Hyperreactivity of Platelets from Spontaneously Hypertensive Rats
Role of External Magnesium

MARYVONNE BAUDOUIN-LEGROS, BÉATRICE DARD, AND PASCALE GUICHENEY

SUMMARY Thrombin-induced serotonin secretion from platelets from age-matched spontaneously hypertensive rats (SHR) and control Wistar-Kyoto rats (WKY) was compared in the presence of different Ca\(^2+\) and Mg\(^2+\) concentrations. Platelets from SHR were more reactive than those of WKY, and the difference was more marked in 11-week-old than in younger rats. The responses to three concentrations of extracellular Ca\(^2+\) and one extracellular Mg\(^2+\) concentration of 1\(\times 10^{-3}\) M were compared. A high external Ca\(^2+\) concentration (2 \(\times 10^{-3}\) M) increased secretion in platelets of both strains without suppressing the difference between them. Platelets from SHR were more sensitive than those from WKY to a low external Ca\(^2+\) concentration (2 \(\times 10^{-3}\) M). Platelet secretion which is independent of external Ca\(^2+\) concentration was higher in platelets from SHR than in those from WKY. External Mg\(^2+\) exerted an inhibitory effect on serotonin secretion in both types of platelets, but platelets from SHR were less sensitive to Mg\(^2+\) than were those from WKY. This inhibitory effect appeared to be complex. It could be observed in the absence of external Ca\(^2+\), and in this case, the difference in reactivity between platelets SHR and WKY depended on the external Mg\(^2+\) concentration (up to 2 \(\times 10^{-3}\) M). Furthermore, a Mg\(^2+\)-induced antagonism of the stimulatory effect of external Ca\(^2+\) concentration appeared at higher concentrations of extracellular Mg\(^2+\) and was more potent in platelets from WKY than in those from SHR. These results suggest that the hyperreactivity of platelets from SHR is related to an increased sensitivity to external Ca\(^2+\) and a decreased sensitivity to external Mg\(^2+\) and that an anomaly in the cellular response to physiological concentrations of Mg\(^2+\) may be important in the pathophysiology of essential hypertension. (Hypertension 8: 694-699, 1986)

KEY WORDS • spontaneously hypertensive rat • platelet • 5-hydroxytryptamine secretion • calcium • magnesium

PLATELETS are of interest in studies on hypertension for two reasons. First, their activation, which occurs in relation to endothelial injury and may therefore be more frequent in hypertensive vessels,\(^1\) induces the formation of thrombi and releases many vasoactive substances (e.g., serotonin, norepinephrine, adenosine 5'-diphosphate [ADP]) that may be involved in arteriolar spasm.\(^2\) Second, platelets are circulating, reactive, easily obtainable cells that, when activated, contract like smooth muscle cells and re-

lease their granular content in a manner similar to that of nerve terminals.

The aim of the present study was to determine whether platelet reactivity was altered in spontaneously hypertensive rats (SHR) and, if so, whether this alteration existed at the prehypertensive stage. In addition, we compared the functional response of platelets from hypertensive and control Wistar-Kyoto rats (WKY) to different external calcium concentrations to define the mechanism (or mechanisms) involved in the difference.

Finally, since Mg\(^2+\) possesses vasorelaxant properties, demonstrated on the whole animal\(^3\),\(^4\) as well as on isolated smooth muscle,\(^5\),\(^6\) and also decreases neuronal release,\(^7\) and since its administration has been shown to exert a small hypotensive action,\(^8\),\(^9\) we tested its properties on platelets from SHR and WKY to determine whether its action was direct or mediated by Ca\(^2+\).
Materials and Methods

Okamoto strain SHR and age-matched normotensive WKY were obtained from Ifa Credo (Les Oncins, France). All experiments were performed on 11-week-old male rats, except for the dose-response curve to thrombin, which was also established in the prehypertensive state in platelets from 3-week-old rats. Mean systolic blood pressure, measured using tail-cuff methods, was 169 ± 3 mm Hg for SHR and 125 ± 2 mm Hg for WKY at 11 weeks of age and 105 ± 4 mm Hg for SHR and 95 ± 4 mm Hg for WKY at 3 weeks of age.

The rats were anesthetized with pentobarbital (40 mg/kg), and blood samples of 10 ml and 5 ml, respectively, for the adult and young animals were collected by carotid catheterization using citrate solution. Platelet-rich plasma was obtained by centrifugation (2250 g for 90 seconds) and then diluted in plasma to reach a concentration of 10^8 cells/ml. This platelet suspension was incubated at 37°C with [3H]serotonin (10^-4 M) for 5 minutes, then centrifuged twice at 450 g for 25 minutes. The washing buffer used for the second centrifugation contained only 0.3% bovine serum albumin as a protective agent. All centrifugations were performed at 20°C.

The SHR have more platelets than do WKY; however, the platelet content in endogenous serotonin is similar in both species, as are the kinetic constants of [3H]-5-hydroxytryptamine (5-HT) uptake. Therefore, the 5-HT contents after standardization of the cell concentration in platelet rich plasma from both strains appeared analogous. The washing procedure led to a decrease in 5-HT contents after standardization of the cell concentration. The washing buffer used for the second centrifugation contained only 0.3% bovine serum albumin as a protective agent. All centrifugations were performed at 20°C.

The cells were then resuspended (10^7 platelets/ml) in 0.22 µm; Sartorius filters, Gottingen, FRG), and the remaining radioactivity was measured on the filters. The platelet suspension was filtered (pore diameter, 770-2-hydroxyethylpiperazine-<emphasis>Y</emphasis>-<emphasis>V</emphasis>-<emphasis>Y</emphasis>'-<emphasis>V</emphasis>'-tetraacetic acid buffer," pH 7.4, containing 10^-4 M chlorimipramine was kindly supplied by CIBA-Geigy (Basel, Switzerland). All salts were "Baker analyzed" reagents.

The platelet concentration and the lack of stirring were chosen to prevent any aggregation and therefore to compare pure secretory processes in platelets from SHR and WKY. The temperature of 30°C, by slowing the reaction, allowed simultaneous manipulation of platelets from SHR and WKY under the different experimental conditions.

The [3H]-5-HT creatinine sulfate, which had a specific activity of 17 Ci/mmol (Amersham, Amersham, England), was isotopically diluted to 1:50 in 5-HT creatinine sulfate (Aldrich, Bonassies, France). Bovine thrombin was purchased from Roche (Paris, France); prostaglandin E1, ADP, and ethylene glycol bis(β-aminoethyl ether)-<emphasis>N</emphasis>,<emphasis>N</emphasis>','<emphasis>N</emphasis>'-tetraacetic acid (EGTA) were obtained from Sigma Chemical (St. Louis, MO, USA). Chlorimipramine was kindly supplied by CIBA-Geigy (Basel, Switzerland). All salts were "Baker analyzed" reagents.

Results

Under basal conditions (extracellular Mg^2+ concentration: 10^-3 M; no added Ca^2+, which corresponds to an external Ca^2+ concentration of [2 x 10^-6 M]), the [3H]-5-HT secretion induced by low concentrations of thrombin (up to 0.5 U/ml) was greater in platelets from SHR (Figure 1a) than in those from WKY (Figure 1b). The initial velocity of the reaction was calculated from the secretion measured during the first 3 minutes of incubation, and the dose-response curve of the platelets from SHR was shifted to the left (Figure 1c), illustrating the greater sensitivity of these platelets to the agonist. Furthermore, when maximal secretion (measured after a 15-minute incubation) was plotted against the initial velocity of the reaction (Figure 1d), the curve from SHR exhibited a distortion when compared with that of the WKY. This finding suggests that some cellular event leading to secretion is also more active in platelets from SHR than in those from controls.

Platelets from young animals also responded differently to thrombin: platelets from SHR were significantly more reactive than those from WKY (Table 1). On the other hand, platelets from adult Wistar rats behaved like those from WKY: thrombin, 0.2 and 0.3 U/ml, respectively, released 26.1 and 41.8% of the initial load of [3H]-5-HT during the first 3 minutes of incubation with these platelets.

Thrombin-induced secretion of 5-HT appears to depend on Ca^2+ concentration (Figure 2). Suppression of external Ca^2+ by the addition of 10^-3 M EGTA decreased 5-HT secretion in platelets from both strains. Although this decrease was greater in platelets from SHR, these cells still secreted an appreciable amount of 5-HT under such conditions, whereas the platelet response in WKY to the same low concentration of thrombin (0.2 U/ml) was very slight.

Addition of 2 x 10^-3 M Ca^2+ stimulated 5-HT secretion in platelets from both SHR and WKY. This response affected mainly the total amount of secreted 5-HT and did not abolish the magnitude of difference.
between the two populations of platelets, despite the fact that this Ca\(^{2+}\) concentration is higher than maximal, since the results obtained in the presence of 10\(^{-3}\) M Ca\(^{2+}\) were identical to those measured with 2 \times 10\(^{-3}\) M Ca\(^{2+}\) (results not shown).

External Mg\(^{2+}\) also modulated thrombin-induced 5-HT secretion (Figure 3). In the absence of added Ca\(^{2+}\), Mg\(^{2+}\) decreased thrombin-induced 5-HT secretion in platelets from both strains and abolished the response at a concentration of 10\(^{-2}\) M. This inhibitory effect of Mg\(^{2+}\) began at 10\(^{-3}\) M in platelets from WKY, but only at 2 \times 10\(^{-3}\) M in platelets from SHR. It was also observed, in both platelet species, in the presence of 2 \times 10\(^{-3}\) M external Ca\(^{2+}\), but in this case an important residual secretion in platelets from SHR still occurred at 10\(^{-2}\) M Mg\(^{2+}\).

In the presence of 10\(^{-2}\) M EGTA the functional difference between platelets from SHR and WKY de-

### Table 1. Thrombin-Induced 5-Hydroxytryptamine Secretion from Platelets of 3-Week-Old WKY and SHR

<table>
<thead>
<tr>
<th>Thrombin concentration (U/ml)</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>13.5±2.3</td>
<td>40.0±3.9</td>
<td>55.5±6.7</td>
<td>59.0±5.5</td>
<td>67.4±5.3</td>
</tr>
<tr>
<td>SHR</td>
<td>26.5±4.0*</td>
<td>60.5±4.0†</td>
<td>69.5±2.5†</td>
<td>69.5±2.5*</td>
<td>77.4±1.5*</td>
</tr>
</tbody>
</table>

Values correspond to [\(^3\)H]5-hydroxytryptamine secretion measured after a 15-minute incubation with thrombin. They are expressed as a percentage of the initial load and represent means ± SEM of four duplicate determinations.

*\(p < 0.01\), †\(p < 0.001\), ‡\(p < 0.05\), compared with values in WKY.
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pended largely on Mg²⁺ concentration. Without Mg²⁺, thrombin-induced secretion was only slightly (nonsignificantly) higher in platelets from SHR. However, 2 × 10⁻³ M Mg²⁺ was necessary to abolish the secretory response in platelets from SHR, whereas the same effect was induced by 10⁻³ M Mg²⁺ in platelets from WKY.

Because washed rat platelets respond poorly to many agonists, particularly under the secretory conditions used in this study (no stirring, no added fibrinogen), the inhibitory effect of external Mg²⁺ could only be studied on [³H]5-HT release induced by 10⁻⁵ M ADP in the presence of 2 × 10⁻³ M Ca²⁺ (Table 2). Platelets from SHR were more reactive to ADP than were platelets from WKY. Mg²⁺ also inhibited the ADP-induced [³H]5-HT release and platelets from SHR were less sensitive to Mg²⁺ than were platelets from WKY under ADP stimulation.

The effect of Mg²⁺ was also tested on the inhibitory action of prostaglandin E₁, which is known to act through the stimulation of adenylate cyclase (Table 3). In the absence of any spontaneous secretion of serotonin, prostaglandin E₁ had to be tested on the thrombin-induced secretion; therefore, it was added to the platelet suspension 3 minutes before thrombin (0.3 U/ml). Prostaglandin E₁, 10⁻⁷ M, did not exert any effect on thrombin-induced secretion of serotonin in the absence of external Mg²⁺ but decreased it (by about 30%) in the presence of 10⁻³ M external Mg²⁺ concentration. At an external Mg²⁺ concentration of 2 × 10⁻³ M, 10⁻⁷ M prostaglandin E₁ completely abolished the secretion induced by thrombin, 0.3 U/ml.

Discussion

These results describe an abnormal functional response of platelets from SHR to external Mg²⁺ and suggest that external Mg²⁺ exerts a physiological modulatory role that may be altered in SHR. The hyperreactivity to thrombin and ADP observed in our washed platelet preparation agrees with the hyperaggregability described in platelet rich plasma of SHR²³ and with the recently described increased aggregation of washed platelets from SHR in response to ADP and collagen.¹⁴ The multiplicity of the agonists strongly suggests that the platelet hyperreactivity in SHR is not linked to any anomaly of agonist receptors. As this platelet hyperreactivity in SHR was observed in the absence of plasma, it seems to reflect a cellular defect. However, it could be linked to the enduring effect of a circulating agent such as the endogenous Na⁺, K⁺–adenosine 5'-triphosphatase (ATPase) inhibiting factor, which has been shown to exist in hypertensive humans.¹⁵ This factor, whose existence was not demonstrated in SHR,¹⁶ would first affect platelet serotonin active uptake. This uptake was indeed decreased in hypertensive humans¹⁵ but not in SHR,¹⁷ a finding that does not favor the hypothesis of the effect of such an endogenous factor on platelet release from SHR.

The hyperreactivity in platelets from SHR could conceivably be a consequence of the elevated blood pressure. This was not the case in the present study, since the same anomaly exists in the platelets of prehypertensive animals. The alteration of platelet function observed in SHR therefore appears to be linked to some cellular defect in this rat strain. Experiments performed in stroke-prone SHR, in both platelet rich plasma¹⁸ and washed platelets,¹⁹ did not provide the same results, however, and may reflect a different cellular alteration in this particular rat strain.

Judging from the evolution of the thrombin-induced secretion of 5-HT under the different Ca²⁺ concentrations studied, it appears that platelets from SHR exhibit a greater sensitivity to a low Ca²⁺ concentration (2 × 10⁻⁶ M). This may result from an increased Ca²⁺ permeability of the cell membrane, which may affect basal passive or thrombin-stimulated Ca²⁺ influx. An increase in passive Ca²⁺ influx was indeed observed in synaptosomes from SHR²⁰ and more recently in platelets from SHR.¹⁴ The higher levels of free cytoplasmic Ca²⁺ measured with the fluorescent indicator quin-2 in platelets²¹ and lymphocytes from SHR²² as well as in platelets from hypertensive patients¹¹,²² may reflect this increased membrane permeability associated with reduced Ca²⁺ storage or Ca²⁺ extrusion capacity, as already described in other tissues from SHR.²⁴

As platelet secretion in SHR still occurred under weak stimulation in the presence of EGTA, it appears that external Ca²⁺ is not the only factor involved in the difference in platelet reactivity between the two
groups. An alteration in intracellular Ca\(^{2+}\) translocation may be involved, and the reduced Ca\(^{2+}\) storage or extrusion already evoked again could explain this result. However, other factors might also be implicated. For example, in the absence of any increase in intracellular Ca\(^{2+}\) concentration, 5-HT secretion may be induced by diacylglycerol produced by polyphosphoinositide breakdown.\(^{25}\) The turnover of these polyphosphoinositides has been shown to be altered in erythrocyte membranes from SHR,\(^{26}\) and our results may therefore reflect the same anomaly in platelets.

An inhibitory effect of external Mg\(^{2+}\) on platelet activity has already been reported in other systems. In human platelets, 1 mM Mg\(^{2+}\) potentiated the EGTA decrease in 5-HT secretion induced by the Ca\(^{2+}\) ionophore A23187,\(^{27}\) while the addition of Mg\(^{2+}\) decreased the thrombin-induced activation of pig platelets.\(^{28}\) External Mg\(^{2+}\) also has been shown to decrease 5-HT release from superfused rat suprachiasmatic area,\(^{7}\) and numerous studies have reported on the Mg\(^{2+}\)-induced relaxation of smooth muscle cells.\(^{24,25}\) Therefore, the difference between responses in WKY and SHR to external Mg\(^{2+}\) observed in the present study may reflect a widespread anomaly of several cells in SHR.

The mechanism of this inhibitory effect of Mg\(^{2+}\) appears to be complex. High concentrations of external Mg\(^{2+}\) appear to suppress the 5-HT secretion induced by thrombin in the presence of a low Ca\(^{2+}\) concentration (2 \(\times\) 10\(^{-6}\) M) in platelets from both species, as well as the ADP-induced and thrombin-induced secretion measured in the presence of high external Ca\(^{2+}\) concentrations (2 \(\times\) 10\(^{-3}\) M) in platelets from WKY. An antagonistic action of Mg\(^{2+}\) at the Ca\(^{2+}\) channel level, as described in other tissues,\(^{5,28-31}\) may therefore also apply to platelets. This effect would be limited and overridden in platelets from SHR by the aforementioned increase in Ca\(^{2+}\) permeability.

Nonetheless, the inhibitory effect of Mg\(^{2+}\) was also observed in the absence of external Ca\(^{2+}\), which suggests the existence of a Mg\(^{2+}\)-sensitive inhibitory system of platelet secretion. As such an inhibitory action of Mg\(^{2+}\) has also been demonstrated in smooth muscle cells in the presence of EGTA,\(^{3}\) this hypothetical inhibitory mechanism might be a rather general pathway of cell activation. In the range of Mg\(^{2+}\) concentration activating this inhibitory system, the stimulation of 5-HT release induced by a high Ca\(^{2+}\) concentration can be shown to increase with external Mg\(^{2+}\) concentration in both species of platelets. This suggests that this Mg\(^{2+}\)-sensitive mechanism would then be overridden by a high Ca\(^{2+}\) concentration, as may the membrane Mg\(^{2+}\)-dependent Ca\(^{2+}\)-ATPase or cyclic nucleotide system.\(^{3}\) The potentiating action of Mg\(^{2+}\) on prostanoid E\(_2\)-induced platelet inhibition favors an effect on cyclic nucleotide regulation, and Mg\(^{2+}\) has been shown to play a crucial role in the regulation of hormonal inhibition of adenylate cyclase in human platelets.\(^{33}\)

Our comparison of platelets from SHR and WKY suggests that this inhibitory mechanism would be less sensitive to external Mg\(^{2+}\) in platelets from SHR than in those from WKY (respective optimal concentrations of Mg\(^{2+}\): 2 \(\times\) 10\(^{-3}\) M and 10\(^{-3}\) M) and therefore less active in SHR (physiological extracellular Mg\(^{2+}\) concentrations. This suggestion agrees with the reduction in membrane Ca\(^{2+}\)-ATPase activity measured in various tissues from SHR\(^{25,24}\) and with the modifications in cyclic AMP metabolism described in platelets from SHR.\(^{34}\) Many enzymatic studies are needed to test these hypotheses, which, if demonstrated, would indicate that, as with the other important cations, Mg\(^{2+}\) is involved in the dysfunction of cells from SHR, as suggested by Altura and Altura.\(^{25}\) The low free internal Mg\(^{2+}\) concentration recently measured in erythrocytes from hypertensive subjects\(^{36}\) and the hypotensive action of Mg\(^{2+}\) therapy suggest that this is also the case in humans.

References

17. Kamal LA, Le Quan-Bui KH, Meyer P. Decreased uptake of 3H-serotonin and endogenous content of serotonin in blood
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M Baudouin-Legros, B Dard and P Guicheney

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