Biochimica et Biophysica Acta, 526 (1978) 479—488

BBA 68544

CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN HEART AND AORTA OF SPONTANEOUSLY HYPERTENSIVE RATS

RAM V. SHARMA and RAMESH C. BHALLA

Department of Anatomy and Cardiovascular Center, College of Medicine, University of Iowa, Iowa City, Iowa 52242 (U.S.A.)

(Received November 14th, 1977)
(Revised manuscript received May 9th, 1978)

Summary

Cyclic AMP and cyclic GMP phosphodiesterase (3′:5′-cyclic-AMP 5′-nucleotidohydrolase, EC 3.1.4.17) activities were compared in soluble (105 000 × *g* supernatant) and particulate (105 000 × *g* pellet of 700 × *g* supernatant) fractions of heart and aorta of hypertensive and normotensive rats at 5 μM and 100 μM substrate concentrations. Cyclic AMP phosphodiesterase activity was significantly higher ($P < 0.05$) in particular fractions of hypertensive rat aorta at 100 μM, while no significant changes were observed in heart. Cyclic GMP phosphodiesterase activity was significantly increased ($P < 0.05$) in particulate and soluble fractions of hypertensive rat heart compared to normotensive rat at both substrate concentrations, but there were no differences in aorta. When phosphodiesterase activities in the soluble fractions of aorta and heart were examined as a function of enzyme concentration, rectilinear rates were observed with micromolar, but not with millimolar concentrations of cyclic AMP or cyclic GMP. Double reciprocal plots for cyclic AMP phosphodiesterase activity were non-linear in heart and aorta supernatants. Similarly, cyclic GMP phosphodiesterase yielded non-linear kinetics in heart supernatant, however, the plots were linear for aorta supernatant. Particulate fractions of heart showed linear kinetics for cyclic AMP and cyclic GMP phosphodiesterase and the K_m values were in the range of high K_m for soluble enzymes. V in hypertensive rats was approximately doubled compared to normotensive rats for cyclic AMP and cyclic GMP phosphodiesterase. These data suggest that altered phosphodiesterase activity could result in a change in cyclic nucleotide index in the cardiovascular tissues of hypertensive rat.

Introduction

It is well accepted that intracellular levels of cyclic nucleotides play an important role in the regulation of contraction-relaxation mechanism of vascu-

lar smooth [1,2] and cardiac muscle [3—5]. Results obtained with agents causing smooth muscle relaxation either by stimulating β-adrenergic receptors or inhibiting phosphodiesterase activity suggest that relaxation of smooth muscle is due to elevated cyclic AMP levels [6,7]. Although the physiological role of cyclic GMP in smooth muscle contraction is not clearly understood, the levels of this nucleotide, however, are increased by agents that cause contraction in smooth muscle [8]. Alternately, increase in cyclic GMP in myocardium results in suppression of cardiac contraction [9] and increase in cyclic AMP stimulates force and rate of contraction of myocardium.

A possible association between cyclic nucleotide metabolism and the hypertensive disease was suggested when low levels of cyclic AMP were observed in the cardiac and vascular tissues of spontaneous, stress, neurogenic and DOCA hypertensive rats [11—15]. Amer [10] proposed that the ratio of cyclic AMP to cyclic GMP rather than their absolute concentration is of biological significance in regulation of contraction-relaxation mechanism of vascular smooth and cardiac muscle in normal and hypertensive rats.

Regulation of cyclic nucleotide levels in biological systems is a function chiefly of both the rate of synthesis and the rate of degradation. The attempts made to study the enzymes responsible for the synthetic and degradative mechanisms in the hypertensive state have resulted in varying results. Thus the decrease in cyclic AMP concentration has been attributed by Amer's group [10—12] to an increase in cyclic AMP phosphodiesterase (3′:5′-cyclic-AMP 5′-nucleotidohydrolase, EC 3.1.4.17), whereas, Ramanathan and Shibata [14] have observed no change in phosphodiesterase activity and have correlated the decrease in cyclic AMP to low levels of adenylate cyclase. Furthermore, Triner et al. [16] and Klenerova et al. [17] have reported no change in phosphodiesterase activity in cardiovascular tissues of hypertensive compared to normotensive rats. Phosphodiesterases have been shown to be present in soluble, as well as particulate, fractions [18], however, all the studies in hypertensive animals, cited above, have been carried out with tissue homogenates using different substrate concentrations. The available data therefore presents an enormous problem in comparing the results of different investigators and also in assessing whether the differences are in soluble or particulate phosphodiesterase in the vascular and cardiac muscle of hypertensive and normotensive rats.

It was, therefore, of interest to reinvestigate the levels, and the kinetics of cyclic nucleotide phosphodiesterase(s) in the soluble and particulate fractions from heart and aorta of hypertensive and normotensive rats using both cyclic AMP and cyclic GMP as substrates. In the present study, we have observed that cyclic AMP phosphodiesterase activity is significantly increased in particulate fractions of aorta when assayed at 100 μM cyclic AMP concentrations in hypertensive rats. Similarly, cyclic GMP hydrolysis is significantly increased in soluble, as well as particulate, fractions of hypertensive rat heart at both 5 μM and 100 μM cyclic GMP.

Experimental procedures

Materials. Dowex 1 X 8 200—400 mesh (Cl^- form), cyclic AMP, cyclic GMP, snake venom (*Crotalus atrox*), Tris base, ethyleneglycol bis(α-aminoethyl-

ether)-*N*,*N*'-tetraacetic acid (EGTA) and theophylline were purchased from Sigma Chemical Co., St. Louis, Mo. Aquasol, cyclic[^{3}H]AMP (3.8 Ci/mmol), and cyclic[^{3}H]GMP (7.8 Ci/mmol), [^{14}C]adenosine (0.05 Ci/mmol), and [^{14}C]-guanosine (0.5 Ci/mmol) were obtained from New England Nuclear, Boston, Mass.

Animals. Age-matched male and female Kyoto Wistar hypertensive rats (SHR) and Kyoto Wistar (WKY) normotensive rats (12—16 weeks), from a colony maintained at the University of Iowa through inbreeding of original strain of Okamoto and Aoki [19] were used throughout this study. Preoperative systolic pressure was determined in the unanaesthetized state by the tail plethysmographic method using an automatic cuff-inflator-pulse reading system manufactured by Technilab Instruments. The values ± S.D. were 136 ± 8.3 mm Hg (n = 83) and 187 ± 18.3 mm Hg (n = 75) for normotensive and hypertensive rats, respectively.

Preparation of cell fractions. The rats were killed by direct heart puncture while they were under light ether anesthesia. The heart ventricles and aorta from arch to bifurcation were removed and cleaned of loose connective tissue and adherent fat. The tissues were washed twice with cold homogenizing buffer (20 mM Tris · HCl, pH 7.4) to wash blood and then homogenized in 7—10 vols. cold buffer using a Polytron (Brinkman) at a rheostat setting of 2.6 for 3 × 10-s pulses with 1 min intervals. The homogenate was filtered through two layers of cheese cloth and was centrifuged at 700 × g for 10 min, the supernatant was then centrifuged at 105 000 × g for 60 min to yield pellet (particulate) and soluble fractions. The pellet was washed once with homogenizing buffer before assaying for activity. All these steps were carried out at 4°C.

Assay for phosphodiesterase. The assay for phosphodiesterase was in principle the same as that described by Butcher and Sutherland [20] except for slight experimental modification. Phosphodiesterase was assayed by a two-step procedure. At the end of the reaction, 1 ml of a Dowex slurry (3 : 1, w/v) prepared, according to Boudreau and Drummond [21], in 3.0 mM acetic acid (pH 3.0) for cyclic AMP and in 115 mM formic acid (pH 2.2) for cyclic GMP assays was added to the reaction mixture and tubes were allowed to stand for 10 min in the cold, centrifuged at 1000 × g for 10 min, then 0.5 ml supernatant of the 1.55 ml total assay volume was withdrawn and added to 10 ml Aquasol and counted in liquid scintillation spectrometer.

Specific activity of phosphodiesterase is defined as nmol cyclic nucleotide hydrolyzed/min per mg protein at 30°C. The data were analyzed for significance by the Student's t-test method and are presented as mean ± S.D. Protein was determined by the method of Lowry et al. [22] with bovine serum albumin as standard.

Results

Cyclic nucleotide phosphodiesterase activity in the heart and aorta of normotensive and hypertensive rats. Results of cyclic nucleotide phosphodiesterase activities are summarized in Tables I and II. Cyclic AMP phosphodiesterase in the particulate fraction of aorta was significantly higher ($P < 0.05$) in the hypertensive than the normotensive animals, when assayed at 100 μM cyclic

TABLE I

CYCLIC AMP AND CYCLIC GMP PHOSPHODIESTERASE ACTIVITY IN THE AORTA OF HYPERTENSIVE AND NORMOTENSIVE RATS

Enzyme concentration in each experiment was 60—80 μg protein for the soluble fraction and 150—200 μg for the particulate fraction. Data are expressed as nmol cyclic nucleotide hydrolyzed/mg protein per min at 30°C. Values are the mean ± S.D. Numbers in parentheses indicate the number of experiments. For each experiment aorta from 5—6 animals were pooled. Each value was derived from duplicate determinations.

Fraction	Substrate concentration (μM)			
	Normotensive		Hypertensive	
	5	100	5	100
Cyclic AMP phosphodiesterase				
Soluble	0.54 ± 0.09 (6)	2.44 ± 0.30 (12)	0.47 ± 0.09 (6)	2.49 ± 0.68 (12)
Particulate	0.23 ± 0.05 (6)	0.47 ± 0.08 (6)	0.24 ± 0.04 (6)	0.63 ± 0.14 * (6)
Cyclic GMP phosphodiesterase				
Soluble	0.89 ± 0.12 (4)	1.65 ± 0.16 (12)	0.86 ± 0.08 (4)	1.55 ± 0.17 (12)
Particulate	0.19 ± 0.04 (4)	0.92 ± 0.12 (4)	0.23 ± 0.05 (4)	1.16 ± 0.18 (4)

* Significantly different from respective normotensive rat control values ($P < 0.05$).

AMP concentration (Table I). Cyclic GMP phosphodiesterase activity in heart was significantly increased ($P < 0.05$) in hypertensive compared to normotensive in soluble, as well as particulate, fractions at 5 μM and 100 μM (Table II). On the other hand, there was no significant difference in cyclic AMP phosphodiesterase in either fraction at both substrate levels, but in particulate fraction cyclic AMP phosphodiesterase activity was consistently higher in hypertensive than normotensive rats.

Effect of enzyme concentration on phosphodiesterase activity. The phospho-

TABLE II

CYCLIC AMP AND CYCLIC GMP PHOSPHODIESTERASE ACTIVITY IN THE HEART OF HYPERTENSIVE AND NORMOTENSIVE RATS

Enzyme concentration in each experiment was 60—80 μg protein for soluble fraction and 150—200 μg for particulate fraction. Data are expressed as nmol cyclic nucleotide hydrolized/mg protein per min at 30°C; values are the mean ± S.D. Numbers in parentheses indicate the number of animals. Each value was derived from duplicate determinations.

Fraction	Substrate concentration (μM)			
	Normotensive		Hypertensive	
	5	100	5	100
Cyclic AMP phosphodiesterase				
Soluble	0.71 ± 0.15 (10)	2.24 ± 0.21 (10)	0.69 ± 0.19 (10)	2.05 ± 0.37 (10)
Particulate	0.27 ± 0.05 (6)	0.94 ± 0.14 (5)	0.35 ± 0.06 (6)	1.12 ± 0.21 (5)
Cyclic GMP phosphodiesterase				
Soluble	0.54 ± 0.08 (5)	1.4 ± 0.25 (7)	0.77 ± 0.04 * (5)	2.1 ± 0.47 * (7)
Particulate	0.16 ± 0.03 (7)	0.72 ± 0.13 (9)	0.24 ± 0.06 * (7)	0.98 ± 0.19 * (9)

* Significantly different from respective WKY control values ($P < 0.05$).

diesterase activity was plotted as a function of protein concentration. The enzyme activity in soluble fraction of heart and aorta for both hypertensive and normotensive rats at low cyclic AMP and cyclic GMP concentrations (5 μM) yielded rectilinear curves. However, at 100 μM cyclic AMP and cyclic GMP concentrations, the phosphodiesterase activity resulted in non-linear plots. Supplementing the reaction mixture with bovine serum albumin did not result in a non-linear plot. In both normotensive and hypertensive rats, the cyclic AMP and cyclic GMP phosphodiesterase activity of aorta soluble fraction expressed per mg protein showed a decline with increasing enzyme concentration, whereas in the soluble fraction of heart the activity for hydrolysis of both nucleotides increased (Fig. 1).

Kinetics of cyclic AMP and cyclic GMP phosphodiesterases. The Lineweaver-Burk plot for cyclic AMP phosphodiesterase activity of soluble fractions from normotensive and hypertensive rat aorta and heart resulted in non-linear curves. Extrapolation of the linear regions gave two apparent K_m values. The double reciprocal plot for cyclic GMP phosphodiesterase in the soluble fraction was linear for aorta and non-linear for heart. On the other hand, the kinetic plots for the heart particulate fraction were linear both for cyclic AMP and cyclic GMP. The points from linear region of the double reciprocal plots were analyzed by Cleland's computor program [23] and the values for different kinetic parameters are assembled in Table III. A double reciprocal plot representing a

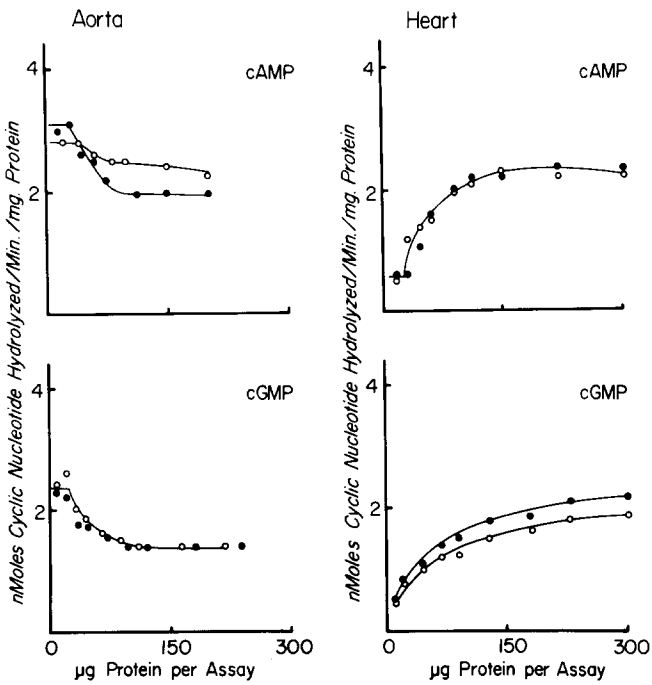

Fig. 1. Specific activity of 105 000 X *g* supernatant phosphodiesterase as a function of protein concentration in normotensive (○) and hypertensive (●) aorta and heart. All assay tubes contained standard reagents (100 μM cyclic AMP (cAMP) or cyclic GMP (cGMP)) and varying amounts of protein. Data are means of duplicate determinations of 2—3 different experiments. For each experiment aorta from 5—6 animals was pooled. For heart, number of experiments indicates number of animals.

TABLE III

KINETIC CONSTANTS OF CYCLIC AMP AND CYCLIC GMP PHOSPHODIESTERASES FROM HEART AND AORTA OF NORMOTENSIVE AND HYPERTENSIVE RATS

The kinetic constants are calculated with a computer program based on the calculations of Cleland [23]. K_m is expressed in μM whereas V is expressed as nmol substrate hydrolyzed/min per mg protein. All results are expressed as mean ± S.E. Numbers in parantheses indicate number of animals used.

Kinetic constants	Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
	Normotensive	Hypertensive	Normotensive	Hypertensive
(A) Heart supernatant				
Low affinity phosphodiesterase				
K_m	28.11 ± 3.20	48.22 ± 6.47 * (6)	14.03 ± 2.85	27.3 ± 3.30 * (6)
V	3.60 ± 0.19	5.22 ± 0.39 *	1.52 ± 0.11	3.58 ± 0.20 *
High affinity phosphodiesterase				
K_m	3.14 ± 0.66	1.42 ± 0.42 * (6)	2.47 ± 0.36	4.35 ± 1.11 * (6)
V	1.07 ± 0.10	0.79 ± 0.08 *	0.65 ± 0.06	1.40 ± 0.10 *
(B) Heart pellet				
Low affinity phosphodiesterase				
K_m	88.57 ± 13.6	67.72 ± 8.14 (4)	12.22 ± 1.96	9.99 ± 1.95 (4)
V	0.96 ± 0.097	1.42 ± 0.10 *	0.73 ± 0.043	1.45 ± 0.096 *
High affinity phosphodiesterase	Absent	Absent	Absent	Absent
(C) Aorta supernatant				
Low affinity phosphodiesterase				
K_m	17.70 ± 2.5	16.15 ± 1.23 (8)	Absent	Absent
V	1.83 ± 0.10	1.71 ± 0.05		
High Affinity phosphodiesterase				
K_m	3.88 ± 0.49	4.48 ± 0.70 (8)	3.84 ± 0.39	4.67 ± 0.54 (8)
V	0.79 ± 0.05	0.84 ± 0.07	1.75 ± 0.045	1.77 ± 0.056

* Significantly different from normotensive control ($P < 0.05$). The results from different experiments were quite reproducible and yielded the same values.

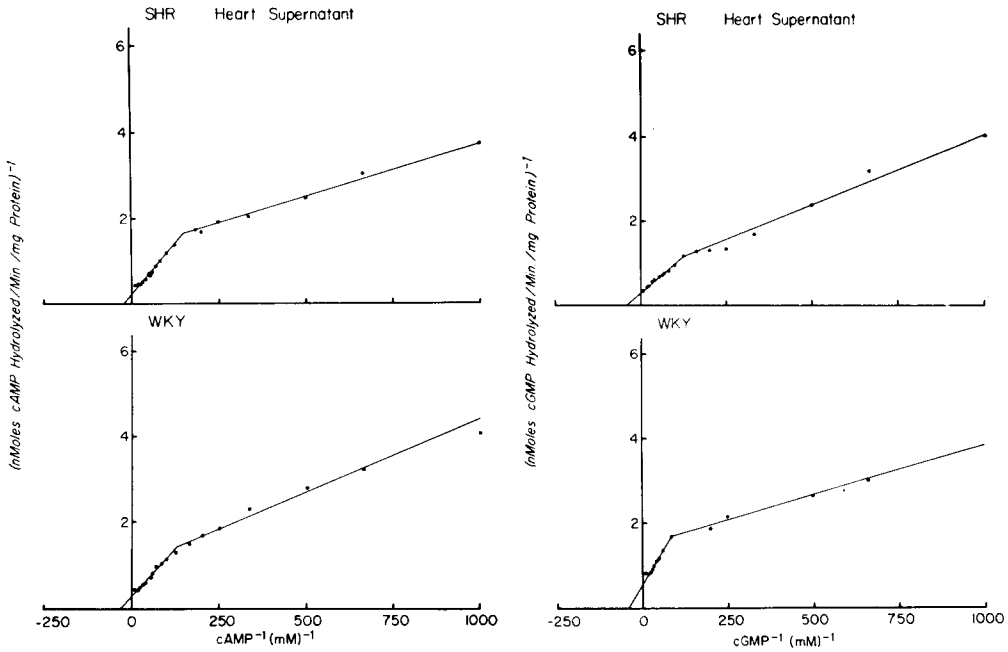

Fig. 2. Lineweaver-Burk plot of cyclic AMP (cAMP) and cyclic GMP (cGMP) hydrolysis by the 105 000 × *g* supernatant of normotensive and hypertensive rat heart. The concentration of cyclic nucleotide varied from 0.5 μM to 1 mM. The enzyme concentration was 60—70 μg protein per assay. The values are the mean of duplicate determinations from two different preparations.

typical experiment for heart supernatant is shown in Fig. 2. In the soluble fraction of heart the K_m and V values for both low and high affinity form were significantly increased ($P < 0.05$) in hypertensive as compared to normotensive rat. On the other hand, the K_m and V values for low affinity cyclic AMP phosphodiesterase were significantly increased ($P < 0.05$) in hypertensive rats, whereas, for high affinity enzyme, both K_m and V were significantly decreased ($P < 0.05$) (Table III). In heart particulate fraction V values for low affinity (the only form present) cyclic AMP and cyclic GMP phosphodiesterases were significantly increased ($P < 0.05$) in hypertensive rats compared to normotensive rats, while no significant changes were observed in K_m values (Table III). In the aorta supernatants, no significant differences were observed for K_m or V values of cyclic AMP and cyclic GMP phosphodiesterases in normotensive and hypertensive rats (Table III).

Inhibition by theophylline. Inhibition of soluble cyclic AMP and cyclic GMP phosphodiesterases by theophylline was studied at substrate concentrations of 5 μM and 100 μM using nine inhibitor concentrations varying from 10 μM to 5 mM. Inhibition constant (K_i) calculated according to Dixon and Webb [24] is given in Table III. Dixon plots revealed that inhibition was competitive in heart for both cyclic AMP and cyclic GMP hydrolysis. However, in aorta biphasic $1/v$ vs S curves were obtained for theophylline inhibition at low substrate concentration (5 μM cyclic AMP or cyclic GMP) which, on extrapolation of two linear regions, gave both competitive and non-competitive inhibition constants. There were no significant differences between hypertensive and normotensive

TABLE IV

INHIBITION CONSTANTS (K_i) OF SOLUBLE CYCLIC AMP AND CYCLIC GMP PHOSPHODIESTERASE INHIBITION BY THEOPHYLLINE

Inhibition constants were determined according to Dixon and Webb [24] as described in the text. Values in parentheses indicate non-competitive inhibition constants. The values are the mean of duplicate determinations from two different experiments.

Tissue	K_i (mM)			
	Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
	Normotensive	Hypertensive	Normotensive	Hypertensive
Heart	0.48	0.42	0.23	0.26
Aorta	0.23 (1.53)	0.28 (1.50)	0.19 (1.36)	0.24 (1.60)

rats in inhibition constants for cyclic AMP or cyclic GMP phosphodiesterases in heart and aorta. However, the K_i for cyclic GMP phosphodiesterase was only half that of cyclic AMP phosphodiesterase (Table IV).

The reaction rates for cyclic AMP and cyclic GMP hydrolysis were linear up to 60 min at 30°C in both tissues. Although in all these experiments percent hydrolysis of substrate was kept between 10 and 40, the rates were linear from 5 to 60% of the substrate hydrolyzed. The pH optima for cyclic AMP hydrolysis was 7.8 and 7.4 for heart and aorta soluble enzyme in 20 mM Tris-HCl, cyclic GMP hydrolysis was maximum at pH 8.2 in both tissues. Both cyclic AMP and cyclic GMP phosphodiesterase activities were activated by Mg^{2+} and Mn^{2+} but not by Ca^{2+}. These results were in agreement with those reported in literature [25] for the porcine coronary artery. There were no specific differences with regard to the pH optima and the divalent cations' requirement for both cyclic AMP and cyclic GMP phosphodiesterases between the hypertensive and normotensive rats in heart or aorta.

Discussion

The results presented in this paper show that the activity of cyclic AMP phosphodiesterase in vascular smooth muscle of the hypertensive rat was significantly increased in the particulate fraction, but not in the soluble fraction as compared to normotensive rat (Table I). Although the total phosphodiesterase activity in the particulate fraction is small compared to the soluble phosphodiesterase, it is possible that the particulate enzyme may play an important role in regulating the cyclic AMP levels at the membrane site. The significance of these results may be particularly pertinent in relation to our previous observations [26] that the phosphorylation and Ca^{2+} uptake in the aortic microsomes of hypertensive rats is significantly reduced as compared to normotensive rats. For hypertensive and normotensive rats, cyclic GMP phosphodiesterase levels were similar in both the soluble and the particulate fractions of vascular smooth muscle (Table I). Therefore, that the altered activity of cyclic nucleotide phosphodiesterases will increase the cyclic AMP to cyclic GMP ratio in hypertensive rats is compatible with Amer's claim [10] that there is increased tone in the hypertensive state due to alteration in the cyclic nucleotide index.

In the soluble, as well as in the particulate, fractions of myocardium, the cyclic GMP phosphodiesterase activity was significantly increased in hypertensive compared with normotensive rat, at both 5 μM and 100 μM substrate (Table II). No significant differences were observed in cyclic AMP phosphodiesterase activity in the hearts of hypertensive and normotensive rats. Amer [10] has reported increased low K_m cyclic GMP phosphodiesterase activity in hypertensive rat heart, and we have observed a similar increase in both low K_m and high K_m phosphodiesterase activity. The rate and force of myocardial contraction appears to be closely related to a decrease in cyclic GMP levels at the onset of contraction accompanied by an increase in cyclic AMP levels [5,27, 28]. In the hypertensive state, the force and rate of heart beat increase [29]. Hence, it seems possible that, in hypertensive rat myocardium the increase in cyclic GMP phosphodiesterase with no significant change in cyclic AMP phosphodiesterase would tend to change the cyclic nucleotide index in a direction which may have an important influence over contraction-relaxation cycle.

Although cyclic AMP phosphodiesterase activity in heart supernatant when tested at 5 μM or 100 μM substrate was not altered in hypertensive rat; a closer examination of the kinetics parameters revealed that both K_m and V values for the low affinity enzyme were significantly increased in hypertension while these values were significantly decreased for high affinity enzyme (Table III). These changes in kinetic parameters in the opposite direction for low and high affinity forms of the enzyme could mask differences in the enzyme activity when measured in crude preparations. At this point it is difficult to assess the importance of low affinity or high affinity form of phosphodiesterase in the hypertensive state because of the lack of knowledge of relative proportions of these two forms in the heart supernatants of normotensive and hypertensive rats. The significant increase in V of cyclic GMP phosphodiesterase in both the soluble and the particulate fractions of heart (Table III) might, at least in part, account for the observed increase in the phosphodiesterase activity in the hypertensive rat as compared to normotensive rat (Table III).

When phosphodiesterase activities in the extracts of normotensive and hypertensive rat heart and aorta 105 000 $\times$ g supernatant were examined as a function of enzyme concentration, rectilinear rates were observed with 5 μM but not with 100 μM cyclic AMP or cyclic GMP. This effect of protein concentration on enzyme activity was more apparent in hypertensive than in normotensive rat aorta for cyclic AMP phosphodiesterase (Fig. 1), although such changes were not observed in heart. One interpretation is that the enzyme exists in different aggregated states which have different specific activities, and that the concentration of enzyme and that of the substrate determine the state of enzyme aggregation. Pichard and Cheung [33] using 1 μM and 2 mM cyclic AMP concentrations have also shown that at 1 μM concentration the curve was rectilinear but at 2 mM concentration the curve was non-linear for cyclic AMP and cyclic GMP hydrolysis for human platelets, bovine brain and rat liver. Recently it has been shown that for yeast particulate enzyme the activity of cyclic AMP phosphodiesterase increased with increasing protein concentration [34]. However, in this report it has been shown that supplementing the reaction mixture with bovine serum albumin results in a linear plot. These varying results could be attributed to the differences in the tissues and species used as

the source of enzyme preparation in these investigations. The observed effects of protein concentration on the enzyme activity warrant the necessity of defining the exact experimental conditions while comparing cyclic nucleotide phosphodiesterase activity between normal and diseased state and also while comparing results from different investigators.

Acknowledgements

This work was supported by NIH Grants HL 19027, HL 14388 and a grant-in-aid from the American Heart Association with funds contributed in part by the Iowa Heart Association. Miss Jeanna Piper participated in some of the initial experiments, as NSF Health Science Program trainee as high school student, the authors acknowledge with great appreciation her contribution and her expert technical help. Thanks to Dr. Diwan Singh for his valuable discussions.

References

1 Andersson, R. (1972) Acta Physiol. Scand., Suppl. 382, 1—59
2 Namm, D.H. and Leader, J.P. (1976) Blood Vessels 13, 24—47
3 Entman, M.L. (1974) Adv. Cycl. Nucl. Res. 4, 163—194
4 Tsien, R.W. (1977) Adv. Cycl. Nucl. Res. 8, 363—420
5 Goldberg, N.D. and Haddox, M.K. (1977) Annu. Rev. Biochem. 46, 823—896
6 Bar, H.P. (1974) Adv. Cycl. Nucl. Res. 4, 195—238
7 Andersson, R., Nilsson, K., Wikberg, J., Johansson, S. and Lundholm, L. (1975) Adv. Cycl. Nucl. Res. 5, 491—518
8 Goldberg, N.D., Haddox, M.K., Nicol. S.E., Glass, D.B., Sanford, C.H., Kuehl, Jr., F.A. and Estensen, R. (1975) Adv. Cycl. Nucl. Res. 5, 307—330
9 George, W.J., Polson, J.B., O'Toole, A.G. and Goldberg, N.D. (1970) Proc. Natl. Acad. Sci. U.S. 66, 398—403
10 Amer, M.S. (1975) in Cyclic Nucleotides in Disease (Wiess, B., ed.), pp. 133—156, University Park Press, Baltimore
11 Amer, M.S. (1973) Science 179, 807—809
12 Amer, M.S., Gomoll, A.W., Perhack, Jr., J.L., Ferguson, H.C. and McKinney, G.R. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4930—4934
13 Amer, M.S., Doba, N. and Reis, D.J. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2135—2139
14 Ramanathan, S. and Shibata, S. (1974) Blood Vessels 11, 312—318
15 Ramanathan, S., Shibata, S., Tasaki, T.K. and Ichord, R.N. (1976) Biochem. Pharmacol. 25, 223—225
16 Triner, L., Vulliemoz, Y., Verosky, M. and Manger, W.M. (1975) Biochem. Pharmacol. 24, 743—745
17 Klenerova, V., Albrecht, I. and Hynie, S. (1975) Pharmacol. Res. Commun. 7, 453—462
18 Wells, J.N. and Hardman, J.G. (1977) Adv. Cycl. Nucl. Res. 8, 119—144
19 Okamoto, K. and Aoki, I. (1963) Jap. Circ. J. 27, 282—285
20 Butcher, R.W. and Sutherland, E.W. (1962) J. Biol. Chem. 237, 1244—1250
21 Boudreau, R.J. and Drummond, G.I. (1975) Anal. Biochem. 63, 388—399
22 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
23 Cleland, W.W. (1970) In The Enzymes (Boyer, P.D., ed.), 3rd edn. Vol. 1, pp. 1—65, Academic Press, New York
24 Dixon, M. and Webb, E.C. (1964) Enzymes, 2nd edn., pp. 315—359, Longmans Green, London
25 Wells, J.N., Baird, C.E., Wu, Y.J. and Hardman, J.G. (1975) Biochim. Biophys. Acta 384, 430—442
26 Bhalla, R.C., Webb, R.C., Singh, D., Ashley, T. and Brock, T. (1978) Mol. Pharmacol. 14, 113—122
27 Woolenberger, A., Babskii, E.B., Krause, E.G., Genz, S., Blohm, D. and Bogdanova, E.V. (1973) Biochem. Biophys. Res. Commun. 55, 446—452
28 Brooker, G. (1975) Adv. Cycl. Nucl. Res. 5, 435—452
29 Genest, J., Nowaczynski, W., Kuchel, O., Boucher, R., Ortega, J.M.R., Constantopoulos, G., Ganten, D. and Messerli, F. (1976) Rec. Prog. Horm. Res. 32, 377—417
30 Appleman, M.M., Thompson, W.J. and Russell, T.R. (1973) Adv. Cycl. Nucl. Res. 3, 65—98
31 Murtaugh, T., Sharma, R.V. and Bhalla, R.C. (1977) 3rd Int. Conf. Cycl. Nucl., p. 65, Raven Press, New York
32 Russel, T.R., Terasaki, W.L. and Appleman, M.M. (1973) J. Biol. Chem. 248, 1334—1340
33 Picherd, A.L. and Cheung, W.Y. (1976) J. Biol. Chem. 251, 5726—5737
34 Londesborough, J. (1977) Biochem. J. 163, 467—476