Phospholipid Metabolism in Platelets from Stroke-Prone Spontaneously Hypertensive Rats and Wistar Kyoto Rats

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Platelets from stroke-prone spontaneously hypertensive rats (SHRSP) show severe hypofunctions accompanied by defective protein (P47) phosphorylation. To examine the mechanism of platelet hypofunctions, phospholipid metabolism in SHRSP was compared with that in Wistar Kyoto rats (WKY). Phosphatidylinositol (PI) content was 20% less in SHRSP than in WKY, but no changes were observed in other phospholipids. Incorporation of [³H]-arachidonic acid (AA) into PI and phosphatidylethanolamine (PE) was 12% and 11% lower, and that into phosphatidylcholine (PC) was 6% higher in SHRSP than in WKY. Thrombin-induced diacylglycerol and phosphatidic acid formation were similar in both groups of platelets. Thrombin-induced release of [¹⁴C]-AA from the labeled platelets and its metabolism to eicosanoids occurred at similar rates. These results suggest that reduced formation of diacylglycerol, an activator of protein kinase C (PKC), does not cause defective phosphorylation of P47, a substrate of PKC, in SHRSP. However it remains unclear how the lower PI content and the altered distribution of AA in PC and PE is related to SHRSP platelet hypofunctions.

Keywords — SHRSP; WKY; platelet; phospholipid

Introduction

With the development of hypertension, thrombin, adenosine diphosphate (ADP), collagen and ionophore A23187-induced platelet aggregation and secretion in stroke-prone spontaneously hypertensive rat (SHRSP) platelets, were markedly reduced compared with those in age-matched Wistar Kyoto rat (WKY) platelets.¹⁾ Concomitantly, thrombin-induced 47 kDa protein (P47) phosphorylation which was mediated by protein kinase C (PKC), was significantly attenuated in SHRSP platelets.^{2,3)} However, stimulation with 12-*O*-tetradecanoyl phorbol 13-acetate (TPA), a direct activator of PKC, phosphorylated P47 of both platelets in a similar degree. Since platelet aggregation was inversely correlated with blood pressure or heart to body weight ratios in various strains of spontaneously hypertensive rats (SHR) and hypotensive treatments did not rectify the aggregability of SHRSP platelets, the hypofunctions in SHRSP platelets appeared to be due to a genetic defect.³⁾ Platelet membrane phospholipids play a critical role in signal transduction.⁴⁾ Di-

acylglycerol (DG), a product of phosphoinositide hydrolysis by phospholipase C (PLC) activates PKC synergistically with cytoplasmic Ca²⁺ increased by inositol-1,4,5-triphosphate (IP₃), another hydrolytic product.⁵⁾ Arachidonic acid (AA) released from phospholipids of stimulated membranes is metabolized by two enzymes, cyclo-oxygenase and lipoxygenase to substances with various biological activities.

In order to gain insight into the mechanism of platelet hypofunctions in SHRSP, the present study was undertaken to investigate phospholipid metabolism in SHRSP and WKY platelets.

Materials and Methods

Chemicals — [³H]-Arachidonic acid ([³H]-AA 83.3 Ci/mmol) and [¹⁴C]-AA (52.0 mCi/mmol) were from NEN, Boston, MA, U.S.A.: [³²P]-orthophosphate from Japan Isotope Co., Tokyo: Thrombin from Midori Cross Co., Osaka: PI (soybean), PIP and PIP₂ (bovine brain), phosphatidic acid (PA) from Sigma, St Louis, MO, U.S.A.: 1, 2 Diolein from Funakoshi, Tokyo: Nordihydroguaiaretic acid

(NDGA) was from Paesel, Borsigallee, FFM: thin-layer chromatography (TLC) plates (Art 5721, 20×20 cm) and high performance-TLC (HPTLC) plates (Art 5641, 10×20 cm) from Merck, Darmstadt, FRG.

Experimental Animals and Preparation of Washed Platelets — SHRSP and WKY were originally provided by professor Okamoto of Kinki University Medical School and have been maintained by brother-sister breeding in our laboratory. Male SHRSP aged 10–17 weeks unless otherwise described and age-matched male WKY were used. Washed platelets from these rats were prepared as previously described.⁶⁾

Analysis of Platelet Phospholipids — Platelet (5×10^8 cells/ml, 1 ml) phospholipids were extracted with 3.75 ml of CHCl_3 –MeOH (1:2) overnight at 4 °C and an addition of 1.25 ml of CHCl_3 and 2 M KCl containing 5 mM ethylenediaminetetraacetic acid (EDTA). The lower phase was evaporated under N_2 . Phospholipid extracts were dissolved in CHCl_3 –MeOH– H_2O (75:25:2) and applied on a HPTLC plate, which was rinsed with a mixture of CHCl_3 –MeOH (2:1) overnight. The plate was developed at 4 °C in a mixture of CHCl_3 –MeOH–40% CH_3NH_2 (63:35:10). Spots were visualized with I_2 vapor and scraped off. Phospholipids were eluted from silica with MeOH and analyzed for phosphorus according to the method of Bartlett.⁷⁾

Analysis of Platelet Inositol Phospholipids — Platelet (2×10^9 cells/ml, 1 ml) inositol phospholipids were extracted with 3.75 ml of CHCl_3 –MeOH–HCl (100:200:2)⁸⁾ overnight at 4 °C. Subsequently 1.25 ml of CHCl_3 and 2 M KCl containing 5 mM EDTA was added. The lower phase was transferred and the upper layer was rinsed twice with 2 ml of CHCl_3 . Consecutive 3 lower phases were combined and the solvent was removed under N_2 . Extracts were dissolved in CHCl_3 –MeOH– H_2O (75:25:2), and were applied on a HPTLC plate precoated with potassium oxalate.⁹⁾ The plate was developed at 4 °C in CHCl_3 –acetone–MeOH–acetic acid– H_2O (40:15:13:12:8).⁹⁾ The plate was heated for 10 h with ammonium bicarbonate in a sealed chamber to develop fluorescence. Spots were visualized under ultraviolet (UV) light

(360 nm) and were scraped off. Inositol phospholipids were extracted from silica with 3 ml of CHCl_3 –MeOH–2 N HCl (5:3:2) for PI,¹⁰⁾ 3 ml of CHCl_3 –MeOH–2.5 N HCl (1:2:0.1) for PIP and PIP₂.⁸⁾ The silica was consecutively extracted with 1 ml of MeOH and 1 ml of MeOH–HCl (95:5). The three supernates were combined and assayed for phosphorus by the method of Hess.¹¹⁾

Preparation of [³H]-AA Labeled Platelets — Platelet rich plasma (PRP, 9 vol) was incubated with [³H]-AA (8.33 $\mu\text{Ci}/0.1$ nmol/ml in 10% bovine serum albumin (BSA), 1 vol) for 60 min at 37 °C.¹²⁾ The platelets were washed with buffer A (25 mM Tris-HCl, 137 mM NaCl, 5.4 mM KCl, 0.2 mM ethylene glycol bis (2-aminoethylether) *N,N,N',N'*-tetraacetic acid (EGTA), 0.2% dextrose, pH 6.5) by centrifugation and were resuspended in buffer B (25 mM Tris HCl, 137 mM NaCl, 5.4 mM KCl, 0.2% dextrose, pH 7.4) to make a suspension (6×10^8 cells/ml).

Measurement of DG Formation — [³H]-AA labeled platelet suspensions (0.9 ml) were incubated with thrombin at 37 °C in the presence of 1.5 mM CaCl_2 . DG formed, was extracted and released on TLC plates according to the method by Rittenhouse.¹²⁾

Measurement of PA Formation — Washed platelets (2×10^9 cells/ml) were incubated for 90 min at 37 °C in equal volumes of neutralized [³²P]- H_3PO_4 (0.5 mCi/ml), and were washed twice by centrifugation. ³²P-Labeled platelets were stimulated with thrombin in the presence of 1.5 mM CaCl_2 (total volume 0.5 ml) at 37 °C. PA formed, was extracted and resolved on TLC plates according to the method of Kennerly *et al.*¹³⁾

Measurement of [¹⁴C]-AA Release — Washed platelets (2×10^9 cells/ml, 9 vol) were suspended in piperidine-*N,N'*-bis-2-ethanesulfonic acid (PIPES) buffer (10 mM PIPES, 145 mM NaCl, 5 mM KCl, 5.5 mM dextrose, 0.2 mM EGTA, pH 6.5) and were incubated with 1 vol of 5 μM [¹⁴C]-AA (0.23 $\mu\text{Ci}/\text{ml}$) in 10% BSA at 37 °C for 60 min. They were washed once with PIPES buffer and were resuspended in *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfate (HEPES) buffer (10 mM HEPES, 145 mM NaCl,

5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 5.5 mM dextrose, 0.2 mg/ml BSA, pH 7.4). [¹⁴C]-AA labeled platelets (5×10^8 cells/ml, 0.95 ml) were preincubated for 2 min with 100 μ M NDGA (25 μ l), and were stimulated with thrombin (25 μ l) at 37 °C. The reaction was stopped by adding 50 μ l of 10% formic acid.

Metabolism of [¹⁴C]-AA — Washed platelets (5×10^8 cells/ml, 475 μ l) in a 25 mM Tris-HCl buffer (pH 7.6) containing 137 mM NaCl, 2.7 mM KCl, 11 mM dextrose, were incubated with 25 μ l of 50 μ M [¹⁴C]-AA (0.7 Ci/mol) at 37 °C. The reaction was stopped by adding 25 μ l of 10% formic acid. AA and its metabolites were resolved on TLC plates.¹⁴⁾

Protein Assay — Platelet protein was measured by the method of Lowry *et al.*¹⁵⁾ BSA was used as the standard.

Statistical Analysis — The significance of observed differences was analyzed by Student's *t*-test.

Results

Phospholipid Contents in Platelets from SHRSP and WKY

Phospholipid composition of platelets from SHRSP and WKY was compared (Table I). Total lipid phosphorus was 8.67 ± 1.71 ($n = 18$) and 8.92 ± 1.65 ($n = 17$) μ g P/mg platelet protein in SHRSP and WKY, respectively, which were not significantly different. Although PI is a minor phospholipid in platelet membranes, its content in SHRSP platelets was 20% lower than that in WKY platelets. There was no significant difference in content of the two major phospholipids (PC and PE).

Inositol Phospholipid Contents in SHRSP and WKY Platelets

To confirm the accuracy of our method for the assay of inositol phospholipids, their content in human platelets were determined and were compared with published values (Table II). On the base of proteins, PI and PIP₂ content of

TABLE I. Phospholipid Class Composition in Platelets from SHRSP and WKY

	Phospholipid composition (% of total)		
	PI	PC	PE
	Mean $\pm$ S.D. (number of samples)		
WKY	5.28 ± 1.27 (14)	42.2 ± 3.01 (13)	24.4 ± 4.04 (13)
SHRSP	4.26 ± 0.96 (16) ^{a)}	43.5 ± 3.40 (17)	25.6 ± 1.33 (16)

Phospholipids in platelets (5×10^8 cells/ml) were extracted by CHCl₃-MeOH (1:2) and were separated by HPTLC using CHCl₃-MeOH-CH₃NH₂ (63:35:10) as a developing solvent.

a) Significance $p < 0.05$ vs WKY.

TABLE II. Contents of PI, PIP and PIP₂ in Platelets from SHRSP and WKY and Human Platelets

	PI	PIP (nmol/mg protein)	PIP ₂
	Mean $\pm$ S.D. (number of samples)		
WKY	10.60 ± 1.59 (9)	1.45 ± 0.29 (15)	0.93 ± 0.16 (18)
SHRSP	8.27 ± 1.33 (9) ^{a)}	1.45 ± 0.30 (14)	1.00 ± 0.15 (18)
Human	7.90 ± 0.54 (7)	1.50 ± 0.11 (5)	0.77 ± 0.08 (5)

Inositol phospholipids were extracted and resolved on HPTLC plates. Each spot was scraped from the plates. Phospholipids were extracted from silica and analyzed for phosphorus as described under the experimental procedure. Platelets (1×10^9 cells) contained 1.60 mg protein in SHRSP, 1.52 mg protein in WKY and 2.51 mg protein in human subjects, respectively. The recoveries of inositol phospholipids from silica were 85.0% for PI, 82.3% for PIP and 81.5% for PIP₂, respectively (mean of 5 determinations).

a) Significance $p < 0.01$ vs WKY.

TABLE III. Incorporation of [^3H]-AA into Platelet Phospholipids

	PI	PC	PE
	% of total ^3H -radioactivity incorporated		
	Mean $\pm$ S.D. (number of samples)		
WKY	11.06 ± 1.16 (15)	54.69 ± 3.11 (15)	23.37 ± 2.75 (15)
SHRSP	9.76 ± 0.78 (14) ^{b)}	58.21 ± 2.67 (14) ^{a)}	20.91 ± 2.48 (14) ^{a)}

PRP (9 vol) from SHRSP and WKY were incubated with [^3H]-AA ($8.33 \mu\text{Ci/ml}$ in 10% BSA, 1 vol) for 60 min at 37 °C. Labeled platelets were rinsed with buffer A and were resuspended in buffer B (5×10^8 cells/ml). Phospholipids were extracted from [^3H]-AA labeled platelets and were resolved on TLC as described in the legend to Table I. Phospholipids were eluted from silica by MeOH and the radioactivity was counted.

a) Significance $p < 0.01$, b) $p < 0.001$ vs WKY.

human platelets was slightly less than that in rat platelets. Their content in human platelets, expressed as nmol/ 10^9 cells, were 19.84 ± 1.35 (PI), 3.76 ± 0.27 (PIP) and 1.94 ± 0.21 (PIP₂), which closely resembles values reported by Wilson *et al.*¹⁶⁾ In the present study our rat platelets, the PIP content was 1/7—1/10 of PI content and PIP₂ was 1/10—1/15. As shown in Table II, PI in SHRSP platelets was 22% less than that in WKY platelets.

Incorporation of [^3H]-AA into Platelet Phospholipids

The incorporation of [^3H]-AA into platelets

(WKY) was 20.2% of the added [^3H]-AA. There was no significant difference in total incorporation of [^3H]-AA into platelets between SHRSP and WKY. Thrombin (0.1 U/ml)-induced aggregation was unaltered by incubation with [^3H]-AA (10 nM) for 60 min.

Although there was no difference in the PC and PE content of platelets from SHRSP and WKY (Table I), incorporation of [^3H]-AA into PC in SHRSP was significantly higher by 6.4% than those in WKY platelets while that into PE was 10.5% lower in SHRSP than that in WKY platelets. [^3H]-AA was incorporated into PI

Fig. 1. Thrombin-Induced DG Formation in Platelets from SHRSP (—●—) and WKY (---○---)

a) Time courses, b) concentration dependency curves. [^3H]-AA prelabeled platelets (6×10^8 cells/ml) were stimulated with thrombin (1 U/ml) for the time indicated in a); with various concentrations of thrombin for 15 s in b). Thrombin (0.1, 0.15, 0.5 U/ml) induced platelet aggregation was shown in insets. DG were extracted by CHCl_3 -MeOH (1:2) and separated by TLC using benzene-diethylether-EtOH-NH₄OH (50:40:2:0.1) as a developing solvent. Each spot was visualized with iodine vapor and scraped off. DG were eluted from silica with ether. Each point and vertical bar represent mean $\pm$ S.D. for 3 pooled platelet preparations. Each experiment was carried out in triplicate. a) Significance $p < 0.01$.

(11.8% less) in SHRSP platelets than in WKY platelets, which would result mainly from the smaller PI content in SHRSP platelets (Table III).

Thrombin-Stimulated DG Formation

Figure 1 shows thrombin (1 U/ml)-induced DG formation in SHRSP and WKY platelets. DG formation in human platelets examined preliminarily, peaked at 15 s and then decreased (Data are not shown). The time course was consistent with that observed by Rittenhouse¹²⁾ and by Kaibuchi *et al.*⁵⁾ However, rat platelets show slightly different time courses. DG formation peaked at 15 s as with human platelets, then decreased slightly at 30 s, gradually increasing afterward. In Fig. 1 a) experiments, a supramaximal thrombin concentration (1 U/ml) for aggregation was used to observe apparent DG formation. Compared with WKY platelets, SHRSP platelets showed decreased formation of DG at 15, 30 and 90 s-stimulation. At this thrombin concentration (1 U/ml), there was no difference in aggregation responses between the two species. Figure 1 b) shows DG formation in

response to various concentrations of thrombin (0.1–3 U/ml) with aggregation tracings depicted simultaneously. Aggregation responses of SHRSP platelets to lower doses of thrombin (0.1 and 0.15 U/ml) were smaller than those in WKY platelets. With these thrombin concentrations, no significant DG formation was observed in either platelet group. Supramaximal thrombin doses (≥ 0.5 U/ml) produced no difference between aggregation of SHRSP and WKY platelets. Whereas DG formation was apparently observed at these concentrations, it was lower in SHRSP platelets than in WKY platelets.

Thrombin-Induced PA Formation

To confirm that the initial platelet activation step is not impaired, PA formation was examined in SHRSP and WKY platelets. As DG produced from inositol phospholipids is quickly transformed to PA, the formation of PA reportedly reflects *in vivo* PLC activity.¹⁷⁾ Figure 2 depicts the time courses of thrombin-induced aggregation and [³²P]-PA formation and concentration-dependent curves. By incubation with [³²P] for 90 min, approx. 25% of

Fig. 2. Thrombin-Induced PA Formation in Platelets from SHRSP and WKY

a) Time courses, b) concentration dependency curves. Washed platelets (2×10^9 cells/ml) were incubated with equal volumes of neutralized [³²P]-H₃PO₄ (0.5 mCi/ml) for 90 min at 37 °C. After two washings, [³²P]-prelabeled platelets (4×10^8 cells/ml) from SHRSP (—●—) and WKY (---○---) were stimulated with thrombin [0.1 U/ml in a), for 30 s in b)]. PA was extracted by CHCl₃–MeOH–HCl (100:200:2) and was resolved by TLC as described in the text. Radioactivity (cpm) uptake into platelet lipids (2×10^8 cells) was $9.9 \times 10^5 \pm 6.6 \times 10^4$ ($n = 16$) in SHRSP and $10.1 \times 10^5 \pm 8.7 \times 10^4$ ($n = 17$) in WKY without significant difference. Each point and vertical bar represent mean $\pm$ S.D. for 3 pooled platelet preparations. Each experiment was carried out in triplicate.

added [^{32}P] was taken up into platelets, and 75% of its activity was found in inositol phospholipids (30% in PI, 20% in PIP and 25% in PIP_2). There was no difference in [^{32}P]-uptake into platelets (2×10^8 cells) between the two strains [SHRSP: $9.9 \times 10^5 \pm 6.6 \times 10^4$ cpm ($n=16$) vs WKY: $10.1 \times 10^5 \pm 8.7 \times 10^4$ cpm ($n=17$)]. When [^{32}P]prelabeled platelets were stimulated with thrombin (0.1 U/ml) for various times (Fig. 2 a), PA formation increased linearly with similar rates in both platelets. The slightly higher rate of PA formation in WKY platelets at 60 and 90 s might result from the higher content of PI in WKY compared to that in SHRSP platelets. Fig. 2 b) shows the concentration-dependency curves of PA formation on stimulation (30 s) and aggregation tracings (Fig. 2 b insets) depicted simultaneously. There was no difference in [^{32}P]-PA formation between WKY and SHRSP at the examined concentrations of thrombin despite the significant difference in the aggregation response at the concentration of 0.13 U/ml thrombin.

Thrombin-Induced [^{14}C]-AA Release from

Prelabeled Platelets

Neither the time courses (3–10 min) of [^{14}C]-AA release from platelet groups stimulated with thrombin (1 U/ml), nor the [^{14}C]-AA release from both platelets stimulated for 10 min with varying concentrations of thrombin (0.1–10 U/ml) was altered.

Metabolism of [^{14}C]-AA in Platelets

Since the rate of thrombin-induced [^{14}C]-AA release from SHRSP and WKY platelets appeared to be similar, the metabolism of AA was compared by incubating exogenously added [^{14}C]-AA (50 μM) with platelet suspensions from SHRSP and WKY (Fig. 3). [^{14}C]-AA was metabolized so rapidly in both platelet groups that it completely disappeared in 3 min. 12-L-Hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), the main metabolite of AA in this system, appeared after 1 min and reached a plateau at 3 min. Approx. 60% of exogenously added [^{14}C]-AA was transformed to 12-HETE in 3 min. Formation of 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) was observed at 30 s and peaked at 3 min. 7% of [^{14}C]-AA on

Fig. 3. Time-Courses of [^{14}C]-AA Metabolism in Platelets from SHRSP and WKY

a) Decrease in [^{14}C]-AA, b) formation of 12-HETE, c) formation of HHT, d) formation of TXB₂. Washed platelets (5×10^8 cells/ml) from SHRSP (—●—) and WKY (---○---) were incubated with 50 μM of [^{14}C]-AA (0.7 Ci/mol) for the indicated times. AA and its metabolites were extracted with CHCl_3 -MeOH (2:1) and resolved by TLC using CHCl_3 -MeOH-acetic acid- H_2O (90:8:1:0.8) as the developing solvent. The radioactivity of each spot was analyzed by densitometry of autoradiographs. Values were expressed as percentage of the radioactivity added in the reaction mixtures. Each point and vertical bar represent mean $\pm$ S.D. for 3 pooled platelet preparations. Each experiment was carried out in triplicate.

the average was changed into HHT. Another product of cyclo-oxygenase, TXB₂, appeared at 1 min but did not increase by further incubation. TXB₂ production was less than 5%. No apparent difference in the metabolism of these four substances was observed between SHRSP and WKY platelets.

Discussion

PI, PIP and PIP₂ are minor constituents of mammalian membranes. Agonist-induced phosphoinositide breakdown functions as a signal-generating system by yielding two second messengers, DG and IP₃.⁴⁾ DG stimulates PKC synergistically with Ca²⁺⁵⁾ while IP₃ is proposed as a second messenger for Ca²⁺ mobilization.¹⁸⁾ It is therefore interesting to investigate whether there are changes of membrane lipids, especially phosphoinositides in SHRSP platelets. PI was 20% less in SHRSP platelets while the contents of PIP and PIP₂ were not significantly different between the two strains. No changes in the contents of PC and PE were observed in SHRSP platelets compared with those in WKY platelets. These results coincided with results observed in erythrocyte membranes from SHR and WKY.¹⁹⁾ However, it is not clear whether the decrease in PI content of these membrane is a common phenomenon observed in hypertensive animals or how it is related to hypertension.

Thrombin-induced DG formation was detected only at the supramaximal thrombin concentration at which there was no difference in aggregation between SHRSP and WKY platelets. At lower concentrations no significant formation of DG was observed although aggregation was induced in SHRSP and WKY platelets to a significantly lesser degree in SHRSP platelets. Therefore, these results indicate that attenuated responses of aggregation, secretion and protein phosphorylation in SHRSP platelets are apparently not related to the degree of DG formation. This assumption is supported by the finding that thrombin-induced PA formation reflecting PLC activity¹⁷⁾ in SHRSP and WKY platelets was not different.

The increase in incorporation of [³H]-AA into PC in SHRSP platelets (Table III) indicates

that the distribution of fatty acids in PC and PE, or the turnover rates of these phospholipids differ in SHRSP and WKY platelets. In relation to this observation, Marche *et al.*²⁰⁾ reported that in quiescent platelets of SHR, the labeling of PC with [³²P] was markedly enhanced in comparison with that of WKY platelets.

Thrombin induced liberation of AA is mainly from PC²¹⁾ and also PE²²⁾ through the activation of PLA₂. Other possible sources of AA are phosphoinositides and phosphatidic acid. Phosphoinositides are degraded by PI-specific PLC to DG,¹²⁾ which is then hydrolyzed by DG lipase to 2-monoacylglycerol²³⁾ to produce AA by monoacylglycerol lipase.²⁴⁾ Hydrolysis of PA by PA-specific PLA₂ liberates AA and lyso-PA.²⁵⁾ Our previous data suggested that despite reduced aggregation and secretion in SHRSP platelets, the PLA₂ pathway seemed to function normally or was enhanced.²⁶⁾ Okamoto *et al.*²⁷⁾ showed that PLA₂ and PLC activities were elevated with age in the renal membrane of SHRSP compared with those in age-matched WKY.

Endogenous 12-lipoxygenase products inhibit PGH₂-induced platelet aggregation.⁶⁾ 12-Hydroperoxyeicosa-tetraenoic acid inhibits TXA₂ synthetase²⁸⁾ and DG lipase activity,²⁹⁾ indicating that lipoxygenase products in platelets may regulate the activation process. Therefore, the release and metabolism of AA in SHRSP and WKY were compared. Although incorporation of [³H]-AA into platelet phospholipids under quiescent conditions was slightly different in SHRSP and WKY platelets (Table III), the release of AA from prelabeled platelets and the metabolism of AA did not differ in SHRSP and WKY platelets. Under stimulated conditions, AA may be incorporated into a separate phospholipid pool sensitive to activated hydrolysis. Thus, under *in vivo* conditions in the presence of various stimulants, the labeling profile may be altered from that in quiescent conditions. There is evidence for a separate, hormone-sensitive phosphatidylinositol pool in the WRK-1 rat mammary tumor cell line, which accounts for 17% of the total cellular PI.³⁰⁾ It is reported that there are two sources of AA for oxidative metabolism through lipoxygenase and

cyclo-oxygenase in mouse peritoneal macrophages, and that the products of AA are dependent upon the nature of the stimulants.³¹⁾ Therefore, it is possible that release of AA from endogenous phospholipids and its metabolism differ in SHRSP and WKY platelets which may have different fatty acid distribution and phospholipid composition. In support of these suggestions, more TXB₂ was formed (with high doses of thrombin) from endogenous phospholipids in SHRSP platelets than in WKY²⁶⁾ although the formation from exogenous AA in SHRSP platelets was similar to that in WKY platelets. It has been reported that the main metabolites of exogenously added AA in rat brain microvessels are PGE₂ and PGD₂ whereas PGI₂ is a major metabolite from endogenous phospholipids.³²⁾

In conclusion, alteration of the phospholipid content and metabolism may partially contribute to SHRSP platelet hypofunction. However, the major defect in SHRSP platelets appears to be an impaired activation of PKC by Ca²⁺ from evidence thus far obtained in our laboratory indicating: 1) that reduced aggregation, secretion and P47 protein phosphorylation is observed in SHRSP platelets stimulated with Ca²⁺ ionophore A23187 (or ionomycin) as well as with thrombin,³⁾ 2) that TPA-induced platelet responses in SHRSP are similar to those in WKY,³⁾ and 3) Ca²⁺ dependency curves of the three platelet responses mentioned above, are shifted towards the right in SHRSP compared to WKY.³³⁾

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