Aberrations of Cyclic Nucleotide Metabolism in the Hearts and Vessels of Hypertensive Rats

(spontaneous hypertension/stress hypertension/desoxycorticosterone acetate hypertension/
nucleotide cyclases/cyclic nucleotide phosphodiesterase)

M. SAMIR AMER, ALLEN W. GOMOLL, JAMES L. PERHACH, JR., HUGH C. FERGUSON, AND
GORDON R. McKinney

Department of Pharmacology, Mead Johnson Research Center, Evansville, Indiana 47721

Communicated by Bernard B. Brodie, September 26, 1974

ABSTRACT  In the aortas and mesenteric arteries from spontaneous hypertensive rats and in the aortas from stress- and desoxycorticosterone-acetate-hypertensive rats, the intracellular cGMP: cAMP ratios were significantly elevated when compared to the ratios in the aortas of the respective controls. Decreases in the intracellular cAMP or cGMP levels were consistently associated with increased activity of the cyclic-nucleotide-specific low K_+ phosphodiesterase (3':5'-cAMP 5' nucleotidohydrolase, EC 3.1.4.17). Increases in intracellular cGMP levels were associated with elevated guanylyl cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] activity. Furthermore, adenylyl cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] activity was less sensitive to stimulation by the β-adrenergic stimulant isoproterenol in both the aortas and the hearts of the hypertensive animals. These changes could provide the biochemical basis for the (a) increased vascular smooth muscle tone and peripheral resistance observed in these animals, (b) increased reactivity to norepinephrine, and (c) decreased ability of aortas from hypertensive rats to relax. The presence of these same effects in different etiologic types of hypertension indicates that this aberration in cyclic nucleotide metabolism may represent a common metabolic defect basic to the hypertensive syndrome irrespective of etiology.

Previous studies from this laboratory have indicated that the aortas from spontaneous and stress hypertensive rats (1) and more recently from neurogenically hypertensive rats (2) contained lesser amounts of 3':5'-cAMP due primarily to increased rates of hydrolysis of the cyclic nucleotide. The decrease in the intracellular levels of cAMP was proposed as a possible mechanism for the increased vascular resistance and the vascular smooth muscle changes associated with the three rat models of hypertension.

In the present work, studies were conducted with tissues from rats made hypertensive by combined desoxycorticosterone acetate (DOCA) and salt administration. The role of 3':5'-cGMP, the other naturally occurring cyclic nucleotide, which is now being considered as an important partner for cAMP in the control of cellular function (3-5), was also investigated.

MATERIALS AND METHODS

Spontaneous and stress hypertensive rats and their respective controls were obtained as previously described (1). DOCA-
hypertensive rats were prepared as described by Stanton and White (6). Systolic blood pressures were determined indirectly by the tail cuff technique and were 192 ± 3, 165 ± 4, and 226 ± 1 mm Hg for the spontaneous, stress, and DOCA-hypertensive rats, respectively. Control rats for those same groups had pressures of 130 ± 2, 125 ± 2, and 132 ± 2 mm Hg, respectively. Values are ± SEM.

Most of the experiments were carried out on the aortas, mesenteric arteries, and hearts removed from the animals immediately after their decapitation. A portion of each tissue (20-30 mg) was immediately frozen in liquid nitrogen and kept frozen at -20°C until analyzed for cyclic nucleotide contents within 2 weeks. The remainder of the fresh tissue was homogenized in 4 volumes of ice-cold isotonic sucrose, kept at 4°C, and assayed for pertinent enzyme activities within 2 hr. The aortas and mesenteric arteries from two to four animals were combined into each sample. cAMP and cGMP levels and phosphodiesterase (PDE, 3':5'-cAMP 5'-nucleotidohydrolase, EC 3.1.4.17) adenylly cyclase [AC, ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] and guanylyl cyclase [GC, GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] activities were determined as described previously (1, 7). The cyclic nucleotide index was calculated according to the formula:

\[
\frac{\text{cAMP content in control}}{\text{cGMP content in control}} + \frac{\text{cAMP content in hypertensive}}{\text{cGMP content in hypertensive}}
\]

RESULTS

In the spontaneously hypertensive rat the aortas contained significantly less cAMP and more cGMP than did the control aortas (Table 1). Aortas of stress hypertensive animals contained significantly less cAMP, while their cGMP contents were similar to the controls. The concentration of cAMP in the aortas from DOCA-hypertensive rats was similar to that in the aortas and controls but the cGMP contents were significantly higher. All three forms of hypertension showed significant elevation of the cyclic nucleotide index above 1, indicating that in the aortas of the hypertensive animals there was a larger cGMP:cAMP ratio than in their respective controls.

The hearts of stress and DOCA-hypertensive rats contained normal quantities of both cyclic nucleotides with index ratios not significantly different from 1. The hearts from the spon-
Table 1. Cyclic nucleotide levels* in the aortas of control and hypertensive rats

<table>
<thead>
<tr>
<th>Type</th>
<th>Cyclic AMP</th>
<th>Cyclic GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hypertensive</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>0.98 ± 0.23</td>
<td>0.45 ± 0.16‡</td>
</tr>
<tr>
<td>Stress</td>
<td>0.60 ± 0.07</td>
<td>0.36 ± 0.05‡</td>
</tr>
<tr>
<td>DOCA</td>
<td>0.58 ± 0.05</td>
<td>0.55 ± 0.04</td>
</tr>
</tbody>
</table>

* pmol/mg of wet tissue; mean of 3 to 12 experiments ± SEM.
† cAMP control ± cAMP hypertensive.
‡ cGMP control ± cGMP hypertensive.
§ Significantly different from respective controls (P < 0.05).
¶ Significantly different from 1 (P < 0.05).

Cyclic nucleotide index ratio was not statistically different from 1.

Table 2 shows that total PDE activity was significantly increased in the aortas of both the spontaneous and stress hypertensive but not in the DOCA-hypertensive rats when cAMP was used as the substrate. More importantly, an increase in % PDE activity present as the low K_m form (PDE II), and possibly the more important form (1) of the enzyme, was evident in the aortas from all types of hypertensive animals. No significant changes in either the total PDE activity or the % of the low K_m form were observed in the aortas obtained from any of the three hypertensive models examined when cGMP rather than cAMP was used as the substrate. The hearts obtained from the spontaneously hypertensive rats contained higher total levels of cyclic AMP-PDE activity and a significantly higher % of the low K_m form. Higher total PDE activity and low K_m enzyme were also apparent when cGMP was used as the substrate. The total cGMP-PDE activity was significantly higher in the hearts of the stress hypertensive rats than the low K_m enzyme was normal. No changes in the PDE activity in the hearts of the DOCA-hypertensive rats were observed irrespective of the substrate used. No differences in the K_m values were observed between the enzyme from the hypertensive and control aortas or hearts.

Studies with AC indicated that the most consistent observation was the decreased sensitivity of enzyme from the three types of hypertensive animals to stimulation by isoproterenol (Table 3). Higher concentrations of isoproterenol produced equivalent maximal stimulation of the enzyme. Sensitivity to sodium fluoride, however, varied from one type of hypertension to the other, being minimal in stress and maximal in the DOCA-hypertensive animals. The basal level of AC activity in aortas of DOCA-hypertensive rats appeared to be significantly higher than the level in the respective control aortas. GC activity appeared to be normal in the aortas of stress but significantly elevated in those from spontaneous and DOCA-hypertensive rats.

Qualitatively similar effects were observed on the AC and GC activities from the hearts of the three hypertensive rat models. On the whole, AC activity appeared to be less sensitive to isoproterenol stimulation but exhibited good sensitivity to sodium fluoride. There were no apparent changes in the basal GC activity in the hearts of any of the hypertensive rat models studied.

As can be seen from Fig. 1, mesenteric arteries from the hypertensive animals contained significantly less cyclic AMP and more cyclic GMP than did their respective controls. The cyclic nucleotide index was also statistically significantly greater than 1. The profile of PDE, AC, and GC activities was similar to that of the aorta. The relative insensitivity of AC to stimulation by isoproterenol in the mesenteric vessels from the spontaneously hypertensive rats is shown graphically in Fig. 2. Although the extent of the stimulation by the β-adrenergic agonist was less than in the aorta, greater concentrations of isoproterenol were needed to produce equivalent stimulation of the enzyme in the hypertensive compared to that of the enzyme from control vessels.

Table 2. Phosphodiesterase activity* in the aortas of control and hypertensive rats

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total activity*</td>
<td>% Low K_m‡</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>6.0 ± 1.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Stress</td>
<td>4.4 ± 0.3</td>
<td>1.95</td>
</tr>
<tr>
<td>DOCA</td>
<td>7.3 ± 0.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Nanomoles cyclic nucleotide hydrolyzed per 5 mg of wet tissue per 10 min at 30°C; mean of 4 to 10 experiments ± SEM.
† Obtained as described previously (1).
‡ Significantly different from respective control (P < 0.05).
§ Significantly different from respective control (P < 0.01).
**RAT MESENTERIC ARTERY**

**Cyclic Nucleotide Contents**  
(Average of 16 determinations)

Cyclic Nucleotide Index

\[
\begin{align*}
\text{cAMP control} & \quad \text{cAMP hypertensive} = a \\
\text{cGMP control} & \quad \text{cGMP hypertensive} = 2.5
\end{align*}
\]

**Phosphodiesterase Activity**  
(Average of 4 determinations)

**Cyclase Activity**  
(Average of 4 determinations)

---

**FIG. 1.** Cyclic nucleotide metabolism in mesenteric arteries from spontaneously hypertensive and control rats. Bars represent the average ± SEM. N = normotensive; H = hypertensive; C = control; * = significantly different from normotensive (P < 0.05); □ = significantly different from 1 (P < 0.05) and ▲ = significantly different from control (P < 0.05).

**DISCUSSION**

The abnormal cyclic nucleotide metabolism found in these three hypertensive rat models is similar to that found in animals made acutely hypertensive by lesions in the nucleus-tractus-solitarii (2). Thus, the aortas from rats with four types of hypertension with widely different etiologies seem to exhibit similar defects in their cyclic nucleotide metabolism. These defects result in a significant increase in the intracellular
The results with the spontaneously hypertensive rat have already been confirmed* (22, 23). The consistent comp-

* J. F. Kuo, personal communication.

<table>
<thead>
<tr>
<th>Type</th>
<th>Adenyl cyclase activity</th>
<th>Basal guanylyl cyclase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Isoproterenol (10 μM)</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>Control</td>
<td>8.33 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>9.44 ± 0.58</td>
</tr>
<tr>
<td>Stress</td>
<td>Control</td>
<td>9.03 ± 2.86</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>11.42 ± 1.93</td>
</tr>
<tr>
<td>DOCA</td>
<td>Control</td>
<td>36.20 ± 1.74</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>55.28 ± 1.06§</td>
</tr>
</tbody>
</table>

* Picomoles cyclic nucleotide formed per mg of wet tissue per min; mean of 4 to 10 experiments ± SEM.
† Significantly different from respective control (P < 0.01).
‡ Significantly different from respective control (P < 0.05).
§ Significantly different from respective basal (P < 0.05).
¶ Significantly different from respective basal (P < 0.01).

... cGMP:cAMP ratio (cyclic nucleotide index >1) in the vasculature. This change in cyclic nucleotide balance in favor of cGMP may therefore represent a final common pathway characteristic of the hypertensive syndrome irrespective of etiology. It may also provide the biochemical basis for the increased vascular smooth muscle tone and the elevated peripheral resistance common in hypertension. cAMP appears to be associated with decreased vascular smooth muscle tone (8), while cGMP appears to mediate smooth muscle contraction (3, 5, 9, 10). In this respect, the ratio of the two cyclic nucleotides, rather than changes in the levels of either alone, appears to be the more important factor.

The results of the present study concerning cyclic nucleotide levels in the hearts of the animals used generally support the absence of excessive changes in cardiac function in the hearts of the hypertensive rats, since the index value was essentially normal. This agrees well with the possible noninvolvement of the heart in neurogenic (11), spontaneous (12), and DOCA-hypertensive rats (13). This does not conflict, however, with the possible role that increased cardiac output may play in the initiation of the hypertensive state, but agrees with the general observation that in maintained hypertension cardiac function is mostly normal (14). It appears that the turnover of cAMP is increased in the aortas of DOCA-hypertensive rats. This may provide an explanation for the special effects of the PDE inhibitor, caffeine, on vessels from these animals. Whereas, in the vessels from normotensive rats caffeine produces a vigorous spike contraction, probably due to its greater selectivity for cyclic GMP-PDE than the cyclic AMP enzyme (9), it produced only relaxation in the vessels from DOCA-hypertensive rats (15).

The significantly elevated AC levels in the aortas of the DOCA-treated rats may be related to the increased levels of angiotensin-II known to be present in these animals. Low concentrations of the polypeptide have been found to augment greatly the response to sympathetic nerve stimulation, which is at least partly mediated via AC stimulation (16). Angiotensin-II may also directly cause the release of catecholamines from nerve endings, the latter in turn stimulating AC activity (17).

The decreased AC sensitivity to isoproterenol in the aortas from all three hypertensive rat models is interesting and may be related to the resistance of the vessels from these hypertensive animals to relaxation owing to their decreased capacity to synthesize cAMP (18, 19). This is especially true since cAMP has been shown to be closely associated with vascular smooth muscle relaxation (8). Similar subresponsivity of AC of the hearts from the hypertensive animals may explain the decreased ability of the hearts from these animals to synthesize cAMP (20) and to react normally to adrenergic stimulants (21).

Decreased sensitivity of AC to stimulation in the vessels of the hypertensive animals may also underlie the greater sensitivity of these vessels to the pressor effects of norepinephrine and angiotensin. Neuronal or extraneuronal norepinephrine would be able only to stimulate GC (9), with the production of the contraction-mediating cGMP, with little or no damping effects of increased cAMP synthesis.

The present studies demonstrate a definite association of cyclic nucleotide aberration with hypertension. Regardless of etiology, specific changes in cyclic nucleotide metabolism, leading to an increased cGMP:cAMP ratio in the vascular bed, combined with decreased sensitivity of AC to stimulation, appear to be associated with the development of hypertension. Our results with the spontaneously hypertensive rat have already been confirmed* (22, 23). The consistent com-

![Fig. 2. The effect of increasing isoproterenol concentrations on adenyl cyclase activity in rat mesenteric arteries from normotensive (•) and spontaneously hypertensive (○) rats. Values are averages of six determinations. * = statistically different from normotensive control (P < 0.05).]
common occurrence of these changes in four different hypertensive rat models is impressive and may represent an important biochemical lesion in hypertension. Whether this biochemical lesion is the cause of or results from the hypertensive state remains to be determined. However, since decreased sensitivity of AC to stimulation is also prevalent in other diseased states (24, 25), this could possibly point to a causative role of that diminished AC sensitivity in the etiology of hypertension.

The authors wish to express their appreciation for the indispensable assistance of Lloyd Allen, Cindy Forman, Carol Gatewood, Gary Gentry, Steve Harris, Jill Sloan, and Margaret Stockton.