Hypersensitivity of Phospholipase C in Platelets of Spontaneously Hypertensive Rats

SOPHIE KOUTOUZOV, ADNANE REMMAL, PIERRE MARCHE, AND PHILIPPE MEYER

SUMMARY Thrombin-induced aggregation and serotonin release were markedly enhanced in platelets from spontaneously hypertensive rats (SHR) when compared with those from normotensive Wistar-Kyoto rats (WKY). Since phosphoinositides are involved in calcium-mediated platelet responses, the metabolism of these lipids was investigated in SHR and WKY by using $^{32}$P-labeled quiescent platelets. In unstimulated cells, both the rate and extent of $^{32}$P incorporation into individual inositol-containing phospholipids and phosphatidic acid were identical in SHR and WKY. This finding suggests that the pool size and basal turnover of phosphoinositides did not differ between the two strains. In contrast, early thrombin-induced phosphoinositide metabolism, when monitored as changes in $[^{32}P]$phosphatidic acid, was significantly higher in SHR than in WKY. For example, a 20-second exposure to thrombin, 0.3 U/ml, induced the formation of 1.6 times more $[^{32}P]$phosphatidic acid in SHR than in WKY. These results provide evidence for a leftward shift of the dose-response and time-course curves of thrombin-induced $[^{32}P]$phosphatidic acid formation in SHR. Moreover, the extent of the difference between SHR and WKY was independent of the extracellular calcium concentration. Following thrombin stimulation, $[^{32}P]$phosphatidic acid formation likely reflects the initial agonist-receptor interaction; therefore, these results suggest that phospholipase C activity is enhanced in platelets of SHR and that the hypersensitivity of phospholipase C in SHR may play a role in the overall alteration of cell calcium handling and, hence, in the platelet responses of SHR.

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KEY WORDS • spontaneously hypertensive rats • platelets • phosphoinositides • serotonin secretion

FREE cytosolic calcium plays an important role in the development of tension in vascular smooth muscle, and hence arteriolar resistance, and may be closely related to the development of primary hypertension (see References 1 and 2 for recent reviews). Altered calcium handling by membranes (reviewed in Reference 3), as well as enhanced intracellular free calcium content, has been reported in various cell types of essentially hypertensive patients and spontaneously hypertensive rats (SHR). However, the mechanism by which intracellular calcium levels become elevated in cells from hypertensive subjects remains unknown. In these subjects, platelets have been shown to display an increased sensitivity toward a variety of agonists as measured by the cell physiological responses (e.g., shape change, aggregation, release reaction, thromboxane formation). Since platelet responses are mainly controlled by Ca$^{2+}$ influx, Ca$^{2+}$ mobilization, or both, these circulating cells appear to be an attractive model for studying the reactivity of cells from hypertensive subjects with respect to calcium metabolism.

A wide range of cell-activating substances that involve Ca$^{2+}$ mobilization in the expression of the cellular response are known to act by stimulating the metabolism of inositol-containing membrane lipids: phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), phosphatidylinositol 4,5-bisphosphate (PIP$_2$; see Reference 17 for review). The agonist-mediated breakdown of PIP$_2$ by phospholipase C is considered the earliest event initiating cell activation and has been demonstrated in platelets on stimulation with thrombin. It can therefore be postulated that an altered metabolism of these lipids might participate...
in the overall alteration of cell Ca²⁺ handling and responsiveness observed in hypertensive subjects. To gain a further understanding of the mechanism(s) accounting for the Ca²⁺-related hyperreactivity of cells from hypertensive subjects, we studied phosphoinositide metabolism in washed platelets from SHR, either in their resting state or after thrombin stimulation. This comparison was performed by measuring the variations in 32P-labeled lipids following platelet incubation with [32P]orthophosphate. Aggregation and serotonin release reaction were also measured. The results were compared with those obtained from washed platelets isolated from normotensive control rats of the Wistar-Kyoto strain (WKY).

**Materials and Methods**

**Animals**

Male SHR of the Okamoto strain and normotensive WKY were obtained from CERJ (St. Berthevin, France) and were studied at 12 to 15 weeks of age. Systolic arterial blood pressure, measured by the tail-cuff method, was 190 ± 3 and 122 ± 2 (SEM) mm Hg for SHR and WKY, respectively (n = 15 rats per strain).

**Preparation of 32P-Labeled and ¹H]Serotonin-Labeled Washed Platelets**

Blood was withdrawn from the abdominal aorta of rats under light ether anesthesia into 10% (vol/vol) 170 mM trisodium citrate/130 mM citric acid/4% dextrose as anticoagulant. Platelet-rich plasma was obtained by centrifugation at 230 g for 15 minutes at room temperature and was further centrifuged at 120 g for 7 minutes to sediment contaminating red and white blood cells. Platelets in platelet-rich plasma (generally 4–5 ml/animal; i.e., ~10⁹ cells/ml) were labeled with either 32P by incubation at 37°C for 2 hours with 32P]orthophosphate, 3 μCi/10⁸ cells (1 mCi/ml; Amersham, Buckinghamshire, UK) or ¹H] by incubation at 37°C for 5 minutes with 10⁻⁶ M serotonin containing [¹H]serotonin, 1 μCi/10⁸ cells (19.8 Ci/mmol; Amersham). Labeled platelets were isolated by centrifugation at 400 g for 15 minutes and resuspended in an equivalent volume of Ca²⁺-free Tyrode’s buffer (NaCl, 137 mM; KCl, 2.6 mM; NaHCO₃, 12 mM; MgCl₂, 0.9 mM; glucose, 5.5 mM; EGTA, 1 mM; gelatin, 0.25%, pH 6.5). Platelets were then washed twice in Tyrode’s buffer by centrifugation at 400 g for 15 minutes. The final platelet pellet was resuspended in a Ca²⁺-free Tyrode’s/HEPES buffer (NaCl, 137 mM; KCl, 2.6 mM; MgCl₂, 0.9 mM; glucose, 5.5 mM; HEPES, 5 mM; gelatin, 0.25%, pH 7.4). All steps following platelet labeling were performed at room temperature. Platelet suspensions from SHR and WKY were adjusted to equal concentrations (2–4 × 10⁹ cells/ml) and, unless otherwise specified, were incubated for a 60-minute equilibration period at 37°C before experimentation. This procedure was judged to yield fully "rested" platelets on the basis of negligible [32P]phosphatidic acid (PA) formation (2%) and low "spontaneous" [¹H]serotonin release from final platelet suspensions.

To measure the parameters under study, platelet suspensions (0.4 ml) were placed into aggregometer tubes, stirred for 2 minutes (1000 rpm) at 37°C in the presence of 1.3 mM CaCl₂ (unless otherwise specified), and then exposed to thrombin or buffer (control). For [¹H]serotonin-labeled platelets, 10⁻⁶ M imipramine was added with CaCl₂ to prevent serotonin reuptake during the release reaction. Tracings of platelet shape change and aggregation were obtained by recording light transmission throughout all experiments.

**Assays**

Following the addition of thrombin (or buffer), platelet serotonin release was determined by assaying the appearance of [¹H]serotonin in the platelet-free suspension, as described previously. Thrombin-induced serotonin release was expressed as the percentage of maximum release obtained by stimulating the platelet suspensions with thrombin (5 U/ml) for 5 minutes. The amount of radioactivity in the supernatants of unstimulated platelet suspensions (i.e., spontaneous release) was subtracted from all values. Throughout the experiments, spontaneous release never exceeded 7% of the maximum serotonin release and no difference was noted between platelets of SHR and WKY.

To measure 32P-labeled lipids following the platelet reaction in aggregometer cuvettes, 32P-labeled platelets were transferred to tubes containing 3.75 volumes of ice-cold chloroform/methanol/concentrated HCl (20:40:1, vol/vol/vol) to terminate the reaction. Lipids were then extracted and analyzed as previously described. The various inositol lipids and PA were separated by one-dimensional thin-layer chromatography on oxalate-impregnated silica gel plates (article 5715; Merck), Darmstadt, West Germany) using chloroform/acetone/methanol/glacial acetic acid/water (40:15:13:12:8, per volume). The 32P-labeled lipids were localized by autoradiography and scraped off the plates, and their radioactivity was counted by scintillation. For identification, standard phospholipids (Sigma Chemical, St. Louis, MO, USA) were submitted to chromatography along with the platelet extracts and visualized by iodine vapor.

**Statistical Analysis**

One animal was used per experiment, and each measurement was performed in duplicate. Variability was expressed as the means ± SEM, and tests of significance were performed using the unpaired Student’s t test.

**Results**

**Thrombin-Induced Physiological Responses**

**Aggregation**

Typical aggregation tracings of SHR and WKY washed platelets were obtained and are shown in Figure 1. In this figure, a decrease in light transmission...
represents the shape change of platelets from disks to spheres while an increase in light transmission reflects platelet aggregation. After a short (20 seconds) period of stimulation by low concentrations of thrombin (up to 0.5 U/ml), the aggregation of platelets of SHR could be readily observed while only the shape change of platelets of WKY was visible. When platelet suspensions from both strains were allowed to aggregate, the velocity of aggregation was higher in SHR than in WKY (91 ± 3 vs 61 ± 6 mm/min; n = 8, p < 0.01, with thrombin, 0.3 U/ml).

Serotonin Release

Preliminary experiments indicated that [3H]serotonin incorporation as well as spontaneous and thrombin-induced serotonin release did not change whether platelet-rich plasma was incubated for 2 hours at 37°C and [3H]serotonin was added for the last 5 minutes (so that the total length of incubation was similar to that for the 32P-labeling procedure) or incubated directly for 5 minutes with the amine. Under the experimental conditions used for [3H]serotonin labeling, SHR and WKY incorporated 89,832 ± 4984 and 105,051 ± 8514 cpm/10^8 platelets (n = 6, NS), respectively. Likewise, the values for maximum serotonin release (expressed as a percentage of the total [3H]serotonin incorporated) were 82.6 ± 3.6 and 83.2 ± 1.3% for SHR and WKY, respectively (NS).

The time course of [3H]serotonin release by platelets stimulated with thrombin, 0.3 U/ml, showed an increase within the first 30 to 60 seconds and a plateau thereafter (Figure 2A). Figure 2 also shows that, at all times studied, from 10 seconds up to 2 minutes, platelets of SHR exhibited release values about 2.5 times higher than the respective values produced by platelets of WKY. When studied as a function of the dose of thrombin, the [3H]serotonin release (after 20 seconds of stimulation) was barely detectable for thrombin concentrations below 0.1 U/ml, but it increased in a dose-dependent manner between 0.2 and 0.5 U/ml and reached a maximum of 70 to 80% at thrombin, 1 U/ml (Figure 2B). When stimulated by thrombin concentrations of 0.2 to 0.5 U/ml, platelets of SHR released significantly more [3H]serotonin than did platelets of WKY; the difference was no more significant at thrombin, 1 U/ml (see Figure 2B).

32P-Labeling of Lipids in Unstimulated Platelets: Kinetics and Distribution

Under the experimental conditions used, five major lipids incorporated the label: PIP_2, PIP, PI, PA, and an as yet unidentified inositol-containing glycerophospholipid designated X by Tysnes et al. The chromatographic separation of 32P-labeled lipids extracted from rat platelets (not shown)
was similar to that published by Tysnes et al., who used the same solvent system for the separation of phospholipids from human platelets.

The $^{32}$P-labeling of rat platelet lipids was first studied as a function of incubation time with $^{[32P]}$orthophosphate. Figure 3 shows that the $^{32}$P radioactivity associated with the lipids increased with time up to 90 to 120 minutes and, except for PI, reached a plateau thereafter. $^{32}$P incorporation into X (not represented for reasons of clarity) exhibited the same pattern as PIP. There was no difference between SHR and WKY in the rate of $^{32}$P-labeling of all platelet lipids.

Preliminary experiments with either rat strain (not shown) revealed that, following preparation of washed platelets, a 1-hour incubation period (see Materials and Methods) allowed $^{[32P]}$PI to be near isotopic equilibrium without affecting the $^{32}$P values associated with the other lipids. Thereafter, all $^{32}$P-labeled lipids (except for PI) remained stable for at least 1 more hour (i.e., the time required for our experimental design). All further experiments were therefore performed using such an equilibration period.

The distribution of $^{32}$P into lipids of unstimulated platelets from SHR and WKY observed under our experimental conditions is summarized in Table 1. PIP$_2$, PIP, PI, and X incorporated approximately 35, 16, 25, and 20% of the total lipid-associated radioactivity, respectively; $^{[32P]}$PA barely exceeded 2%. The $^{32}$P distribution in these lipids did not differ between SHR and WKY. Moreover, Table 1 clearly shows that the amount of $^{32}$P associated with each lipid was similar in both rat strains.

![Figure 3](image-url)

**Figure 3.** Kinetics of $^{32}$P-labeling of plaquelet phosphoinositides. Platelet-rich plasma was incubated with $^{[32P]}$orthophosphate, 3 µCi/10$^8$ cells. At the designated times, duplicate 3-ml aliquots were removed, washed platelets prepared, and $^{32}$P-labeled lipids analyzed as described in Materials and Methods. Curves are representative of two experiments. PI = phosphatidylinositol; PIP = PI 4-phosphate; PIP$_2$ = PI 4,5-bisphosphate; PA = phosphatidic acid.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>WKY (n = 12)</th>
<th>SHR (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIP$_2$</td>
<td>3219 ± 179</td>
<td>(34.9 ± 1.5)</td>
</tr>
<tr>
<td>PIP</td>
<td>1487 ± 175</td>
<td>(16.8 ± 0.6)</td>
</tr>
<tr>
<td>X</td>
<td>1849 ± 147</td>
<td>(19.9 ± 1.1)</td>
</tr>
<tr>
<td>PI</td>
<td>2499 ± 198</td>
<td>(26.6 ± 1.1)</td>
</tr>
<tr>
<td>PA</td>
<td>162 ± 11</td>
<td>(1.7 ± 0.1)</td>
</tr>
<tr>
<td>Total</td>
<td>9299 ± 529</td>
<td>(100)</td>
</tr>
</tbody>
</table>

Results are means ± SEM. Values in parentheses represent the percentage of radioactivity associated with each class of lipid over the total lipid-associated radioactivity. Platelets were $^{[32P]}$labeled by incubating platelet-rich plasma with $^{[32P]}$orthophosphate for 2 hours. $^{32}$P-labeled platelets were then isolated by washing, and the resulting platelet suspension was further incubated for a 60-minute equilibration period, before analysis of $^{32}$P-labeled lipids (see Materials and Methods).

PI = phosphatidylinositol; PIP = PI 4-phosphate; PIP$_2$ = PI 4,5-bisphosphate; PA = phosphatidic acid; X = unidentified inositol-containing glycerophospholipid.

**Table 1.** $^{32}$P-Labeling of Phospholipids in Unstimulated Platelets of Adult WKY and SHR

**Thrombin-Induced $^{32}$P-Labeled Lipid Changes**

Figure 4 represents the comparison between SHR and WKY of $^{32}$P-labeled phosphoinositide changes brought about by exposure to thrombin, 0.3 U/ml, for various times. A significant loss (10–15%) of the radioactivity associated with PIP$_2$ was constantly observed within 20 to 30 seconds after stimulation and was followed by an increased incorporation of the label at longer times. There was no significant difference between SHR and WKY in the extent of the $^{[32P]}$PIP$_2$ breakdown. At 20 seconds $^{[32P]}$PIP$_2$ represented 88 ± 3 and 88 ± 4% (n = 9) of values in unstimulated samples for SHR and WKY, respectively. By contrast, after the first 30 seconds of stimulation, the resynthesis of $^{[32P]}$PIP$_2$ was significantly accelerated for SHR. At 60 seconds, $^{[32P]}$PIP$_2$ represented 115 ± 5 and 98 ± 3% (n = 4, p < 0.05) of control values (obtained in the absence of thrombin) for SHR and WKY, respectively. Within the first 60 seconds of thrombin stimulation, $^{[32P]}$PIP increased regularly, and from 20 to 60 seconds, this increase was significantly higher in SHR than in WKY. At 30 and 60 seconds, $^{[32P]}$PIP values represented respectively 132 ± 2 and 143 ± 3% of control values for SHR versus 115 ± 2 and 128 ± 3% of control values for WKY (n = 3, p < 0.01). During the first minute of activation, neither $^{[32P]}$PI nor $^{[32P]}$X (not shown) varied significantly from their respective basal values (without thrombin). In contrast with the slight variations in $^{[32P]}$PIP$_2$ and $^{[32P]}$PIP (expressed in terms of both $^{32}$P-labeled lipid changes and comparison of SHR vs WKY), there was considerable variation in the early thrombin-induced increase of $^{[32P]}$PA between the two strains.

The kinetics of $^{[32P]}$PA formation obtained with SHR and WKY when platelets were stimulated with thrombin, 0.3 U/ml, are represented in Figure 5A, which shows that $^{[32P]}$PA formation increased regular-
Figure 4. Time course of thrombin-induced changes in \(^{32}\text{P}\)-labeled lipids of platelets of SHR and WKY. \(^{32}\text{P}\)-labeled washed platelet suspensions obtained as described in Materials and Methods (see also the footnote to Table 1) were challenged with thrombin, 0.3 U/ml, for the designated times before lipid analysis. Results are expressed as the percentage of \(^{32}\text{P}\)phosphatidylinositol (PI), \(^{32}\text{P}\)PI 4-phosphate (PIP), and \(^{32}\text{P}\)PI 4,5-bisphosphate (PIP\(_2\)) in stimulated samples compared with that in respective controls (see Table 1). Single (p<0.05) and double (p<0.01) asterisks indicate significant difference compared with values for WKY. Bars represent SEM from three to nine independent experiments performed on individual animals.

Figure 5. Kinetic (A) and dose-response (B) studies of thrombin-induced phosphatidic acid (PA) formation in platelets of SHR and WKY. \(^{32}\text{P}\)-labeled washed platelet suspensions from SHR and WKY were stimulated with thrombin (0.3 U/ml) or buffer (control) for the designated times (A) or with various doses of thrombin for 20 seconds (B). Results were expressed as the percentage of \(^{32}\text{P}\)PA in stimulated samples compared with that in controls. Single (p<0.01) and double (p<0.001) asterisks indicate significant difference compared with values for WKY.
ly within 60 seconds. Moreover, our data clearly indicate that at all times studied (from 10 to 60 seconds), the increase in [32P]PA was significantly higher in platelets of SHR than in platelets of WKY. Thus, at 20 seconds, [32P]PA values for platelets of SHR were about 1.5 times higher than those for platelets of WKY. [32P]PA formation was further studied as a function of thrombin concentration after 20 seconds of stimulation. When expressed as a percentage of control values (i.e., the [32P]PA value obtained in unstimulated platelets), [32P]PA increased in a dose-dependent manner up to 0.5 U/ml and reached a plateau thereafter to attain a maximum of about 600% at 1 U/ml (Figure 5B). Comparison of the dose-response curves obtained with platelets of SHR and WKY reveals that at concentrations up to 0.5 U/ml the thrombin-induced [32P]PA increase was significantly higher (about 1.5 times) in SHR than in WKY (see Figure 5B). At 0.5 U/ml, although [32P]PA values were higher in SHR than in WKY, the difference was not statistically significant. The [32P]PA increases obtained after platelet stimulation with thrombin, 1 U/ml, were similar in SHR and WKY.

The influence of calcium ions on thrombin-induced [32P]PA formation was investigated by adding to the platelet suspensions either 1.3 mM CaCl2 or 0.2 mM EGTA (final concentrations) 2 minutes before thrombin (or buffer) addition. The results showed that the absence of Ca2+ in the extracellular medium 1) did not modify basal values (i.e., in unstimulated cells) of each [32P]-labeled lipids (not shown) and 2) did not significantly affect either the [32P]PA formation elicited by various doses of thrombin or the SHR/WKY ratio of the [32P]PA increase (Table 2).

**Discussion**

The present findings demonstrate that, in terms of physiological responses (i.e., aggregation and release reaction), washed platelets of SHR were more reactive than those of WKY. This finding is consistent with previous studies that reported a hyperreactivity of platelets of SHR to a variety of stimuli, suggesting a defect distal to membrane receptors. Moreover, our results show that enhanced secretion of serotonin in platelets of SHR could not be accounted for by differences either in the total platelet serotonin content or in the maximum release capacity between the two strains. Since platelet serotonin secretion has been reported to be identical in SHR and WKY, the impaired serotonin secretion we observed in SHR probably is related to abnormalities in calcium influx or mobilization, or both, on stimulation. Since phosphoinositides play an integral role in these mechanisms, their metabolism was investigated in platelets of SHR and WKY.

This end was achieved by using 32P-labeled and quiescent platelets whose lipids were at isotopic equilibrium. Since resting platelets contain very small quantities of diacylglycerol (DG),28 they should exhibit very low levels of PA29 and, hence, of [32P]PA. Such requirements appear to be fulfilled with our experimental design since [32P]PA values were less than 2% of total 32P-labeled lipids (see Table 1).

Unstimulated platelets of SHR and WKY behaved similarly with respect to both the rate and extent of 32P-labeling of each lipid (see Figures 3 and Table 1). The actual values of [32P]PA suggest that platelets from both rat strains were in a similar quiescent state. These observations suggest that the actual pool size of the phospholipids labeled with 32Porthophosphate and the turnover of phosphoinositides in resting platelets (i.e., the basal lipid metabolism) did not differ between the rat strains.

With respect to thrombin-induced 32P-labeled lipid changes, platelets of SHR and WKY behaved like human platelets. Platelet activation by thrombin induces a transient increase in DG, which is rapidly phosphorylated into PA.24,28 Thus, the [32P]PIP2 decrease and the concomitant [32P]PA increase that we observed early after thrombin stimulation probably resulted from the agonist-induced cleavage ofPIP, by phospholipase C. However, from our data, participation of a phosphodiesterase cleavage of PIP and PI, which would also lead to DG formation and thereby to [32P]PA, cannot be excluded.6 Since soon after platelet stimulation the low extent and the variability of [32P]PIP and [32P]PIP2 labeling were accelerated in the hypertensive strain, this finding was compared with regard to variations in [32P]PA. Our results clearly show a leftward shift of the dose-response and time-course curves, which was associated for the latter with higher equilibrium levels of thrombin-induced [32P]PA formation in SHR. Taken together, our data strongly suggest that, on stimulation, phosphoinositide turnover is enhanced in platelets of SHR. Indeed, Figure 4 shows that both [32P]PIP and [32P]PIP2, labeling were accelerated in the hypertensive strain. Since [32P]PA formation is widely held as the most sensitive index of platelet phospholipase C activity,31,32 our results also suggest that thrombin-stimulated phospholipase C displays hyperreactivity in platelets of SHR. The similar extent between

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**Table 2. Lack of Effect of Calcium on Thrombin-Induced [32P]Phosphatidic Acid Formation in Platelets of Adult Rats**

<table>
<thead>
<tr>
<th>Thrombin (μ/ml)</th>
<th>WKY (n = 3–4)</th>
<th>SHR (n = 3–4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−Ca2+ + Ca2+</td>
<td>−Ca2+ + Ca2+</td>
</tr>
<tr>
<td>0.1</td>
<td>141 ± 3</td>
<td>143 ± 16</td>
</tr>
<tr>
<td>0.2</td>
<td>240 ± 16</td>
<td>227 ± 16</td>
</tr>
<tr>
<td>0.3</td>
<td>304 ± 29</td>
<td>270 ± 15</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM and are the percentage of [32P]phosphatidic acid after thrombin stimulation for 20 seconds compared with the value in unstimulated controls (as mentioned in Table 1). For the measurements, the platelets were suspended in buffer containing either 0.2 mM EGTA (−Ca2+) or 1.3 mM CaCl2 (+Ca2+) for 2 minutes before thrombin addition (see Materials and Methods).

*p < 0.01, †p < 0.01, §p < 0.05, compared with values for the WKY calcium-deprived group. †p < 0.05, ‡p < 0.05, compared with values for the WKY calcium-containing group.
SHR and WKY in the early [32P]PIP breakdown does not necessarily contradict this hypothesis. Thrombin-induced variations in [32P]PIP appear from simultaneous degradation by phospholipase C and regeneration by PI and PIP phosphorylation. Therefore, [32P]PIP breakdown does not reflect quantitatively the extent of phospholipase C activation. The hypersensitivity of phospholipase C in SHR is likely a reflection of a cellular defect, since the experiments were performed with washed platelets. However, the lasting effect of some circulating substance(s) cannot be excluded.

In platelets, phospholipase C hydrolysis of PIP induces two second messengers, DG and inositol 1,4,5-trisphosphate (IP3). DG stimulates a specific Ca2+ -activated, phospholipid-dependent protein kinase called protein kinase C (see Reference 34 for review), which phosphorylates specific proteins implicated in platelet degranulation, while IP3 is believed to be responsible for Ca2+ mobilization from the dense tubular system. The cellular events activated by DG and IP3 interact synergistically to trigger rapid and complete platelet responses. It is therefore conceivable that protein kinase C activity would be enhanced in thrombin-stimulated platelets of SHR, in which a hyperactivity of phospholipase C would result in an increased production of both DG and IP3. In support of this hypothesis, a recent article reported an enhanced protein kinase C activity in the supernatant of platelets of SHR, as compared to those of WKY.13 Takken together these data strongly suggest that, on stimulating thrombin also promotes a Ca2+ influx.39 Although in the absence of extracellular Ca2+, thrombin-induced serotonin release was markedly reduced in both SHR and WKY, platelets of SHR still exhibited an elevated serotonin secretion compared with those of WKY.15 Taken together these data strongly suggest that, on stimulation with thrombin, a higher Ca2+ mobilization is likely to be involved in the platelet hyperreactivity of SHR. It is therefore tempting to assume that an increased capacity to mobilize Ca2+ from internal stores, thrombin also promotes a Ca2+ influx.39 Although in the absence of extracellular Ca2+, thrombin-induced serotonin release was markedly reduced in both SHR and WKY, platelets of SHR still exhibited an elevated serotonin secretion compared with those of WKY.15 Taken together these data strongly suggest that, on stimulation with thrombin, a higher Ca2+ mobilization is likely to be involved in the platelet hyperreactivity of SHR. It is therefore tempting to assume that an increased

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