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ALTERED HORMONE SENSITIVITY OF ADENYLATE CYCLASE IN MYOCARDIAL SARCOLEMMA OF RENAL HYPERTENSIVE RATS*

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Abstract—Adenylate cyclase activity was studied in the myocardial sarcolemmal membranes from sham-operated control and renal hypertensive rats (RHR). Basal adenylate cyclase activity was not significantly different in RHR as compared to control rats. The stimulation of adenylate cyclase by adenosine, epinephrine and norepinephrine was diminished in RHR, whereas dopamine-sensitive adenylate cyclase was almost completely abolished. The decreased responsiveness of adenylate cyclase to catecholamines was associated with a decrease in the V_{max}. Furthermore, the stimulation of enzyme activity by F^- and forskolin was also decreased. The data indicate that, in renal hypertension, the responsiveness of adenylate cyclase to various hormones and to agents (forskolin and F^-) which do not act through receptors is impaired.

The adenylate cyclase/cAMP system has been reported to be one of the biochemical mechanisms participating in the regulation of arterial tone and reactivity [1]. The reduced levels of cAMP in the cardiovascular tissues have been implicated in the pathogenesis of hypertension [2, 3]. There are various reports in the literature which indicate an increase [4], a decrease [5] or unaltered [6] adenylate cyclase activity in spontaneously hypertensive rats. However, very few studies have been performed thus far in renal hypertension, and those also are controversial [7, 8]. A decrease in catecholamine-sentitive adenylate cyclase activity in myocardial membranes was demonstrated in renal hypertension which was associated with a decrease in the number of receptors [7–9]. On the other hand, Giachetti *et al.* [8] have shown that, in renal hypertension, the number of β-adrenergic receptors was not altered but the affinity of [^{3}H]DHA* binding was decreased as compared to control rats. Therefore, we have undertaken the present studies to explore: (1) if there is an alteration in adenylate cyclase activity in renal hypertension induced by the partial ligation of the aorta, (2) if the responsiveness of adenylate cyclase to β-adrenergic and dopaminergic agonists is altered in a similar fashion, (3) whether adenosine receptors coupled to adenylate cyclase which have been shown to be tightly coupled in rat brain striatum [10, 11] are also affected, and (4) whether the catalytic subunit of adenylate cyclase is also impaired.

We have demonstrated that in renal hypertension not only was the number of hormone receptors reduced but the catalytic moiety of the cyclase was also affected.

MATERIALS AND METHODS

Materials. Adenosine deaminase, GTP, GMP-P(NH)P [guanyl-5′-yl-(β-γ-imino)diphosphate], cyclic AMP, L-epinephrine bitartrate, L-norepinephrine bitartrate, and dopamine were purchased from the Sigma Chemical Co., St. Louis, MO. Creatine kinase (EC 2.7.3.2) and myokinase (EC 2.7.4.3) were purchased from Boehringer Mannheim, Montreal, Canada. Forskolin was obtained from Calbiochem-Behring Corp., San Diego, CA, and [α-^{32}P]ATP was purchased from Amersham, Ontario, Canada. *N*-Ethylcarboxamide adenosine was a gift from Dr. Hans P. Baer of the University of Edmonton, who obtained it from the Byk Gulden Co., Konstanz, Germany.

Animals and surgery. Surgery was performed on female Sprague–Dawley rats (Charles River, Quebec, Canada), with a mean initial body weight of 200 g (range: 190–210 g), under ether anesthesia on day 1 of the experiment, as described previously [12]. Surgery consisted of partial ligation of the aorta between the renal arteries using a silk thread and the style (diameter, 0.103 mm) of a No. 30 injection needle. Removal of the style, once the ligation was made, produced a severe but partial constriction of the aorta. Control rats used in these studies were sham-operated. On days 10, 20 and 30 after surgery, the rats were killed by decapitation, hearts were removed from both groups of rats, and sarcolemmal membranes were isolated.

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* Abbreviations: [^{3}H]DHA, [^{3}H]dehydroalprenolol; NECA, *N*-ethylcarboxamide adenosine; GMP-P(NH)P, guanyl-5′-yl-(β-γ-imino)diphosphate; Fsk, forskolin; RHR, renal hypertensive rats; and DA, dopamine.

Table 2. PCB congeners as inducers of hepatic drug metabolizing enzymes: effects on liver weight, liver protein and microsomal enzymes

Treatment		% Liver wt of body wt	mg protein g liver^{-1}	DMAP N-Demethylase*	B[a]P Hydroxylase†	Aldrin Epoxidase*	EROD†
Corn Oil (Control) (n=10)		5.33 ±0.53	27.6 ±5.2	5.65 ±1.08	0.406 ± 0.256	2.72 ± 0.84	117.6 ± 45.3
Phenobarbitone (PB) (n=10)		6.11 ±0.59	39.3 ±4.8[1]	14.8 ±3.2[1]	1.03 ±0.769	16.3 ± 6.0[1]	203.8 ± 107.5
3-Methylcholanthrene (MC) (n=10)		6.91 ±0.86[1]	29.4 ±6.3	6.06 ±1.57	6.84 ±2.39[1]	0.914± 0.032	4498 ±1063[1]
PB + MC (n=10)		8.12 ±1.55[1]	44.4 ±6.0[1]	13.2 ±2.9[1]	6.42 ±2.26[1]	15.1 ± 5.9[1]	5540 ±1328[1]
2,2',3,4',5,6-HexaCB	100umol/kg (n=4)	5.56 ±0.78	30.9 ±6.7	10.9 ±0.4[1]	0.610 ± 0.044	16.0 ± 0.8[1]	119.3 ± 20.3
	20umol/kg (n=4)	5.02 ±0.21	35.2 ±2.8	9.17 ±0.86[1]	0.648 ± 0.076	13.4 ± 0.9[1]	110.9 ± 57.5
2,3,4',5,6-PeCB	100umol/kg (n=4)	5.94 ±1.09	32.0 ±2.7	9.12 ±1.58[1]	0.591 ± 0.072	14.6 ± 3.5[1]	114.2 ± 27.0
	20umol/kg (n=4)	5.00 ±0.25	27.8 ±1.4	9.02 ±1.30[1]	0.267 ± 0.038	4.49 ± 0.95	83.00 ± 10.90
2,2',3,5,6-PeCB	100umol/kg (n=4)	5.75 ±1.40	23.0 ±13.6	6.04 ±0.58	0.575 ± 0.093	4.44 ± 0.62	77.30 ± 32.41
2,2',3,4,4',5-HexaCB	100umol/kg (n=4)	6.04 ±0.47	39.4 ±3.4[1]	15.4 ±2.1[1]	0.888 ± 0.082	19.3 ± 2.3[1]	463.9 ± 52.8
	20umol/kg (n=4)	6.03 ±1.48	28.1 ±6.6	10.3 ±0.5[1]	0.350 ± 0.199	6.64 ± 1.55[1]	176.9 ± 38.2
2,3,4,4',5-PeCB	100umol/kg (n=4)	9.12 ±1.73[1]	36.1 ±8.2[1]	8.45 ±0.93[1]	6.73 ±0.50[1]	13.3 ± 8.5[1]	5188 ±690[1]
	20umol/kg (n=4)	6.16 ±0.38	30.1 ±1.6	6.46 ±0.82	3.97 ±0.14[1]	2.11 ± 0.78	3446 ±758[1]
2,2',3,4,5-PeCB	100umol/kg (n=4)	4.68 ±0.42	34.4 ±3.6	4.26 ±0.55	0.465 ± 0.356	4.15 ± 1.56	205.9 ± 157.4
2,2',4,4',6-PeCB	100umol/kg (n=4)	4.67 ±0.22	28.9 ±7.8	9.41 ±0.49[1]	0.880 ± 0.440	9.20 ± 0.33[1]	140.9 ± 27.9
	20umol/kg (n=4)	5.23 ±0.59	28.8 ±3.0	5.75 ±1.22	0.175 ± 0.107	2.79 ± 0.94	53.32 ± 25.95
2,4,4',6-TetraCB	100umol/kg (n=4)	4.91 ±0.57	36.9 ±4.0	4.76 ±0.66	0.234 ± 0.122	4.98 ± 1.68	82.39 ± 35.54
2,2',4,6-TetraCB	100umol/kg (n=4)	4.82 ±0.53	32.3 ±2.1	4.70 ±0.59	0.214 ± 0.073	4.34 ± 1.68	72.47 ± 22.04
2,2',3,4,4',6-HexaCB	100umol/kg (n=4)	5.97 ±1.45	31.5 ±3.3	11.1 ±2.6[1]	0.546 ± 0.129	6.04 ± 1.60[1]	180.3 ± 76.4
	20umol/kg (n=4)	5.06 ±0.34	32.6 ±9.4	7.72 ±1.68	0.046 ± 0.089	4.06 ± 0.46	88.85 ± 24.20
2,3,4,4',6-PeCB	100umol/kg (n=4)	4.88 ±0.28	30.4 ±8.4	9.73 ±1.29[1]	0.965 ± 0.082	12.6 ± 3.7[1]	869.0 ± 210.1
	20umol/kg (n=4)	4.59 ±0.20	27.3 ±5.6	7.82 ±1.08	1.02 ±0.273	2.67 ± 0.91	160.4 ± 107.9
2,2',3,4,6-PeCB	100umol/kg (n=4)	4.76 ±0.66	28.8 ±5.3	4.75 ±1.18	0.22 ±0.030	3.18 ± 0.77	99.49 ± 38.88
2,2',3,4,4',5,6-HeptaCB	100umol/kg (n=4)	5.35 ±0.29	36.8 ±6.3[1]	10.7 ±0.9[1]	0.180 ± 0.073	18.0 ± 1.6[1]	123.6 ± 21.3
	20umol/kg (n=4)	5.92 ±1.05	29.6 ±7.9	8.91 ±0.38[1]	0.517 ± 0.052	12.3 ± 1.5[1]	83.10 ± 2.71
2,3,4,4',5,6-HexaCB	100umol/kg (n=4)	8.29 ±1.75[1]	33.2 ±5.2	9.62 ±0.97[2]	0.860 ± 0.183	11.3 ± 1.6[2]	2709 ±569[2]
	20umol/kg (n=4)	6.85 ±1.53[2]	29.1 ±6.0	10.4 ±1.4[1]	0.675 ± 0.080	14.8 ± 2.5[1]	217.3 ± 76.7
2,2',3,4,5,6-HexaCB	100umol/kg (n=4)	4.53 ±0.50	24.8 ±1.7	4.13 ±1.33	0.106 ± 0.072	3.00 ± 1.42	44.61 ± 16.9
2,2',4,4'-tetrachlorobiphenyl	100umol/kg (n=4)	5.45 ±0.57	37.06 ±6.24[2]	12.58 ±2.30[2]	0.456 ± 0.149	6.97 ± 1.75[2]	1.01 ± 0.24

* nmol product formed/mg protein/min.
† nmol substrate metabolized/mg protein/min.
[1,2] Different from control at the 5% (=0.05) and 1% (=0.01) level of significance, respectively.

Fig. 1. Portions of sarcolemma fixed with glutaraldehyde alone and stained with phosphotungstic acid at low pH (0.3). The thick sarcolemmal cell coat is intensively reactive. Inset: Portion of sarcolemma fixed with glutaraldehyde, post-fixed with OsO_4 and stained with uranyl acetate and lead citrate. The membrane (arrow) is darkly stained while the cell coat is only moderately reactive.

(data not shown). The decrease in hormone-responsive adenylate cyclase activity has also been reported in different models of hypertension by several investigators [1, 2, 21–23].

Since adenylate cyclase activity has been shown to be regulated by various modulators such as divalent cations and guanine nucleotides, we studied the effects of Mg^{2+}, Mn^{2+} and GTP on adenylate cyclase in myocardial membranes from RHR and control rats; the results are shown in Table 2. Although

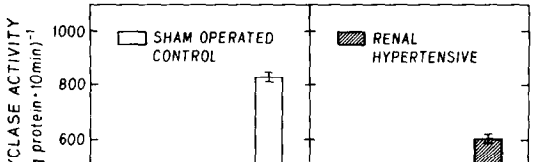

Fig. 2. Effects of various agonists on myocardial adenylate cyclase activity from sham-operated control (□) and renal hypertensive rats (▨). Adenylate cyclase activity was determined in the absence or presence of 50 μM epinephrine (EPI), 50 μM norepinephrine (N-EPI), 100 μM dopamine (DA), 10 mM fluoride (F^-) and 50 μM forskolin (FSK) as described in Materials and Methods. Values are the means ± S.E.M. of triplicate determinations from one of three separate experiments.

Mg^{2+}, Mn^{2+} and GTP all stimulated adenylate cyclase activity in a concentration-dependent manner in both RHR and control rats, the enzyme activities were not significantly different in RHR as compared to control rats. Our data support the observations reported earlier in different models of hypertension [9] but are not in agreement with the recent report of Sharma *et al.* [7] who have shown a significant decrease in basal activity in myocardium and aorta from renal hypertensive rats.

To determine whether the diminished stimulation of adenylate cyclase by catecholamines in RHR is associated with a decrease in the number of receptors or an increase in the activation constant (K_a) to stimulate adenylate cyclase, the effects of various concentrations of epinephrine and norepinephrine on adenylate cyclase were studied in RHR and control rats. The results are shown in Fig. 3. Epinephrine (3A) and norepinephrine (3B) stimulated adenylate cyclase in a concentration-dependent manner in both RHR and control rats. However, the extent of stimulation by these agents ($V_{catecholamines}/V_{control}$) at all concentrations was higher in control rats than in RHR. At 100 μM, epinephrine and norepinephrine stimulated adenylate cyclase by ~3- and 2.7-fold, respectively, in control rats which was decreased to about 2- and 1.7-fold in RHR. The observed diminished stimulation of adenylate cyclase by epinephrine and norepinephrine is associated with a decrease in V_{max} but not in K_a, suggesting that the number of hormone receptors is decreased in RHR.

Since dopamine has been shown to stimulate adenylate cyclase through separate (dopaminergic)

Table 2. Effect of divalent cations and GTP on adenylate cyclase in myocardial sarcolemmal membranes from control and renal hypertensive rats*

Additions	Adenylate cyclase activity [pmoles cAMP (mg protein·10 min)$^{-1}$] Control	Renal hypertensive rats
Mg^{2+} (mM)		
	11 ± 3	10 ± 1
0.5	21 ± 2	22 ± 1
1.0	28 ± 1	30 ± 2
5	79 ± 5	78 ± 4
10	114 ± 5	129 ± 2
Mn^{2+}† (mM)		
	40 ± 2	36 ± 2
0.1	43 ± 3	47 ± 3
0.5	60 ± 3	68 ± 2
1	127 ± 3	135 ± 5
2	126 ± 2	130 ± 2
GTP (μM)		
	55 ± 4	50 ± 3
0.1	65 ± 5	60 ± 6
1.0	85 ± 3	81 ± 3
5	97 ± 6	90 ± 5
10	121 ± 2	108 ± 5

* Adenylate cyclase activity was determined as described in Materials and Methods. The values represent the mean ± S.E.M. of triplicate determinations from one of three experiments.

† 0.5 mM MnATP was used.

receptors, it was of interest to investigate if dopamine receptors are also reduced in renal hypertension. Figure 4 shows the concentration curve of dopamine in RHR and control rats. Dopamine stimulated adenylate cyclase in a concentration-dependent manner. The about 2-fold stimulation of adenylate cyclase observed at 100 μM dopamine in control rats was almost completely abolished in RHR. These data suggest that dopamine receptors may be affected more easily than epinephrine or norepinephrine receptors in renal hypertension.

Adenosine-sensitive adenylate cyclase activity. The presence of adenosine receptors coupled to adenylate cyclase has been demonstrated in cardiac membranes [24], cultured vascular smooth muscle cells [16] and cultured cardiocytes from neonatal rats [17]. In the present studies, we also demonstrate the existence of adenosine-sensitive adenylate cyclase in myocardial sarcolemma from rats. *N*-Ethylcarboxamide adenosine (NECA) stimulated adenylate cyclase in a concentration-dependent manner (Fig. 5). When the effect of NECA was studied on adenylate cyclase in myocardial sarcolemma from RHR, an inhibition in adenylate cyclase activity was observed at all the concentrations of NECA used. NECA at 10 μM stimulated adenylate cyclase activity by about 25% in control rats, whereas only 10–12% stimulation was observed in RHR. The data suggest that adenosine-sensitive adenylate cyclase activity is also inhibited in renal hypertension.

To establish further that the stimulation of adenylate cyclase by NECA was mediated by adenosine "R-sites", the effects of methylxanthines were determined in both RHR and control rats; the results are shown in Table 3. The stimulatory effect of NECA (at 10 and 100 μM) was blocked completely by 3-isobutyl-1-methylxanthine (IBMX) and theophylline

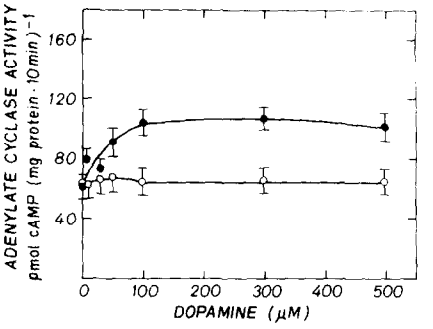

Fig. 4. Effect of various concentrations of dopamine on myocardial adenylate cyclase activity from sham-operated control (●—●) and renal hypertensive rats (○—○). Adenylate cyclase activity was measured as described in Materials and Methods. Values are the means ± S.D. of triplicate determinations from one of two separate experiments.

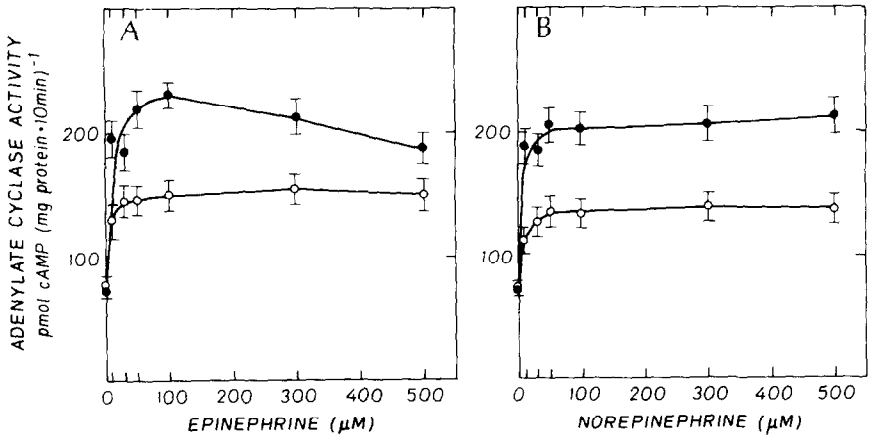

Fig. 3. Effects of various concentrations of epinephrine (A) and norepinephrine (B) on myocardial adenylate cyclase activity from sham-operated control (●—●) and renal hypertensive rats (○—○). Adenylate cyclase activity was measured as described in Materials and Methods. Values are the means ± S.D. of triplicate determinations from one of two separate experiments.

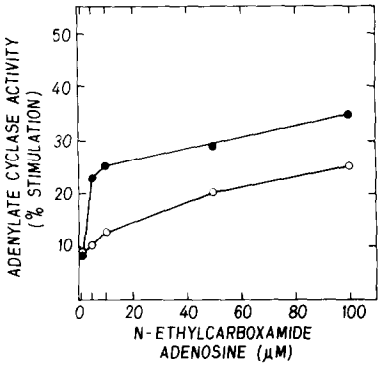

Fig. 5. Effect of various concentrations of *N*-ethylcarboxamide adenosine (NECA) on myocardial adenylate cyclase activity from sham-operated control (●—●) and renal hypertensive rats (○—○). Adenylate cyclase activity was measured in the presence of 1 mM $MnCl_2$ and 0.5 M MnATP as described in Materials and Methods. Values are the means of triplicate determinations from one of three separate experiments.

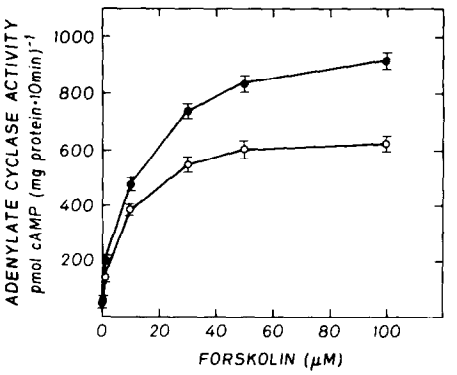

Fig. 6. Effect of various concentrations of forskolin on myocardial adenylate cyclase activity from sham-operated control (●—●) and renal hypertensive rats (○—○). Enzyme activity was measured as described in Materials and Methods. Values are the means ± S.D. of triplicate determinations from one of three separate experiments.

in control rats. On the other hand, in RHR, IBMX and theophylline completely blocked the NECA-stimulated activity when the concentration of NECA was 10 μM but, when the concentration of NECA was increased to 100 μM, theophylline inhibited the stimulation by about 50% but IBMX completely blocked it.

Effect of forskolin. Forskolin, a diterpene, has been shown to activate adenylate cyclase in numerous tissues [25]. The stimulation of enzyme activity by forskolin appears to be mediated via its direct interaction with the catalytic subunit or a component closely associated with it [25–28]. To determine if the catalytic subunit of the enzyme in renal hypertension is also impaired, the effect of forskolin on adenylate cyclase was studied in control and renal hypertensive rats, and the results are shown in Fig. 6. Forskolin stimulated adenylate cyclase activity in a concentration-dependent manner in both control and hypertensive sarcolemma, but the extent of stimulation was decreased in the latter case at all the concentrations of forskolin. At 100 μM forskolin, about 12-fold stimulation of adenylate cyclase was observed in RHR as compared to about 20-fold stimulation seen in control myocardial membranes.

DISCUSSION

Data presented in this paper demonstrate that basal adenylate cyclase activities in myocardial sarcolemma from renal hypertensive rats were not significantly different than their respective controls. These results are in agreement with those of others who did not show any change in the basal adenylate cyclase activity in different models of hypertension [6, 9]. However, our results are in contrast with the recent report of Sharma *et al.* [7] who showed a significant decrease in basal enzyme activity in myocardium and aorta from Grollman one-kidney renal hypertension. In addition, we did not see any significant change in basal adenylate cyclase activity in renal hypertensive rats when the activity was studied under various assay conditions such as varying concentrations of Mg^{2+}, Mn^{2+} or GTP (Table 2). The basal adenylate cyclase activity was also not different in myocardial membranes isolated from renal hypertensive rats after 10, 20 or 30 days of surgery as compared to their sham-operated controls. The apparent discrepancies in basal enzyme activity might be due to the fact that we are dealing with a different model of hypertension.

Table 3. Effects of methylxanthines on NECA-stimulated adenylate cyclase*

Additions	NECA (M)	Adenylate cyclase activity [pmoles cAMP (mg protein·10 min)$^{-1}$]	
		Control	Renal hypertensive
None		180 ± 5	175 ± 5
NECA	10^{-5}	220 ± 2	191 ± 1
NECA + IBMX (1 mM)	10^{-5}	142 ± 4	139 ± 2
NECA + theophylline (1 mM)	10^{-5}	166 ± 2	166 ± 3
NECA	10^{-4}	230 ± 3	210 ± 2
NECA + IBMX (1 mM)	10^{-4}	167 ± 3	173 ± 4
NECA + theophylline (1 mM)	10^{-4}	182 ± 2	193 ± 1

* Adenylate cyclase activity was determined in the presence of 1 mM $MnCl_2$ and 0.5 mM MnATP as described in Materials and Methods. Values are the means ± S.E.M. of triplicate determinations from one of two experiments.

The decreased enzyme activity and diminished stimulation of adenylate cyclase by various concentrations of β-adrenergic agonists (Fig. 3, A and B) in myocardial sarcolemma from RHR as compared to control rats suggest that the number of β-receptors coupled to adenylate cyclase is reduced in renal hypertensive rats. Our results are consistent with earlier reports in the literature on different models of hypertension [1, 2, 21–23]. However, Giachetti *et al.* [8] have shown that in renal hypertension the number of β-adrenergic binding sites is not altered but the affinity of the antagonist ([^{3}H]DHA) is decreased. Since these authors did not determine catecholamine-stimulated adenylate cyclase activity in their studies, it is possible that the total number of β-receptors may not be altered but only a small number of receptors are coupled to adenylate cyclase due to some alterations in the membrane integrity in hypertension. Our results on dopamine-stimulated adenylate cyclase are very intriguing. Dopamine-stimulated enzyme activity was abolished almost completely in renal hypertensive rats, suggesting that dopaminergic receptors are more easily affected than those of other catecholamine receptors. We have shown previously [10] that phospholipids are required for the expression of dopamine-sensitive adenylate cyclase. Any alteration in phospholipid composition of the membrane will result in the loss of dopamine-sensitive adenylate cyclase activity. In our present studies, a complete abolition of dopamine-sensitive adenylate cyclase may be due to such alterations in membrane phospholipid composition which might occur in hypertension.

We have also shown the presence of adenosine receptors coupled to adenylate cyclase in our present studies. The stimulation of adenylate cyclase by NECA, and the inhibition of NECA-stimulated enzyme activity by IBMX and theophylline suggest that the adenosine receptors are of the "Ra" type. The diminished stimulation of adenylate cyclase by NECA in myocardial sarcolemma from RHR as compared to control rats suggests that adenosine receptors coupled to adenylate cyclase may also be reduced in renal hypertension.

Forskolin, a diterpene, is a positive inotropic and antihypertensive agent [29, 30]. It has been shown to activate adenylate cyclase in numerous tissues [25]. The stimulation of adenylate cyclase by forskolin appears to be mediated through its direct interaction with the catalytic subunit or a component associated with it [25–28]. The inhibition of forskolin- and F^--stimulated adenylate cyclase activities in RHR suggests that, in renal hypertension, the responsiveness of the catalytic subunit of the enzyme to agents which do not require receptor input is also impaired. The complete inhibition of F^--stimulated adenylate cyclase activity has also been reported in spontaneous hypertension [6].

It is concluded from the present studies that, in acute malignant renal hypertension, not only was the sensitivity of adenylate cyclase to various hormones altered but the responsiveness of the catalytic subunit was also impaired. It would be interesting to explore further if alterations in membrane phospholipid composition are responsible for the decreased stimulation of adenylate cyclase by various hormones.

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