theophylline ethylenediamine and propranolol affect the max dp/dt by the mediation of cyclic AMP, but digoxin effects it by some other mechanism.

It was concluded that the increase of cyclic AMP concentration caused by various coronary vasodilators is due to the inhibition of PDE activity, at least in cases of theophylline ethylenediamine, dipyridamole and prenylamine.

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