Cyclic Nucleotides and Aggregation in Platelets of Spontaneously Hypertensive Rats

PAVEL HAMET, JACQUES FRAYSSE, AND DOUGLAS J. FRANKS

SUMMARY Abnormalities in the metabolism of cyclic nucleotides have been observed in human as well as in experimental hypertension. This study, conducted in spontaneously hypertensive rats (SHR), established that abnormalities of cyclic nucleotides may be observed in platelets, an easily obtainable tissue which possesses features in common with vascular smooth muscle. Basal levels of cyclic AMP and cyclic GMP were similar in platelets from SHR (Okamoto) and control (Kyoto-Wistar) rats. Prostaglandin E$_1$ (PGE$_1$) increased cyclic AMP significantly more (in time- and dose-response) in SHR than in control rats, but epinephrine increased cyclic GMP concentrations less in platelets from hypertensive rats. The most significant differences in cyclic nucleotide concentrations were observed in 12-week-old rats; smaller differences were present at the ages of 6 and 24 weeks. Abnormalities in platelet aggregation were observed after addition of the divalent cation ionophore A-23187, a finding compatible either with abnormalities in calcium transport or its function in SHR. Furthermore, although an increase of cyclic AMP was always accompanied by inhibition of aggregation, changes in cyclic GMP concentrations did not correlate with aggregation; the 10-fold increase of cyclic GMP produced by epinephrine was not accompanied by aggregation, whereas the ionophore produced irreversible aggregation in both strains without any change in cyclic GMP concentration. The higher cyclic AMP concentrations observed in SHR in response to PGE$_1$ may be due to an increased activity of adenylate cyclase. It is concluded that: (1) abnormalities in aggregation of platelets and metabolism of cyclic nucleotides exist in SHR, and (2) platelets may be a suitable tissue for studies of the regulation of hormonal responsiveness in hypertension.

Several abnormalities in cyclic nucleotide metabolism have been observed in hypertension in both man$^{1,3}$ and experimental animals.$^{4-10}$ These include abnormal tissue concentrations of cyclic nucleotides and differences in the activities of the enzymes that control these levels. However, there are many discrepancies between the numerous studies performed on experimental animals. It is not clear whether the abnormalities in metabolism of cyclic nucleotides seen in hypertension are merely a consequence of the continued elevation of blood pressure or whether they form part of the pathophysiological mechanism of the disease.

We have investigated the metabolism of cyclic nucleotides in platelets, a tissue in which pathophysiological changes are intimately related to those of blood vessels, and which can be readily obtained from both human and animal sources. Platelets offer a number of advantages over other tissues that have been studied in hypertension. They can be obtained as a single cell type by separation from other blood components. They are viable in vitro, and the criterion of platelet viability, aggregation, can be monitored. They show sensitivity to hormones, and cyclic nucleotides are involved in their physiological responses. Furthermore, many analogies exist between platelets and vascular smooth muscle: both tissues contain contractile proteins, and, in both tissues, calcium plays an important regulatory role.$^{12}$

We report here that notable differences in aggregation and cyclic nucleotide metabolism exist between washed platelets obtained from normal and those from spontaneously hypertensive rats (SHR).

Methods

Nucleotides, L-epinephrine, dithiothreitol, phosphoenol pyruvate, pyruvate kinase, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), and prostaglandin E$_1$ (PGE$_1$) were obtained from Sigma Chemical Co., and labeled nucleotides were from Amersham/Searle. Neutral Al$_2$O$_3$ and Dowex AG 50W-X8 (hydrogen form, 100–200 mesh) were from Bio-Rad Laboratories (Canada) Ltd.; the ionophore A-23187 was donated by Eli Lilly, and Nembutal was purchased from Abbott Laboratories.

SEVERAL abnormalities in cyclic nucleotide metabolism have been observed in hypertension in both man$^{1,3}$ and experimental animals.$^{4-10}$ These include abnormal tissue concentrations of cyclic nucleotides and differences in the activities of the enzymes that control these levels. However, there are many discrepancies between the numerous studies performed on experimental animals. It is not clear whether the abnormalities in metabolism of cyclic nucleotides seen in hypertension are merely a consequence of the continued elevation of blood pressure or whether they form part of the pathophysiological mechanism of the disease.

We have investigated the metabolism of cyclic nucleotides in platelets, a tissue in which pathophysiological changes are intimately related to those of blood vessels, and which can be readily obtained from both human and animal sources. Platelets offer a number of advantages over other
Animals and Procedures

Rats from the Kyoto-Wistar strain were used as control animals for SHR (Okamoto-Wistar). The rats originally were purchased from commercial sources (Charles River or Laboratory Supply Co.) or were obtained as a gift from Dr. Jaroslav Kyncl, Abbott Laboratories Ltd., and then bred in our facilities. The general guidelines used for breeding, care, and use were those recommended by the Committee on Care and Use of Spontaneously Hypertensive Rats. Only male rats were used in the present study, and efforts were made to use animals of control and hypertensive strains born in our facilities within ±2 days. The systolic blood pressure in conscious rats was recorded with an occluding cuff, pulse transducer, and electrophysymomanometer (Narco Bio-Systems) after a warming period and in a quiet environment. Pulse rate was obtained from blood pressure recordings. The mean of four readings of systolic blood pressure is presented. Rats of both strains were weighed and their blood pressure and pulse rate recorded during the afternoon preceding the day of the experiment. The experiments were performed on one strain in the morning and on the other strain in the afternoon, in a randomized sequence.

Preparation of Washed Platelets

Rats were anesthetized by intraperitoneal injection of Nembutal (60 μg/g of body weight), and the external jugular vein and carotid artery were catheterized. Blood was drawn from the carotid into a syringe containing 20 mg of EDTA in 3 ml of 0.9% NaCl. Simultaneously, saline was infused into the jugular vein. This procedure permitted an average of 10–15 ml of blood to be obtained from each rat. The preparation of washed platelets was based on procedures described by Haslam and Rosson. The blood was centrifuged at 500 g for 5 minutes at room temperature to separate the platelet-rich plasma from erythrocytes. The platelet-rich plasma was removed and an equal volume of “washing medium” (containing 135 mM NaCl, 13 mM sodium citrate, 5 mM glucose, and 2 mM EDTA, pH 6.5) was added to the packed erythrocytes and centrifuged again at 500 g. The procedure was repeated twice to obtain the maximum yield of platelets. Accumulated platelet-rich plasma and “washing medium” were then centrifuged at 1800 g for 10 minutes, and the platelet sediment was washed twice. The platelets thus obtained were resuspended in "suspension medium" (containing 135 mM NaCl, 2 mM EDTA, 5 mM glucose, and 15 mM TES, pH 7.5). Platelet preparations for the study of adenylate cyclase were obtained by the same procedures, except that the final suspension was made in the medium indicated in the enzyme assay. Platelets were counted by the method of Brecher and Cronkite. All tubes and equipment employed for platelet separation were made either of plastic or of siliconized glass.

Platelet Aggregation and Incubation

The degree of aggregation of platelets was measured by the turbidimetric method described originally by Born. An aggregometer AG 11 (Briston Mfg. Ltd.) and a Fisher Recordall 6000 (Fisher Scientific Co.) served as instruments for this procedure. To 1 ml of platelet suspension (adjusted to contain 200,000 platelets/mm3) CaCl2 was added to give a final concentration of 2 mM in excess of EDTA. Fibrinogen was added to give a concentration of 0.25 mg/ml. The aggregating agents were added after 1 minute of incubation at 30°C with constant stirring at 950 rpm, and changes in turbidity were recorded. The rate of aggregation was determined by the method of Baumgartner and Born. The rate was measured as a tangent to the steepest slope in the light transmittancy recordings during the first 30 seconds of aggregation and expressed in mm/30 sec. The incubation of platelets for measurement of cyclic nucleotides was performed identically except that the incubation was terminated, at the time indicated, by the addition of 1 ml of 0.5 M perchloric acid and the preparation was frozen at −15°C until the assay.

Measurement of Cyclic Nucleotides

Samples for measurement of cyclic nucleotides were quickly thawed and 2.3 nCi of cyclic AMP [3H] (specific activity, 26 Ci/mmol) were added. Samples were then treated sonically at an amplitude of 12 μm for 10 seconds and centrifuged at 40,000 g for 10 minutes at 4°C prior to chromatography. The chromatographic procedures are based on the method suggested by Jakobs et al. The supernatant liquid from samples was applied to a column of neutral Al2O3 (0.7 × 3 cm). The column was first washed with 8 ml of water, and cyclic nucleotides were then eluted with 3 ml of 0.3 M ammonium formate, pH 6.2. The eluate was collected directly on a second column containing Dowex-50 (0.7 × 9 cm). Cyclic nucleotides were then eluted with water: cyclic GMP elutes between the 2nd and 5th ml and cyclic AMP between the 6th and 16th ml. The eluates were then lyophilized. Cyclic GMP was resuspended in 0.5 ml of 10 mM TES, pH 7.5, and cyclic AMP in 0.5 ml of 50 mM sodium acetate, pH 4.5. The recovery of cyclic AMP was estimated by determining the radioactivity in 100 µl of each sample, and each result was corrected for its own fractional recovery. The mean recovery of cyclic AMP was 73.7 ± SD 10.7% (n = 210). Since the required sensitivity for the determination of cyclic GMP was such that the addition of cyclic GMP [3H] would add a substantial blank value to the unknown sample, the recovery of this nucleotide was calculated as a mean recovery (63.9 ± 5.8%, n = 20) from each 10th column run in parallel with each assay, to which an artificial sample containing 5 nCi of cyclic GMP [3H] was applied.

Cyclic AMP was measured by the protein-binding methods of Gilman as modified by Brostrom and
Cyclic GMP was measured by the method of Steiner et al. with the following modifications: the incubation medium was 10 mM TES, pH 7.5; the dilution of the antibody (with 0.1% γ-globulin) was 1:60,000. A two-step incubation was performed: the antibody was incubated with a cyclic GMP standard or with the unknown sample for 30 minutes at room temperature; samples were then placed on ice and the second incubation was initiated by the addition of 125I-labeled succinyltyrosine methyl ester of cyclic GMP and incubated at 0°C for 60 minutes. This two-step incubation substantially increased the sensitivity of the assay (50% displacement was achieved with 10–20 fmol of cyclic GMP). The "bound" and "free" nucleotide were separated by means of 1 ml of 12% polyethylene glycol in 50 mM sodium acetate, pH 6.2. Samples were then centrifuged at 3000 g for 30 minutes at 4°C, the sediment was washed, and the procedure was repeated. The blank values obtained contained less than 100 counts/min. The multiple dilution of samples, verification of specificity, blanks, and coefficient of variation—applied to both assays—were as described previously.

**Measurement of Adenylate Cyclase Activity**

The method used measures the conversion of ATP(α-32P) into cyclic AMP(32P) and has been described elsewhere. The reaction mixture was 25 mM TES, pH 7.5, 5 mM MgCl2, 1 mM dithiothreitol, 1 mM cyclic AMP + 0.01 μCi cyclic AMP [3H], and 0.5 mM ATP to which 0.5 μCi of ATP(α-32P) was added, an ATP-regenerating system (consisting of 5.4 mM phosphoenol pyruvate and 4 μg of pyruvate kinase) and 15–30 μg of particle protein, all in a total volume of 0.1 ml. Platelet suspensions for measurement of adenylate cyclase activity were cooled on ice for 5 minutes, treated sonically at an amplitude of 6 μm for 10 seconds, recooled for 15–30 seconds, and resuspended for 10 seconds. The preparation was then centrifuged at 20,000 g for 10 minutes at 4°C. The supernatant fluid was discarded and the particulate fraction was resuspended in 10 mM TES, pH 7.5, containing 1 mM dithiothreitol. The washing procedure was repeated twice and the final resuspension was dispersed by three strokes of a small glass Dounce homogenizer. The activity of platelet adenylate cyclase was linear with time and the protein concentrations used.

Statistical analyses were performed using Student's t-test.

**Results**

**Basic Data for Rats**

The weights, systolic blood pressures, and pulse rates of rats used in the present study are included in Table 1. The control strain (Kyoto-Wistar) and SHR (Okamoto) demonstrated no differences in weights for any of three age groups used. The systolic blood pressure was substantially higher in the 12- and 24-week-old SHR while, at 6 weeks, the difference was small but significant. The only significantly higher pulse rate was observed in the youngest SHR.

**Effect of Epinephrine on Platelet Aggregation and Cyclic Nucleotide Concentrations**

In rats, epinephrine (10 μM) induced hardly any aggregation of washed platelets (Fig. 1). It caused a transitory decrease in cyclic AMP within 2 seconds in control rats, but not in SHR, the difference being significant only at 2 seconds (Fig. 2). An extremely rapid increase in cyclic GMP was observed in both strains of rat in response to epinephrine, with a maximum observed at 2 seconds. As seen in Figure 2 and Table 2, the increase was greater in normotensive rats than in SHR, but the difference was statistically significant only for 12-week-old rats. However, it is impossible to determine whether the increase of cyclic GMP was actually lower in SHR or whether the peak occurred earlier, since the concentration could not be measured sooner than at 2 seconds. There was no significant difference between basal levels of either cyclic nucleotide in the two strains (Fig. 2, Table 2).

**Effect of Ionophore on Aggregation and Cyclic Nucleotide Concentrations**

The divalent cation ionophore A-23187 is the most powerful aggregating agent we used. Figure 3 (left) shows the aggregation response of washed platelets to different concentrations of ionophore, and Figure 3 (right) shows the dependence of that aggregation on the presence of extracellular calcium. Since the very low concentration (0.1 nM) results in a qualitatively different shape of aggregation curve, it is conceivable that at this dose some nonspecific effects were observed (such as potentiation of spontaneous aggregation).

Routinely, 100 nM ionophore was used in aggregation studies. As seen in Figure 1 and Table 3, the rate of aggregation was higher in SHR than in control rats, the difference being significant at 12 weeks of age. Ionophore, like epinephrine, decreased platelet cyclic AMP concentration in normotensive rats (at 2 and 5 seconds), while no effect, but large variation, was observed in SHR. In contrast to epinephrine, the ionophore had no effect on cyclic GMP concentrations under the same conditions.

**Effect of PGE1 on Aggregation and Cyclic Nucleotide Concentrations**

PGE1 alone did not cause any aggregation of washed platelets. However, as shown in Figure 1...
and Table 3, when 100 nM PGE$_1$ was added to suspension of washed platelets, there was a substantial reduction in the aggregation induced by the subsequent addition (15 seconds later) of ionophore. The addition of PGE$_1$ resulted in a large rapid increase in cyclic AMP in washed platelets, and this increase was greater in SHR (Table 4). Experiments with various concentrations of PGE$_1$ (Fig. 4) revealed differences in individual responses between 12-week-old control and SHR. In additional experiments with rats of different ages, we observed that the differences at 12 weeks were: 73%, 110%, 122%,

### Table 1: Basic Characteristics of Kyoto (Control) and Okamoto (SHR) Wistar Rats

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Number of rats</th>
<th>Body weight (g)</th>
<th>Blood pressure* (mm Hg)</th>
<th>Pulse rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyoto Wistar (control)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6.3 ± 0.6</td>
<td>25</td>
<td>106 ± 30</td>
<td>95 ± 13</td>
<td>423 ± 51</td>
</tr>
<tr>
<td>12.2 ± 1.1</td>
<td>14</td>
<td>236 ± 43</td>
<td>114 ± 14</td>
<td>391 ± 33</td>
</tr>
<tr>
<td>24.0 ± 2.0</td>
<td>3</td>
<td>381 ± 15</td>
<td>109 ± 23</td>
<td>428 ± 66</td>
</tr>
<tr>
<td>Okamoto Wistar (SHR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.3 ± 0.6</td>
<td>22</td>
<td>105 ± 33</td>
<td>111 ± 23</td>
<td>464 ± 51</td>
</tr>
<tr>
<td>12.3 ± 0.7</td>
<td>13</td>
<td>260 ± 35</td>
<td>175 ± 16</td>
<td>420 ± 49</td>
</tr>
<tr>
<td>24.0 ± 2.0</td>
<td>3</td>
<td>381 ± 45</td>
<td>204 ± 18</td>
<td>416 ± 80</td>
</tr>
</tbody>
</table>

Results are means ± SD.
* Systolic blood pressure of nonanesthetized rats taken as described in Methods.
† $P<0.05$ relative to the corresponding groups in the control strain.

**Figure 1** Time course of aggregation in response to epinephrine (10 μM), ionophore A-23187 (0.1 μM), and PGE$_1$; ionophore (0.1 μM, 0.1 μM each)—typical response observed in 12-week-old normotensive rats and SHR.

**Figure 2** Time course of concentrations of cyclic nucleotides in response to epinephrine (10 μM) in control (Kyoto) and SHR (Okamoto) 12-week-old rats. Concentrations of cyclic nucleotides are means ± SD from 6 rats in each strain.

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*P<0.05
and 167% with 0.1, 0.5, 1, and 5 μM PGE_1, respectively, while smaller differences were observed at 24 weeks when it was 27%, 44%, 41%, and 54%, respectively. As in experiments with various concentrations of PGE_1, the time course of the increase in cyclic AMP concentrations by PGE_1 resulted in higher levels of the nucleotide in SHR than in control rats. Basal concentrations of cyclic AMP were stable and similar in both strains.

### Activity of Adenylate Cyclase

To investigate by which mechanisms PGE_1 raised cAMP concentration, we performed some preliminary studies on adenylate cyclase. The activity of adenylate cyclase depended on the experimental conditions used (Table 5); no difference between control rats and SHR was seen in freshly prepared EDTA-treated platelets, although the tissues responded to both PGE_1 and NaF by a substantial increase in activity. However, when platelets were first incubated for 90 seconds under conditions required for aggregation and those used for measurements of cyclic nucleotides, namely, stirred for 60 seconds (950 rpm) at 30°C in the presence of 2 mM excess of Ca^{2+}, an increase in activity was still observed in the presence of PGE_1, but there now was a significantly greater stimulation of the enzyme from SHR by both PGE_1 and NaF, even though the basal activity was the same in both strains. It can also be seen that this type of “preincubation” decreased the basal as well as the PGE_1-stimulated activity in both strains.

### Discussion

Over the past decade, the strain of spontaneously hypertensive rat originally developed by Okamoto and Aoki has become a useful model of essential hypertension. Numerous anomalies in the control of cardiovascular homeostasis have been described for these animals, including abnormal responses to adrenergic stimulation and abnormal contractility following the exposure to different cations. Studies by Shibata and Kurahashi and by Bohr suggested that there was an alteration in the calcium-binding properties of vascular smooth muscle in these animals; Webb and Bhalla demonstrated a lower sequestering ability of sarcoplasmic vesicles in SHR; and Wei et al. described lower calcium accumulation by membrane fractions in these rats. The regulation of smooth-muscle contractility by calcium was recently investigated with the use of the divalent cation ionophore A-23187. The contractile activity of this agent observed in smooth muscle is analogous to its aggregating activity in human and rat platelets (Fig. 3, left). The absence of extracellular calcium partially inhibits the contractile response to the ionophore in vascular smooth muscle.
TABLE 4  Effect of Exposure of Platelets (30 sec, 30°C) to 100 nm PGE₁ on Concentrations (pmol/10⁶ Platelets) of Cyclic AMP

<table>
<thead>
<tr>
<th></th>
<th>Basal level</th>
<th>PGE₁</th>
<th></th>
<th>Basal level</th>
<th>PGE₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.2 ± 14.8</td>
<td>143.2 ± 16.8</td>
<td>26.4 ± 3.9</td>
<td>96.6 ± 25.8</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>28.4 ± 9.2</td>
<td>267.5 ± 52.3*</td>
<td>27.3 ± 8.9</td>
<td>245.5 ± 80.1†</td>
<td></td>
</tr>
</tbody>
</table>

Results are means ± SD.

* P < 0.01
† P < 0.05 relative to the control strain.

smooth muscle. Similarly, the aggregation of platelets by ionophore requires the presence of extracellular Ca²⁺ [31, 32, 33] (Fig. 3, right). The qualitative differences in aggregation in response to A-23187 observed in SHR in the present study therefore may indicate the presence of anomalies in the calcium transport system in platelets of hypertensive animals. This possibility is further supported by recent observations of Wei et al. [34] that the decrease in Ca²⁺ uptake induced by ionophore A-23187 in subcellular fractions of mesenteric artery was more pronounced in SHR. The interpretation of the significance of anomalies in aggregation observed in vitro nevertheless is extremely difficult. Hornstra [35] demonstrated that, in experimental arterial thrombosis, quite opposite anomalies in platelet aggregation may be observed in vitro and in vivo. It therefore is important to restrict the interpretation of our observations to that of an “anomaly” without attributing any qualitative importance to the rates of aggregation observed. Some other studies indicated an “increase” in platelet aggregation [36] and adhesion in human hypertension, [37] while a decreased responsiveness to ADP was reported in old SHR. [38]

Intracellular concentrations and the metabolism of cyclic nucleotides were recently investigated by several groups [4-10]. Whereas all studies agreed that anomalies existed in the “second messenger system,” substantial discrepancies exist between these studies. Thus a normal [5, 6], decreased [7, 9], or even increased [8] basal activity of vascular adenylate cyclase has been described. Similarly, qualitative discrepancies in cyclic AMP phosphodiesterase activity have been observed [5-9]. These discrepancies are somewhat similar to those reported for vascular contractile responsiveness to catecholamines where an increased [2, 26] normal, [39] or even decreased [20] contractility was observed in SHR. An unsatisfactory choice of control strain [9, 12, 39], different methodology, the use of animals of different age and stage of hypertension, or a particular type of breeding may at least partially explain this disparity.

Since cyclic AMP [41, 42] and cyclic GMP [43, 45] seem to be involved in the regulation of platelet function, we made several measurements of the metabolism
of cyclic nucleotides in platelets isolated from control (Kyoto) and from SHR (Okamoto) rats. We observed similar basal levels of cyclic nucleotides in the two strains which corresponded to the levels observed in normal man and rats. Inhibition of platelet aggregation was always accompanied by an increase of cyclic AMP in these and other studies. The induction of aggregation in rat fails to correlate with changes in cyclic GMP levels, whereas, in human platelets, aggregating agents increase the concentrations of cyclic GMP. In contrast, in the present study, the 10-fold increase of cyclic GMP caused by epinephrine was not accompanied by aggregation, whereas the irreversible aggregation induced by the ionophore A-23187 did not result in any observable change in cyclic GMP concentration. The lack of effect of epinephrine on aggregation in rat platelets also has been observed by others. The inhibition of ionophore-induced aggregation by PGE, as demonstrated in our study, previously was observed in human platelets and was attributed to an increase in cyclic AMP concentration. According to Rodan and Feinstein, the ionophore inhibits the stimulation of adenylate cyclase by PGE, but in the present study, this agent was added after PGE, when the maximum stimulation of cyclic AMP synthesis had already occurred. Our studies suggest that the aggregation of washed rat platelets in response to ionophore A-23187 does not necessarily involve changes in cyclic nucleotides, but we cannot exclude either an extremely rapid change of enzyme activities or “compartmentalization” of cyclic nucleotides within platelets, since only the total (intra- and extracellular) cyclic nucleotides were measured.

The ionophore induced higher aggregation in SHR (Table 3), yet the degree of inhibition of aggregation by PGE, appears to be dependent on the age as much as on the strain of the rats. Thus, in 6-week-old SHR, the degree of inhibition is greater than in controls (75% vs. 59%), a phenomenon attributable to the larger increase of cyclic AMP in response to PGE, On the other hand, in 12-week-old SHR, the degree of inhibition was similar (70% vs. 77%) in spite of the higher cyclic AMP response to PGE, probably due to the greater increase in the rate of aggregation caused by ionophore alone.

The most significant difference between control and SHR was seen in the concentrations of cyclic AMP after stimulation by PGE, The time course of the increase in cyclic AMP concentration induced by PGE, and the dose-response curve indicated that in SHR, PGE, caused a much greater elevation of cyclic AMP. These differences appeared early in the life of hypertensive rats (6 weeks) and increased with age. To ascertain the possible reasons for these increased levels, we studied activities of adenylate cyclase in platelets of both strains. In the cell-free system, changes in activity were found which could account for the increased cyclic AMP levels. The fact that the demonstration of differences in adenylate cyclase activation between SHR and controls depends on the experimental conditions suggests an involvement of regulatory factors (such as Ca++) which deserve further study. In addition to adenylate cyclase, cyclic nucleotide phosphodiesterase could be responsible for anomalies of cyclic nucleotide levels. Since preliminary observations indicated that differences in phosphodiesterase activity did exist, a more detailed study of this enzyme is currently being undertaken in our laboratory. The increased responsiveness of adenylate cyclase to PGE, in SHR is of interest because of the possible involvement of prostaglandins in the pathogenesis of essential hypertension. The decreased release of prostaglandin-like activities from renal medulla has been described for hypertensive rats, and an enhanced hypotensive effect of PGE, was demonstrated in SHR.

Since PGE, induced a substantial elevation in adenylate cyclase activity and cyclic AMP levels, it may be important to consider whether or not such an increase is of relevance to protein kinase activity.
We have demonstrated that full dissociation of protein kinase occurred in rat platelets at a concentration of 1 μM PGE_{1}, i.e., the level at which differences are already apparent between control rats and SHR.

The manner in which the anomalies seen in SHR (levels of c cyclic AMP, adenylyl cyclase responsiveness) change with the age of the rat is of interest. Pfeffer and Frohlich demonstrated an evolution of changes in SHR from an increase in cardiac output in young animals to an increase of peripheral resistance in old ones. In cardiovascular and other tissues of the rat, a decrease in adenylate cyclase from different chronic exposure to stimulating agents since such possible "defects" may be reversed by in vitro manipulation, such as preincubation, multiple washings, etc., it may represent yet another source of the discrepancies reported in enzyme studies. With these limitations in mind, we nevertheless believe that studies on the transmission of hormonal information in platelets may contribute to our understanding of pathophysiological mechanisms of experimental and human hypertension.

Acknowledgments

We acknowledge the expert technical assistance of Sock Laine Leung.

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Cyclic nucleotides and aggregation in platelets of spontaneously hypertensive rats.

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doi: 10.1161/01.RES.43.4.583
_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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