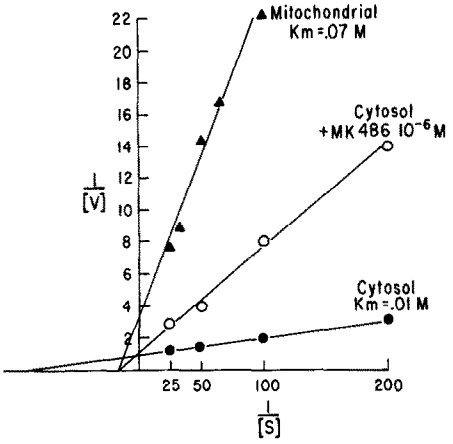

Fig. 2. Kinetic studies of 3-*O*-methyldopa aminotransferase activity. Enzyme source was rat liver. Procedure was as described for Fig. 1, using L-3-*O*-methyldopa concentrations from 0·005 to 0·04 M. Velocity is expressed as absorbance at 380 nm. The figure illustrates the results from a typical experiment; each point represents one data point. At least four such experiments were conducted.

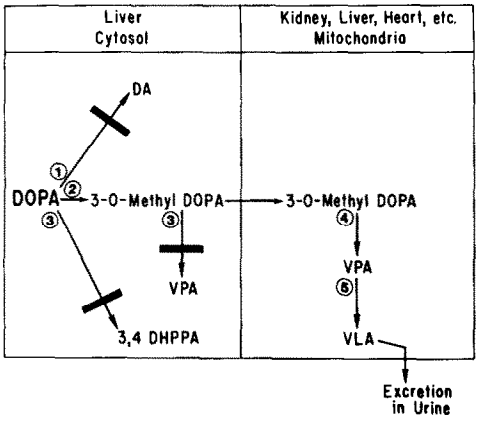

Fig. 3. Alteration of dopa metabolism by therapeutic levels of MK 486. Black bars indicate inhibited pathways. Enzymes are: (1) aromatic amino acid decarboxylase (EC 4.1.1.28); (2) catechol-*O*-methyl transferase (EC 2.1.1.6); (3) tyrosine aminotransferase (EC 2.6.1.5); (4) mitochondrial aminotransferase (EC 2.6.1.1); and (5) aromatic keto-acid reductase (lactic dehydrogenase) (EC 1.1.1.27) [15]. Dopa = 3,4-dihydroxyphenylalanine; DA = 3,4-dihydroxyphenylethylamine; DHPPA = 3,4-dihydroxyphenylpyruvate; 3-*O*-methyldopa = 3-methoxy-4-hydroxyphenylalanine; VPA = 3-methoxy-4-hydroxyphenylpyruvate; and VLA = 3-methoxy-4-hydroxyphenyllacetate.

Thus, MK 486 alters at least two important avenues of metabolism—decarboxylation leading to dopamine and transamination leading ultimately to trihydroxyphenylacetate. With the disruption of both these pathways by the inhibitor, *O*-methylation becomes the principal pathway, giving rise to large amounts of 3-*O*-methyldopa which is a substrate for the relatively uninhibited ubiquitous mitochondrial transaminase. These observations would account for the reported urinary metabolic patterns treated with dopa and the inhibitor MK 486 and also illustrate how misleading it may be to label drugs with such definitive labels as "decarboxylase inhibitors."

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Cyclic AMP metabolism in the cardiac tissue of the spontaneously hypertensive rat

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A strain of spontaneously hypertensive rats (SHR) developed by Okamoto and Aoki [1] is used as a model system for studying the pathogenesis of essential hypertension. Several investigations have been carried out on the properties of the various tissues of SHR animals and their responses to several drugs in comparison to normotensive Wistar rats (NWR). The atria from SHR have been shown to have a greater amount of intrinsic developed tension than NWR atria [2]. The inotropic and chronotropic responses of SHR atria to isoproterenol are reported to be less than those of NWR atria [2]. Since adenosine-3′,5′-monophosphate (cyclic AMP) has been reported to be involved in

Table 1. Cyclic AMP content of the cardiac muscle of SHR and normotensive animals*

SHR		NWR				
		Hawaii		Kyoto		
(30 days)	(90 days)	(30 days)	(90 days)	(30 days)	(90 days)	Cross-bred NWR (90 days)
0·53 (5) ± 0·05	0·73 (5) ± 0·24	1·11 (5) ± 0·23	2·40 (5) ± 0·06	1·30 (5) ± 0·27	2·80 (5) ± 0·05	2·12 (3) ± 0·21

* Cyclic AMP content is expressed as nmoles/g wet wt. Numbers in parentheses indicate the number of animals used.

the inotropic and chronotropic responses of the cardiac tissue to catecholamines, it was of interest to study the metabolism of cyclic AMP in SHR and NWR animals. Lower levels of cyclic AMP in the cardiac and vascular smooth muscles from SHR animals have been reported by Amer [3], Amer and McKinney [4] and Ramanthan and Shibata [5]. We have now studied the cyclic AMP content and the enzymes phosphodiesterase and adenylate cyclase that control the cyclic AMP level in the cardiac tissue of SHR, pre-hypertensive SHR and cross-bred animals and wish to present the data in this report.

Hypertensive and normotensive rats of either sex, weighing 200–300 g, about 90 days in age, were used in these experiments. SHR animals derived from stock received from the N.I.H. animal center (Dr. C. T. Hansen), inbred in our colony to produce a sizable population for experimental use, were used. For control animals, we used two strains of normotensive Wistar rats (NWR), Kyoto NWR and Hawaii NWR, supplied by the animal production center of the University of Hawaii. Young SHR, 30–35 days after birth, that had not developed hypertension yet (pre-hypertensive SHR) and matched control NWR (in age and weight) were also used. The mean systolic and diastolic blood pressures were measured under anesthesia with sodium pentobarbital (50 mg/kg i.m.) by a Stathman P23 pressure transducer connected by cannula to the femoral artery. Also, in order to check the effect of anesthesia, systolic blood pressure of each of five NWR and SHR was measured by use of a Sphygmomanometer (NARCO Biosystem, Inc.), without anesthesia; no significant difference was observed in the blood pressure measured by the two methods. The mean blood pressure of SHR, Hawaii NWR and Kyoto NWR was 201 ± 8 mm Hg (N = 30), 128 ± 6 mm Hg (N = 30) and 130 ± 6 mm Hg (N = 30) respectively. The mean blood pressure of pre-hypertensive SHR was 131 ± 6 mm Hg (N = 30) as compared to 125 ± 7 mm Hg (N = 30) of control Kyoto NWR of the same age and weight. A third type of control animal, cross-bred normotensive animals (offsprings of SHR and Kyoto NWR parents), was also used in our experiments. The blood pressure of these animals was 136 ± 7 mm Hg (N = 20).

Determination of cyclic AMP. The cardiac tissue was quickly excised from the animals, sacrificed by guillotining and was frozen immediately in liquid nitrogen. A weighed amount (25–50 mg) was homogenized in 6% trichloroacetic acid in the cold. The supernatant after centrifugation was extracted with ether (eight times) to remove the trichloroacetic acid, lyophilized and the residue was dissolved in buffer and a suitable aliquot was used for cyclic AMP determination.

The cyclic AMP content was determined by a protein binding assay. The binding protein was prepared from bovine adrenal glands and the assay was carried out essentially as described by Wombacher and Körber [6] and Tsang *et al.* [7].

Adenylate cyclase assay. The enzyme was prepared by homogenizing the cardiac tissue, excised quickly and cleaned from animals sacrificed as above, in ice-cold Tris buffer, containing theophylline, pH 7·4. The assay was carried out as described by Amer [3] and included an ATP-regenerating system. The cyclic AMP formed was purified on Dowex 50 columns and assayed by the protein binding method described above.

Assay of phosphodiesterase (PDE). The cardiac tissue from the animals was homogenized in Tris buffer (0·1 M) containing magnesium acetate (10^{-3} M), pH 7·4, in the cold. The homogenates were then centrifuged at 105,000 *g* for 1 hr and the supernatant was used for enzyme assay. The assay was carried out by incubating a suitable aliquot of the supernatant, with ^{3}H-labeled cyclic AMP as described by Pöch [8]. The PDE activity was expressed as

Table 2. Adenylate cyclase activity of the cardiac muscle of SHR and normotensive animals*

	SHR	NWR	Cross-bred NWR	Pre-hypertensive SHR (30 days)	NWR (30 days)
Basal (untreated)	35·47 (7)†,‡ ± 4·77	77·17 (5) ± 1·20	60·69 (5) ± 6·58	55·80 (3)§ ± 1·09	73·90 (3) ± 2·14
Isoproterenol (10 μM)	40·22 (7) ± 12·13	81·75 (5) ± 4·92	90·15 (5) ± 11·54	84·47 (3) ± 15·37	83·91 (3) ± 6·85
Sodium fluoride (8 mM)	167·67 (7) ± 14·58	264·81 (5) ± 22·59	297·09 (5) ± 12·52	202·85 (3) ± 11·09	287·05 (3) ± 9·74

* Adenylate cyclase activity is expressed as pmoles cyclic AMP formed/mg of protein/10 min. Number in parentheses indicate the number of animals used.

† SHR significantly lower than NWR (Student's *t*-test, $P < 0·005$).

‡ SHR significantly lower than cross-bred NWR (Student's *t*-test, $P < 0·005$).

§ Pre-hypertensive SHR significantly lower than NWR (Student's *t*-test, $P < 0·005$).

pmoles cyclic AMP hydrolyzed/mg of protein/min. Protein was determined by the method of Lowry *et al.* [9]. The enzyme was incubated in our experiments at room temperature (24°) and the substrate concentration was $30{\cdot}12 \times 10^{-9}$ moles/liter.

The cyclic AMP content of the cardiac tissue of SHR and pre-hypertensive animals was significantly lower than that of the respective control animals. No significant difference was observed in the cyclic AMP level between the cardiac tissue of SHR and pre-hypertensive animals. The cyclic AMP content of the cardiac tissue of the cross-bred animals resembled that of NWR (Table 1).

Table 2 incorporates the results of the basal and stimulated activities of the adenylate cyclase of the cardiac tissues from SHR, pre-hypertensive SHR, NWR and cross-bred animals. The mean basal adenylate cyclase activity of SHR is significantly lower than that of the control animals. Although the mean basal activity of the cross-bred NWR was significantly lower than that of the corresponding NWR, the activity of the cross-bred NWR was significantly higher than in the SHR. The mean basal activity of the pre-hypertensive animals was lower than that of the corresponding control NWR.

No significant stimulation of the adenylate cyclase activity was observed with isoproterenol (10 μM) in the cardiac muscle of SHR and NWR. Isoproterenol (10 μM) stimulated the adenylate cyclase activity of the cardiac tissue of cross-bred NWR and pre-hypertensive SHR significantly. On the other hand, significant stimulation of the adenylate cyclase activity of the cardiac tissue by sodium fluoride (8 mM) was observed in all cases.

The phosphodiesterase activity of the cardiac tissue indicates lower activity in SHR than in NWR. The results are included in Table 3.

The importance of selecting appropriate controls in studies on SHR has been discussed by Clineschmidt *et al.* [10] and Shibata *et al.* [11]. In the experiments described here, two types of normotensive controls are included, one Kyoto NWR strain from which SHR was derived and another NWR from the University of Hawaii animal colony. In addition, we have included cross-bred animals as a third type of control, as the blood pressure of these animals was similar to that of NWR.

No significant stimulation of the adenylate cyclase activity of the cardiac tissue of SHR and NWR was observed with isoproterenol (10 μM), while a slight (approximately 1·5-fold) but significant stimulation was observed in cross-bred NWR and pre-hypertensive SHR. Caron and Lefkowitz [12]. Amer [3] and Amer and McKinney [4] have reported a large increase of activity of adenylate cyclase by isoproterenol in canine and rat hearts. On the other hand, Triner *et al.* [13] have reported lack of stimulation by isoproterenol in rat aorta. Robison *et al.* [14] report that broken cell preparations are somewhat less sensitive to hormonal stimulation than the whole preparations.

Table 3. Phosphodiesterase activity of the cardiac muscle of SHR and normotensive animals*

SHR (90 days)	NWR		Cross-bred NWR (90 days)
	Kyoto (90 days)	Hawaii (90 days)	
7·26 (8) ± 0·93	20·3 (8) ± 3·40	16·3 (5) ± 1·78	15·1 (12) ± 3·72

* Phosphodiesterase activity is expressed as pmoles cyclic AMP hydrolyzed/mg of protein/min. Numbers in parentheses indicate the number of animals used.

Sodium fluoride, on the other hand, caused a significant increase in the adenylate cyclase activity of the cardiac tissue from all the animals. However, Amer [3] and Amer and McKinney [4] report that the adenylate cyclase from the SHR aorta and cardiac tissue was less sensitive to isoproterenol and sodium fluoride than NWR. No difference in the basal activity of this enzyme between SHR and NWR was reported.

The blood vessels of SHR also contain lower levels of cyclic AMP and lower adenylate cyclase and PDE activities [15]. On the basis of the data presented here, we suggest that the overall metabolism of cyclic AMP in the cardiac muscle of SHR and pre-hypertensive SHR is lower than in NWR. The lower intracellular level of cyclic AMP in SHR and pre-hypertensive SHR is attributable to the lower basal activity of the enzyme adenylate cyclase. It is also possible to conclude that changes can occur in tissues during the process of hypertension rather than as a result of high blood pressure. It is interesting to note that the metabolism of cyclic AMP in cross-bred NWR resembles more closely that of NWR.

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