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ADENYLATE CYCLASE ACTIVITY IN MYOCARDIUM OF SPONTANEOUSLY HYPERTENSIVE RAT: EFFECT OF ENDOGENOUS FACTORS AND SOLUBILIZATION

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SUMMARY

The isoproterenol- and glucagon-stimulated adenylate cyclase activities in the myocardial membranes of hypertensive rat were consistantly lower as compared with normal controls. Addition of cytosolic fraction (100,000 xg supernatant) to the particulate preparation had an additive effect for glucagon and Gpp(NH)p stimulated enzyme activity and a synergistic effect for isoproterenol stimulation. Cytosolic fraction of normal control animals did not bring the adenylate cyclase activity in SHR equivalent to the control values. The basal and F^--stimulated enzyme activity of solubilized adenylate cyclase was reduced by about 30% in SHR as compared with WKY, which could be due to a decrease in the actual amount of adenylate cyclase in the myocardium of SHR.

Earlier reports from this laboratory (1) and from others (2-4) have demonstrated a loss of sensitivity of adenylate cyclase (EC.4.6.1.1.) stimulation by catecholamines in cardiovascular tissues of spontaneously hypertensive rat (SHR). The molecular mechanisms resulting into the loss of hormone sensitivity in SHR have not been elucidated, although several possibilities exist which deserve consideration, These include (i) altered affinity or number of β-adrenergic receptors, (ii) a decrease in the amount of adenylate cyclase enzyme molecules present (iii) changes in the amount of an adenylate cyclase modulator(s), (iv) alterations in the kinetic parameters of the enzyme.

The presence of an endogenous factor which stimulates adenylate cyclase activity in the myocardial membranes has been recently demonstrated (5). In

Abbreviations: SHR, Spontaneously hypertensive rat; WKY, Normal Kyoto Wistar rat; Gpp(NH)p, guanylylimidodiphosphate; PEP, phosphoenolpyruvate; cyclic AMP, adenosine-3',5'-monophosphate; ATP, adenosine 5'-triphosphate.

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addition, the importance of microenvironment of membrane in adenylate cyclase activity has been shown (6). Therefore, we have examined the effect of homologous (SHR) and heterologous (Kyoto Wistar normotensive control, WKY) cytosol on hormone-sensitive adenylate cyclase enzyme activity in myocardium of SHR. Further, the importance of membrane structure in relation to decreased adenylate cyclase activity in SHR was tested by solubilizing the enzyme from membrane by using non-ionic detergents. We report, that addition of homologous or heterologous cytosol to the SHR myocardial particulate preparation did not increase the enzyme activity to the level of WKY. The basal and fluoride-stimulated enzyme activity of the soluble preparation of SHR was reduced by about 30% as compared to WKY. These observations suggest either a decrease in the amount of adenylate cyclase enzyme molecules or alterations in the kinetic properties of the enzyme in the myocardium of SHR.

MATERIALS AND METHODS

Materials:

Adenosine 5'-triphoshate (ATP: sodium salt from equine muscle), adenosine 3', 5'-monophosphate, 3-isobutryl-1-methyl xanthine, Dowex 50W-x8 (200-400 mesh, 8% cross linked, H+ form) and guanylylimidodiphosphate (Gpp(NH)p) were obtained from Sigma Chemical Co., St. Louis, Missouri, 2 phosphoenol pyruvate (PEP, trisodium salt) and pyruvate kinase (from rabbit muscle) were obtained from Calbiochem, La Jolla, California [^{32}P]-ATP (TEA salt) 10-30 Ci/mmole, [^{3}H]-cyclic AMP (NH_4 salt 4 Ci/mmole) and Aquasol were purchased from New England Nuclear, Boston, Massachusetts.

Adult Kyoto Wistar spontaneously hypertensive rats (SHR), and Kyoto Wistar normotensive rats (WKY) 12-16 weeks old were used. The SHR maintained at the University of Iowa are inbred descendents of the hypertensive Wistar strain developed by Okamoto and Aoki (7). The control rats were raised under conditions identical to those used for SHR. Preoperative systolic blood pressures were determined in the unanesthetized state by the tail plethysmographic method using an automated cuff inflator-pulse reading system manufactured by Technilab Instruments. The blood pressure for WKY and SHR mean $\pm$ SEM was 130 $\pm$ 2 (n=30) and 192 $\pm$ 2 (n=31) respectively.

Preparation of myocardial membranes:

The rats were killed by direct heart puncture while they were under light ether anesthesia. The ventricles were dissected free of atria and washed with ice-cold homogenizing buffer (0.25 M sucrose, 0.05 M Tris-Hcl, pH 7.4) to get rid of blood. The tissue was homogenized in 7 volumes of cold homogenizing buffer using a polytron (Brinkman) at a rheostat setting of 3 for 10 second. The homogenate was filtered through two layers of cheese cloth and centrifuged at 3000 g for 15 minutes to yield the pelleted crude plasma membranes. Pelleted membrane fractions were washed once with 0.05 M Tris-Hcl pH 7.4 and the pellet was suspended in this buffer before assaying for adenylate cyclase activity. The cytosol used was 100,000 xg supernatant of the myocardial homogenate. For

solubilization of adenylate cyclase, the particulate preparations were incubated for half an hour on ice in a buffer containing 1% detergent, 1 mM EDTA, 1 mM $MgCl_2$ and 0.05 M Tris-HCl pH 7.4. The detergent suspensions were centrifuged at 40,000 xg for 30 minutes. The resulting supernatant was immediately assayed for adenylate cyclase activity. The ratio of detergent to protein was maintained between 1:3 and 1:4. All the enzyme preparations were made fresh each day and assayed within 1 hour of preparation.

Adenylate cyclase assay:

Adenylate cyclase activity was measured by the method of Krishna et al. (8), using PEP-pyruvate kinase ATP regenerating system. The incubation volume of 200 µl contained 200-400 µg protein 1mM 3-isobutryl-1-methyl xanthine, 10 mM PEP, 12.5 IU/ml pyruvate kinase, 1 mM cyclic AMP, 1.0 mM ATP ^{32}P (10-15 cpm/pmole), 2 mM Mg^{++}, 0.05 M Tris-HCl, pH 7.4 and 20 µl of the drug or reagent tested. The reaction was initiated by the addition of enzyme preparation. The incubation was carried out at 30°C for 10 minutes after which the reaction was stopped by the addition of 100 µl of a diluting solution which contained 40 mM ATP, 0.05 M Tris-HCl buffer, pH 7.4 and 12 mM ^3H Cyclic AMP (approximately 17 cpm/nmole to monitor recovery). The tubes were then immediately placed in a 100° dry bath for 3 minutes. Cyclic AMP formed was separated by sequential chromatography on columns of Dowex cation exchange resin and aluminum oxide (9). The activity present in assays conducted without tissues was subtracted from the experimental values. Protein was determined by the method of Lowry et al. (10) using bovine serum albumin as a reference. All assays were performed under conditions of linearity with respect to protein concentration and time of incubation

RESULTS

Addition of cytosol (90 µg protein), Gpp(NH)p (10 µM), isoproterenol (10 µM) and glucagon (10 µM) increased adenylate cyclase activity in particulate preparations of SHR and WKY hearts. The isoproterenol, and glucagon-stimulated activities were consistantly lower in SHR as compared with WKY (fig. 1). The cytosol (100,000 xg supernatant) used as source of adenylate cyclase modulator(s) had no adenylate cyclase activity. As shown in Fig. 1, addition of homologous cytosol to the particulate preparations had an additive effect for glucagon and Gpp(NH)p stimulated enzyme activity. Isoproterenol stimulated adenylate cyclase activity of the particulate preparations increased progressively as the concentration of supernatant was increased, the response was linear up to 80 µg of cytosol protein added (data not shown). In the control rats, isoproterenol (10 µM) increased adenylate cyclase activity 150%, and addition of cytosol (90 µg) alone 70%; however isoproterenol in the presence of cytosol increased the activity 500% suggesting a synergistic effect of the cytosol with isoproterenol Similar results were obtained in the hypertensive animals. Addition of cytosol

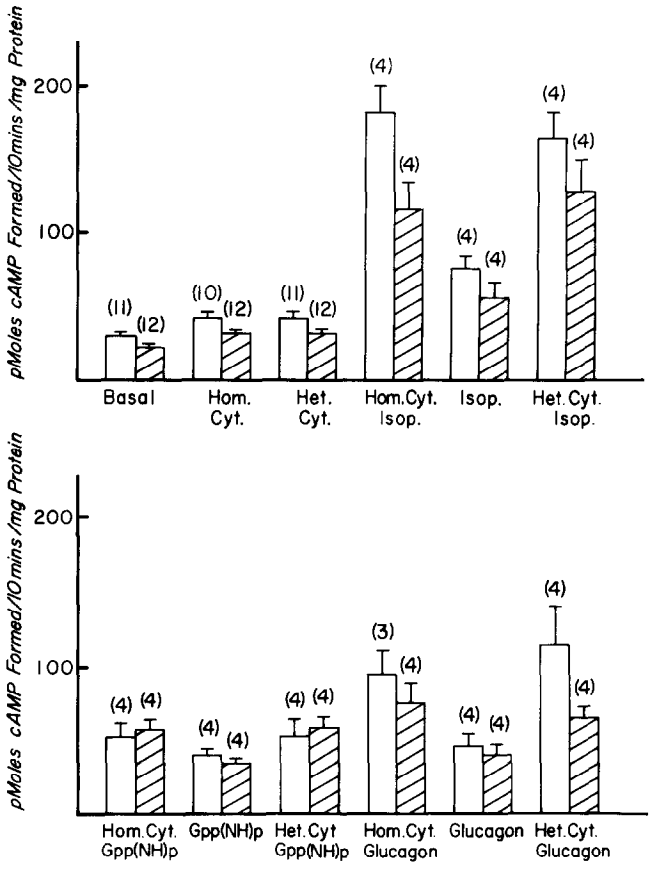

Fig. 1. Effect of cytosolic fraction on the isoproterenol-, glucagon- and Gpp(NH)p-sensitive adenylate cyclase activity in the myocardial membranes of SHR ▨ and WKY □. The assay was performed, as described under "Materials and Methods". Hom. Cyt. = Homologous cytosolic fractions and Het. Cyt. = Heterologous cytosolic fraction. Each value is the mean ± SEM. (n) represents the number of experiments from which the mean has been derived.

had no significant effect on NaF- stimulated enzyme activity (data not given).

Mixing experiments, utilizing WKY cytosol with SHR particulate preparations did not bring the basal, hormone-stimulated or Gpp(NH)p- stimulated enzyme activity of SHR equivalent to WKY (fig. 1). However, it was interesting to note that in the presence of heterologous cytosol the magnitude of the difference between SHR and WKY was reduced for isoproterenol-stimulated enzyme activity. This effect was not observed for glucagon or Gpp(NH)p - stimulated enzyme activity.

The influence of membrane environment on adenylate cyclase activity was

Table 1. Solubilized adenylate cyclase activities of myocardium from spontaneously hypertensive rat (SHR) and Kyoto Wistar normotensive control rat (WKY).

Detergent	Adenylate cyclase activities		
	Sample	Basal	Fluoride
Triton X-100	WKY (12-16 weeks)	259 ± 10	1202 ± 51
	SHR (12-16 weeks)	166 ± 19	772 ± 100
Lubrol-PX	WKY (12-16 weeks)	312 ± 20	1857 ± 134
	SHR (12-16 weeks)	198 ± 25	1348 ± 102
	WKY (28-30 days)	277 ± 10	2700 ± 318
	SHR (28-30 days)	197 ± 23	1880 ± 352

Membranes were solubilized as described under "Experimental Procedures". Adenylate cyclase assay conditions are given in the Methods section. Values are the mean ± SE. The means are from 4 experiments for Triton X-100 and 3 experiments for Lubrol-PX.

tested by solubilizing the enzyme using nonionic detergents, Lubrol-PX and Triton X-100. The protein yield of the extract solubilized with Triton-X100 was about 30-40% more than Lubrol PX. However, the specific activity of the enzyme extracted with Lubrol-PX was greater than those of Triton-X100. These data suggested that Lubrol-PX is superior to Triton X-100 for solubilization of myocardial adenylate cyclase. The protein yeild, after solubilization with both these detergents, was similar in SHR and WKY. The adenylate cyclase activity was linear up to 600 µg protein in each assay.

As shown in Table 1, the basal and fluoride-stimulated enzyme activity in SHR was reduced by about 30-40% as compared with WKY. Similar results were obtained during the prehypertensive state (28-30 day old rats). Stimulation by isoproterenol was lost in the solubilized enzyme preparation. On the other hand, stimulation over the basal value by F^- in the solubilized preparation was of the same order as in the intact membranes.

DISCUSSION

In this communication, the activities of adenylate cyclase in the particulate preparations and the solubilized preparation from the heart of SHR and WKY are compared. Catecholamines and glucagon have pronounced effects on cardiac contractile force and rate. Some of these effects appear to be mediated by stimulating the formation of cyclic AMP (11,12). The loss of hormone-sensitivity in SHR, observed in these studies, has important clinical and pharmacological implications. Our findings support the earlier contention (5) that endogenous factor(s) may influence the adenylate cyclase activity in the particulate preparation of myocardium. Pecker and Hanoune (13) observed the need for addition of low concentration of GTP to the medium along with the cytosolic fraction to obtain enhancement of basal and glucagon-sensitive adenylate cyclase activity of hepatic plasma membranes. Our studies and also those of Sanders, Thompson and Robison (5) indicate that the requirement of GTP is not necessary along with cytosol fraction to achieve the enhancement of adenylate cyclase activity of myocardial plasma membranes. Our studies also suggest a separate mechanism by which the cytosolic factor may regulate isoproterenol- and glucagon-sensitive adenylate cyclase actiivty in rat heart. The cytosolic fraction had additive effect for glucagon and Gpp(NH)p-stimulated enzyme activity, and synergistic effect for isoproterenol-stimulated enzyme activity. Addition of WKY cytosol to the SHR myocardial membranes did not increase the hormone-sensitive adenylate cyclase activity equivalent to the levels of WKY. These data suggest that loss of hormone sensitivity of adenylate cyclase in SHR may not be due to the alterations in the endogenous regulatory factor(s).

The results of our studies indicate that the specific activities of adenylate cyclase enzyme removed from membrane enviornment and transferred to a common detergent environment was reduced in SHR by about 30-40% as compared with WKY. Similar results were obtained in young animals (28-30 days old) during the prehypertensive stage, suggesting that the altered function of

adenylate cyclase may be associated with the pathogenesis of disease. Although differential solubilization of adenylate cyclase from these two sources cannot be rigorously excluded, the precentage of total membrane protein solubilized from hypertensive and WKY heart particulate preparations was identical with either detergent. The solubilized adenylate cyclase enzyme could be stimulated by F^--ions but not by isoproterenol suggesting that isoproterenol and F^--ions activate adenylate cyclase by different mechanisms. Solubilization resulted in loss of isoproterenol stimulation suggesting a disruption of receptor enzyme complex. It seems likely that F^--ions may activate adenylate cyclase by directly interacting with the catalytic subunit of the enzyme complex. Therefore, the reduction in basal and F^--stimulated activities after solubilization suggest that total amount of enzyme molecules may be reduced in SHR as compared with WKY. However, it does not exclude the possibility of altered kinetic properties of the adenylate cyclase activity. Experiments are in progress to answer definitively whether the number of adenylate cyclase molecules is reduced or the kinetic properties of the enzyme are altered in SHR.

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