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Activity of Cyclic AMP-Dependent Protein Kinase in Heart and Aorta of Spontaneously Hypertensive Rat (40918)

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Abstract: The activity of cyclic AMP-dependent protein kinase was studied in heart and aorta of spontaneously hypertensive rats (SHR) and their controls (Kyoto–Wistar). Although the basal activities were comparable, the response to exogenously added, as well as endogenously synthesized, cyclic AMP was significantly decreased in hearts from 5-week-old SHR. This decrease was not observed at the age of 18 weeks. On the other hand, a normal response to exogenous cyclic AMP was observed in the aorta of young animals, while reduced activity was demonstrated in adult SHR. It is conceivable that these biochemical abnormalities are related to disturbances of cardiovascular functions in SHR, thus an increased heart rate is observed at the age of 5 weeks, while normal heart rate and high blood pressure are present in 18-week-old rats. These studies underline the need for careful age related studies of pathophysiological events in spontaneous hypertension in the rat, particularly at the onset of the disease.

Although the exact role of cyclic AMP in the heart and vascular smooth muscle is still largely unclear (1), the accumulated evidence implicates these nucleotides in the regulation of the contractile function of these organs. Several anomalies of cyclic nucleotides and their regulatory enzymes were demonstrated to parallel these abnormalities of contractile function leading to hypertension in humans (2–4) and in animals (5–14).

Only few studies concerning hypertension were performed on cyclic AMP-dependent protein kinase (11–13), an enzyme thought to be the sole mediator (15) of the action of cyclic AMP. We have undertaken a study of cyclic AMP-dependent protein kinase in heart and aorta of SHR and control rats. In addition, we have evaluated the activity of these enzymes in the early course of the development of hypertension.

Methods. Animals. Male spontaneously hypertensive rats (SHR, Okamoto–Wistar) and the normotensive rats used as control animals (Kyoto–Wistar) were bred in our animal facility according to standardized conditions previously described (14). The systolic blood pressure of conscious animals was measured using an occluding cuff, pulse transducer, and electrosphygmomanometer (Narco Bio-Systems), the day before sacrifice of the animal. Mean values of four recordings of systolic blood pressure are given. Pulse rate was determined from the same recordings.

Preparation of tissues. Heart and aorta were quickly removed from anesthetized animals (Nembutal 60 $\mu g \cdot kg^{-1}$) after complete exsanguination (14), also described previously, and plunged into liquid nitrogen. The frozen tissues were pulverized and the obtained powder was transferred into 10 m*M* Pipes buffer, pH 6.8, at 4°, containing 10 m*M* EDTA and 0.5 m*M* Mix. The tissues were sonicated at 11 μm and the resulting homogenates maintained at 4° until the assay of protein kinase activity.

In several experiments a fraction of 1.5 ml of heart homogenate was centrifuged at 30,000 *g* for 30 min. The supernatant was removed and the pellet was resuspended and sonicated briefly at 5 μm in 1.5 ml of the above homogenizing medium.

Preparation and incubation of heart slices. Anesthetized animals were exsan-

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guinated (14) and their hearts removed. Isolated ventricles were quickly plunged into a cold Krebs–Henseleit solution (NaCl 118 m*M*, KCl 4.7 m*M*, $CaCl_2$ 1 m*M*, $MgSO_4$ 1.2 m*M*, KH_2PO_4 1.2 m*M*, EDTA 0.5 m*M*, $NaHCO_3$ 25 m*M*, glucose 1 mg·ml^{-1}) saturated with 95% O_2, 5% CO_2. Slices of the ventricle were prepared with a Stadie–Riggs tissue slicer and further divided into 2 × 2-mm squares using a razor blade. The slices thus obtained were suspended in Krebs–Henseleit buffer at 4° and washed three times with 10 ml of the same medium. Slices (25–50 mg of tissue) were then transferred into individual vessels containing 1.9 ml of Krebs–Henseleit buffer and preincubated at 30° for 30 min under a saturated atmosphere of O_2–CO_2. At 10 and 20 min the preincubation medium was aspirated and replaced by fresh buffer saturated with O_2–CO_2 at 30°. The incubation was started by the addition of the stimulating agent (isoproterenol and 1-methyl-3-isobutylxanthine [Mix]) and incubated for 6 min. The incubation was terminated by the aspiration of the medium and slices were frozen using a clamp precooled at the temperature of liquid nitrogen. The tissue was pulverized under liquid nitrogen and stored at −70° until assayed for protein kinase or cyclic AMP levels.

Standard assay of protein kinase. Protein kinase activity was measured by determining the amount of ^{32}P incorporated into histone (Type II-A from Sigma) according to a previously described procedure (16), using the principle of the method described by Keely *et al.* (17) and Rubin *et al.* (18). The incubation mixture contained in a final volume of 120 μl: 10 m*M* Pipes buffer, pH 6.8, $MgCl_2$ 10 m*M*, $\gamma^{32}P$-ATP 0.3 m*M* (10–30 cpm·pmol^{-1}) cyclic AMP as indicated, and 5–40 μg of enzyme preparation. Incubation was carried out at 30° for 8 min with the enzyme preparation from heart and for 5 min with the enzyme preparation from aorta. The reaction was terminated by the addition of 2 ml of cold 10% trichloracetic acid (TCA) followed by the addition of 0.2 mg of bovine serum albumin. The precipitate was then collected by centrifugation and dissolved in 0.1 ml of ice-cold NaOH. The protein was reprecipitated by 1 ml of cold 10% TCA and boiled for 10 min, and the precipitate transferred onto a glass fiber filter. The tube was rinsed with 4 ml of cold 5% TCA and the filter was further washed with an additional 20 ml of cold 5% TCA. Filters were finally transferred into 10 ml of distilled water and the Čerenkov radiation measured in a liquid scintillation counter. Under the present experimental conditions, the incorporation of ^{32}P into substrate histones was linear with respect to time and enzyme concentrations used. The activity was expressed as pmol of ^{32}P transferred to histones·min^{-1}·mg protein^{-1}. The blank values (zero time of incubation) were less than 100 cpm. Proteins were determined using the method of Lowry *et al.* (19). With the condition used, the addition of protein kinase modulator inhibited the protein kinase activity by 95 and 96% in absence and presence of cyclic AMP, respectively. The degree of the inhibition suggests that major portion of protein kinase measured was cyclic AMP-dependent type of enzyme. Determination of cyclic AMP levels were performed using the protein-binding procedure of Gilman (20) as described previously (14).

Results. Basic data for control and SHR rats. Table I shows the basic characteristics of animals used in these studies. In the group of 5-week-old rats, there was no significant difference in the systolic blood pressure between the two strains of rats, while the pulse rate of the SHR animals was significantly higher. On the contrary, in the group of 18-week-old rats the systolic blood pressure had become much higher in the SHR (Okamoto) strain, but the pulse rates were the same as those of the normotensive strain.

Protein kinase activity in heart. Protein kinase activity was measured in hearts of 5- and 18-week-old rats in the absence of cyclic AMP (so-called cyclic AMP-independent protein kinase activity, which in fact may also contain already dissociated free catalytic subunits), and in the presence of increasing concentrations of cyclic AMP (cyclic AMP-dependent protein kinase activity).

As seen in Fig. 1a, the protein kinase activity in heart homogenates from 5-week-

TABLE I. BLOOD PRESSURE AND PULSE RATE OF CONTROL (KYOTO–WISTAR) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

Strain	Age (weeks)	Number of rats	Systolic blood pressure (mm Hg)	Pulse rate (min^{-1})
Control	5.4 ± 0.2[a]	7	99 ± 4	453 ± 12
	18.3 ± 1.8	5	111 ± 6	410 ± 22
SHR	5.4 ± 0.2	7	114 ± 8	514 ± 19[c]
	18.2 ± 1.9	5	174 ± 8[b]	410 ± 26

[a] Data represent means ± SE.
[b] $P < 0.001$, [c] $P < 0.02$ relative to age-matched group in the control strain.

old rats was lower in SHR at all concentrations of cyclic AMP which produced an activation of the enzyme, but the maximum activity (total activity) was obtained in the same range of concentrations (0.75 to 3.3 μM) of cyclic AMP in the two strains of rats. About the same K_a (50 nM in SHR and 45 nM in control) could be determined from the curves in Fig. 1a. The difference observed in total activity (measured in the presence of a concentration of exogenous cyclic AMP yielding maximum activity of protein kinase) was found to be statistically significant (Table II). It was observed that the apparent V_m for ATP was decreased in SHR animals (479 instead of 637 pmole of ^{32}P transferred · mg protein^{-1} in control animals) while the apparent K_m for ATP was similar in the two strains (59.4 μM in SHR and 55.3 μM in the control strain). The so-called cyclic AMP-independent activity (as observed in the absence of added exogenous cyclic AMP) was also lower in SHR although not significantly different from control animals (Table II). As in the whole homogenate, the cyclic AMP-dependent protein kinase activity in the 30,000 g supernatant was significantly lower in SHR. In the 30,000 g pellet, the total and the cyclic AMP-independent activity were the same (Table II).

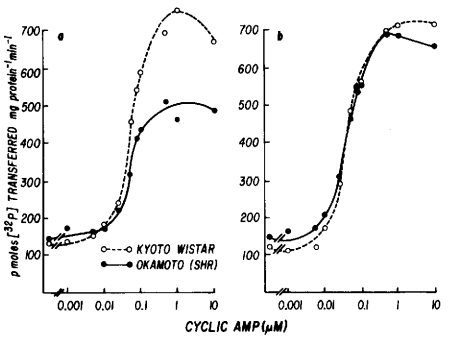

FIG. 1. Stimulation of protein kinase activity in rat heart homogenates by various concentrations of cyclic AMP in 5-week-old (a) and 18-week-old (b) control (Kyoto–Wistar) and SHR (Okamoto) strains.

In contrast to what has been observed in 5-week-old rats, no statistically significant difference in protein kinase activity was seen in heart homogenates of 18-week-old rats (Table III). The total activity (an increase in SHR and a decrease in control strain) thus became the same with increasing age. Figure 1b shows that in both strains of 18-week-old rats the maximal activity was obtained using a concentration of cyclic AMP between 0.75 and 3.3 μM (optimal concentration), the same range that produced a maximal activation of protein kinase in heart homogenates of 5-week-old animals. There was also no difference in the K_a (41 nM in SHR and 47 nM in control) for cyclic AMP between the two strains of 18-week-old animals nor between 5- and 18-week-old rats.

Protein kinase activity in aorta. Different results were obtained in studies on protein kinase activity in rat aorta. In the 5-week-old rats the cyclic AMP-independent activity, the total activity, the optimal cyclic AMP concentration, and the K_a (55 nM in SHR and 48 nM in control) for cyclic AMP were all similar in both strains (Table IV, Fig. 2a). In adult (18-week-old) animals, a substantial decrease of cyclic AMP-

TABLE II. PROTEIN KINASE ACTIVITY IN HEART OF YOUNG (5-WEEK-OLD) CONTROL AND SHR RATS

	Control				SHR			
Cyclic AMP[a] (μM)	0	0.033	0.1	1	0	0.033	0.1	1
Homogenate	118 ± 7	285 ± 14	435 ± 12	508 ± 21	106 ± 7	251 ± 12	366 ± 11[d]	405 ± 19[c]
Supernatant[b]	315 ± 47	782 ± 55	1267 ± 134	1330 ± 93	309 ± 35	679 ± 23	1070 ± 15	1163 ± 53[c]
Pellet[b]	80 ± 22	155 ± 12	207 ± 10	223 ± 3	64 ± 16	152 ± 8	202 ± 5	223 ± 3

[a] Concentration of exogenous cyclic AMP added during the measurement of protein kinase activity. Activity in the absence of added cyclic AMP expresses cyclic AMP-independent protein kinase.

[b] Supernatants and pellets were obtained by centrifugation of heart homogenate at 30,000g for 30 min. Activities are expressed in pmole of ^{32}P transferred · mg protein^{-1} · min^{-1} and means ± SE of three experiments (including 2 animals of each strain) are shown.

[c] $P < 0.001$, [d] $P < 0.02$ relative to corresponding stimulation in control rats (using Student paired t test). Analysis of variance by ANOVA demonstrated a highly significant difference ($P < 0.001$) in response to various doses of cAMP and a highly significant difference between control and SHR in the whole homogenate ($P < 0.0005$) and supernatant ($P < 0.025$).

independent activity and cyclic AMP stimulated activity was observed in both SHR and control (Table IV, Fig. 2b). However, a much lower level of total activity was observed in hypertensive rats so that the total activity in SHR animals is only 68% of that found in the normontensive strain. This difference was statistically significant (Table IV). The optimal concentration of cyclic AMP was the same and did not differ from that in 5-week-old rats. However, the K_a was higher in adult SHR (110 nM in SHR and 70 nM in control).

Effect of isoproterenol on cyclic AMP level and protein kinase activity in heart slices. In order to evaluate whether endogenous cyclic AMP would result in differential stimulation of cyclic AMP-dependent protein kinase, heart slices from young (5-week-old) animals of both strains were incubated with isoproterenol (a β-stimulatory agent) in the presence of Mix (a phosphodiesterase inhibitor). As demonstrated in Table V isoproterenol (0.1 μM) with Mix (10 μM) caused a highly significant increase in cyclic AMP levels under the conditions used for incubation of heart slices. No significant difference between the increase observed in control (54%) and SHR (62%) was seen. Similarily to the levels of cyclic AMP, the basal level of protein kinase was slightly but not significantly lower in SHR. Stimulation with isoproterenol (in the presence of Mix) induced a similar degree of increase in activity in the absence of added cyclic AMP in control (50%) and SHR (41%) but the resulting activity was significantly lower in

TABLE III. PROTEIN KINASE ACTIVITY IN WHOLE HEART HOMOGENATE OF ADULT (18-WEEK-OLD) SHR AND CONTROL RATS

	Cyclic AMP (μM)[a]			
	0	0.033	0.1	1
Control	81 ± 6	200 ± 21	396 ± 24	466 ± 24
SHR	84 ± 10	232 ± 46	422 ± 42	462 ± 83

[a] Concentration of exogenous cyclic AMP added during the measurement of protein kinase activity. Activity in the absence of added cyclic AMP expresses cyclic AMP-independent protein kinase. Activities are expressed in pmole of ^{32}P transferred · mg protein^{-1} · min^{-1} and means ± SE of three experiments (including two animals of each strain) are presented. Analysis by ANOVA demonstrated highly significant difference ($P < 0.0001$) in response to various doses of cAMP but no significant difference between control and SHR.

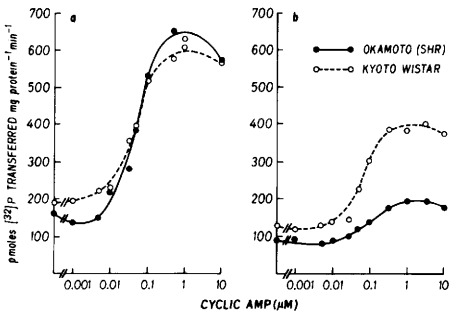

FIG. 2. Stimulation of protein kinase activity in homogenates of rat aorta by various concentrations of cyclic AMP in 5-week-old (a) and 18-week-old (b) control (Kyoto–Wistar) and SHR (Okamoto) strains.

TABLE IV. PROTEIN KINASE ACTIVITY IN AORTA HOMOGENATE OF 5- AND 18-WEEK-OLD SHR AND CONTROL RATS

	Cyclic AMP (μM)[a]			
	0	0.033	0.1	1
Young (5 week old)				
Control	169 ± 9	345 ± 9	527 ± 28	585 ± 50
SHR	161 ± 19	305 ± 26	507 ± 62	638 ± 70
Adult (18 week old)				
Control	97 ± 12	155 ± 16	266 ± 18	354 ± 45
SHR	98 ± 3	148 ± 15	191 ± 18[b]	241 ± 20[b]

[a] Concentration of exogenous cyclic AMP added during the measurement of protein kinase activity. Activities are expressed in pmol of ^{32}P transferred · mg protein^{-1} · min^{-1} and means ± SE of at least three experiments (including two animals of each strain) are presented.

[b] $P < 0.005$ to age-matched group of control. Analysis by ANOVA demonstrated highly significant difference ($P < 0.0001$) in response to various doses of cAMP in both age groups and highly significant difference ($P < 0.004$) between control and SHR in 18-week-old animals.

hypertensive animals (Table VI). The total activity (in the presence of added cyclic AMP) was always lower in SHR but the difference was not statistically significant.

Discussion. Although the pathophysiology of spontaneous hypertension in the rat remains as unknown as that in human, several recent studies suggested evolutionary changes in the course of the disease. Thus, Pfeffer and Frohlich (21) have suggested that the increase in heart rate in young animals corresponds to an increase in cardiac output, the subsequent increase in blood pressure being due to an increase in total peripheral resistance. A different view is offered by Dietz *et al.* (22) who reported a decreased blood volume as the reason behind the increased heart rate in young animals and the accumulation of sodium leading to the subsequent increase in blood pressure. Notwithstanding the mechanism, it is a general observation that the heart rates of SHR are increased in very young animals (about 5 week old) at a time at which the blood pressure is still relatively normal. This observation was confirmed in our previous (14) as well as in the present studies.

TABLE V. EFFECT OF ISOPROTERENOL ON CYCLIC AMP LEVELS IN HEART SLICES OF 5-WEEK-OLD SHR AND CONTROL RATS

Additions	N	Cyclic AMP level[a]	
		Control	SHR
—	6	2.6 ± 0.1	2.3 ± 0.2
Mix 10 μM	3	2.8 ± 0.3	2.5 ± 0.3
Isoproterenol 0.1 μM + Mix 10 μM	3	4.0 ± 0.2[b]	3.8 ± 0.3[b]

[a] Data are expressed in pmole of cyclic AMP · mg protein^{-1} and means ± SE are presented. N = number of separate experiments, each including two animals of each strain.

[b] $P < 0.001$ relative to the level observed without any additions in within each strain.

We have observed a decreased protein kinase activity in hearts of 5-week-old SHR in response to the added (Table II, Fig. 1) or endogneously increased (Table VI) cyclic AMP. Limas and Cohn (23) have observed a reduction in cyclic AMP-dependent phosphorylation of the cardiac sarcoplasmic reticulum in SHR. These authors observed an accentuation of the differences between SHR and control rats with age. It is difficult to evaluate whether these results differ from ours since our data is limited to homogenates and crude supernatants using exogenous substrates for phosphorylation. Similarly to the present study, Kuo *et al.* (12) observed no differences in cyclic AMP-dependent protein kinase activity in adult SHR as compared to controls. It would be premature to conclude from these initial studies to any basic involvement of protein kinase anomalies in the increased heart rate observed in very young SHR. It is also unclear whether or not the anomalies

TABLE VI. EFFECT OF ISOPROTERENOL ON PROTEIN KINASE ACTIVITY IN HEART SLICES FROM 5-WEEK-OLD SHR AND CONTROL RATS

		Control			SHR		
Addition	N	protein kinase activity[a] − cAMP	+ cAMP	Activity ratio (−cAMP/ +cAMP)	protein kinase activity[a] − cAMP	+ cAMP	Activity ratio (−cAMP/ +cAMP)
—	6	126 ± 11	444 ± 23	0.29 ± 0.01	109 ± 9	415 ± 17	0.26 ± 0.01
Mix 10 μM	3	136 ± 12	410 ± 32	0.33 ± 0.02	115 ± 17	384 ± 32	0.30 ± 0.03
Isoproterenol + 0.1 μM Mix μM	3	190 ± 16[b]	424 ± 20	0.45 ± 0.03[b]	160 ± 21[b,c]	398 ± 40	0.40 ± 0.03[b]

[a] Activities were measured in homogenate of heart slices with or without 1 μM of exogenous cyclic AMP. Data are expressed in pmole of ^{32}P transferred · mg protein^{-1} and means ± SE are presented. N = number of different experiments, each including two animals of each strain.

[b] $P < 0.05$ relative to activity observed without any addition within each strain, as estimated by unpaired t test.

[c] $P < 0.05$ relative to the activity in control strain, as established by paired t test.

observed reflect any changes in specific areas such as sino-atrial node. It is nevertheless conceivable that since the cyclic AMP-dependent protein kinase seems to be involved in the regulation of the heart relaxation rate (24) that this anomaly may be a reflection of an abnormal regulation of heart concentration. It is also clear that before any comprehension of the pathophysiology may be possible we need a better elucidation of the still unresolved mysteries of the physiology of the heart cycle and its neurohumoral regulation (25).

In contrast to the observation in heart, the responsiveness of protein kinase to exogenous cyclic AMP was normal in the aorta of young SHR. The activity decreased with age in all animals, but significantly more in hypertensive animals (Fig. 2, Table IV). A similar decreased cyclic AMP-dependent protein kinase (and calcium uptake) has been reported by Bhalla *et al.* (13). These authors have suggested that the observed anomalies in microsomes of SHR leads to a higher smooth muscle tone. In variance to our observations, however, these authors have observed a decrease of cyclic AMP-dependent protein kinase already at the age of 30 days. On the other hand, Sands *et al.* (11) have observed an elevated level of activities of this enzyme in adult SHR. These several discrepancies may be due to methodological differences, the handling of the tissue, etc., but also to the variations between spontaneously hypertensive animals of different origin, age, and sex studied (26). Similar discrepancies exist in the literature concerning phosphodiesterase and adenylate cyclase (6–10). The existence of these discrepancies make very difficult the establishment of any hypothesis for pathophysiological involvement of these observations in the genesis of hypertension. The data presented here underline the need for the study of the evolution of hypertensive disease, as well as the need for further research into the basic physiological mechanism of smooth muscle contraction.

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1. Hardman, J. G., and Kramer, G. L., Cyclic Nucleotides and Their Biosynthetic and Degradative Enzymes, *in* "Handbook of Physiology" (D. F. Bohr, ed.) in press.
2. Hamet, P., Kuchel, O., and Genest, J., J. Clin. Endocrinol. Metabol. 36, 218–226 (1973).
3. Lowder, S. C., Hamet, P., and Liddle, G. W., Circ. Res. 38, 105–108 (1976).
4. Messerli, F. H., Kuchel, O., Hamet, P., Tolis, G., Guthrie, G. P., Fraysse, J., Nowaczynski, W., and Genest, J., Circ. Res. 38, (Suppl. II), 42–47 (1976).
5. Largis, E. E., Allen, D. O., CLark, J., and Ashmore, J., Biochem. Pharmacol. 22, 1735–1744 (1973).

6. Amers, M. S., Gomoll, A. W., Perhach, J. L. Jr, Ferguson, H. C., and McKinney, G. R., Proc. Nat. Acad. Sci. USA **71**, 4930–4934 (1974).
7. Triner, L., Vulliemoz, Y., Verosky, M., and Manger, W. M., Biochem. Pharmacol. **24**, 743–745 (1975).
8. Ramanathan, S., and Shibata, S., Blood Vessels **11**, 312–318 (1974).
9. Klenerova, V., Albrecht, I., and Hynie, S., Pharmacol. Res. Commun. **7**, 453–462 (1975).
10. Ramanathan, S., Shibata, S., Tasaki, T. K., and Ichord, R. N., Biochem. Pharmacol. **25**, 223–225 (1976).
11. Sands, H., Sinclair, D., and Mascali, J., Blood Vessels **13**, 361–373 (1976).
12. Kuo, J. F., Davis, C. V., and Tse, J., Nature (London) **261**, 335–336 (1976).
13. Bhalla, R. C., Webb, R. C., Singh, D., Ashley, T., and Brock, T., Mol. Pharmacol. **14**, 468–477 (1978).
14. Hamet, P., Fraysse, J., and Franks, D. J., Circ. Res. **43**, 583–591 (1978).
15. Kuo, J. F., and Greengard, P., Proc. Nat. Acad. Sci. USA **64**, 1349–1355 (1969).
16. Coquil, J.-F., Dupuis, M., and Hamet, P., Union Med. Canad. **106**, 457–464 (1977).
17. Keely, S. L., Corbin, J. D., and Park, C. R., J. Biol. Chem. **250** (13), 4832–4840 (1975).
18. Rubin, C. S., Erlichman, J., and Rosen, O. M., Methods Enzymol. **38**, 308–315 (1974).
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265–275 (1951).
20. Gilman, A. G., Proc. Nat. Acad. Sci. USA **67**, 305–312 (1970).
21. Pfeffer, M. A., and Frohlich, E. D., Circ. Res. **32/33** (Suppl. 1), 28–38 (1973).
22. Dietz, R., Schömig, A., Haebara, H., Mann, J. F. E., Rascher, W., Lüth, J. B., Grünherz, N., and Gross, F., Circ. Res. **43** (1), I98–I105 (1978).
23. Limas, C. J., and Cohn, J. N., Circ. Res. **40** (Suppl. 1), I62–I69 (1977).
24. Kirchberger, M. A., and Tada, M., J. Biol. Chem. **251** (3), 725–729 (1976).
25. Keely, S. L. Jr., Lincoln, T. M., and Corbin, J. D., Amer. J. Physiol. **234**, H432–H438 (1978).
26. Sands, H., and Jorgensen, R., Artery **4**, 536–544 (1978).

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