Salt-Induced Plasma Factor That Inhibits Platelet Thromboxane A₂ Release and Renal Prostaglandin E₂ Production in Rats

YOSHIO UEHARA, MASAO ISHII, TOSHIHIKO ISHIMITSU, AND TSUNEAKI SUGIMOTO

SUMMARY This study examined the relationship between a plasma factor (or factors) that inhibits the release of thromboxane A₂ from platelets and excessive salt intake in rats. The plasma factor, termed platelet inhibitory factor, was also characterized. The release of thromboxane A₂ from thrombin-activated platelets was reduced in Wistar rats that were uninephrectomized and given 2% saline for a week, but not in rats with acute volume expansion. Platelet inhibitory factor was extracted from the plasma of these uninephrectomized and saline-loaded rats and partially purified using membrane sieves, reverse-phase high performance liquid chromatography (HPLC), modified straight-phase HPLC, and gel-permeation column chromatography. The molecular weight of the factor was about 4300 daltons by gel filtration method. The partially purified platelet inhibitory factor decreased the release not only of thromboxane A₂, but also of prostaglandin E₂ and prostaglandin D₂ from thrombin-activated platelets. The factor inhibited the aggregation of human platelets induced by adenosine 5'-diphosphate (ADP), collagen, and thrombin, but not that by arachidonate. The platelet inhibitory factor reduced the activities of phospholipases A₂ and C but did not affect the conversion of arachidonate to thromboxane A₂. Furthermore, platelet inhibitory factor decreased prostaglandin E₂ production in cultured renal cells, and platelet inhibitory factor-like activity was detected in kidney extract from the salt-loaded rats. These results suggest that platelet inhibitory factor is produced by chronic salt intake and involved in the functional alterations of the platelets and probably the kidneys, mainly through its inhibitory action on the liberation of arachidonate.

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KEY WORDS • platelet inhibitory factor • salt intake • thromboxane A₂ • renal cells • phospholipases

THE mechanism of salt-induced hypertension is still controversial. In addition to the extracellular volume expansion following excessive salt intake, the increase in sympathetic nervous activity,¹ the deterioration of the vasodepressor system,² or the induction of some humoral factors that facilitate the transmembrane transport of ionized calcium³ have all been proposed to account for the elevation of blood pressure. Dahl et al.⁴ and Tobian et al.⁵ have demonstrated the possibility that some humoral pressor factor (or factors) occurs in the circulating blood of Dahl salt-sensitive (S) rats, which are considered to be the animal model for the role of salt sensitivity in human hypertension. In this context, Uehara et al.⁶ have found one or more unusual constituents in Dahl S rat plasma that inhibit the release of thromboxane A₂ from thrombin-activated platelets, and have also found that a trace amount of this constituent is detected even in the plasma of Dahl salt-resistant (R) rats fed a high salt diet. Thus, this plasma factor, tentatively termed platelet inhibitory factor (PIF), seems to appear following high salt intake.

In the present study, we expanded our previous investigation of PIF by examining the relationship between PIF and salt intake in rats, extracting PIF from the plasma of salt-loaded rats, and attempting to characterize the effects of PIF.

Materials and Methods

Experiment 1

In Experiment 1, we examined the release of thromboxane A₂ (TXA₂) from thrombin-activated platelets in rats after acute or chronic salt loading. To measure TXA₂ release after acute salt loading, 14 eight-week-
SALT-INDUCED INHIBITION OF THROMBOXANE A\textsubscript{2} RELEASE/Uehara et al. III-7

old male Wistar rats were anesthetized with 80 mg/kg of thiobutabarbital (Inactin). A physiological saline solution equal in weight to 2.5% of body weight was infused for 1 hour. One hundred microliters of blood was obtained before and at the end of the infusion without any change in blood pressure. The blood specimens were used for assaying the release of TXA\textsubscript{2} from platelets and the hematocrit.

To measure the effects of chronic salt loading, 27 seven-week-old male Wistar rats were uninephrectomized or sham-operated under pentobarbital anesthesia (25 mg/kg of body weight) and divided into three groups: sham-operated rats given tap water (Group 1), uninephrectomized rats given tap water (Group 2), and uninephrectomized rats given 2% saline (Group 3). Seven days later, they were anesthetized with pentobarbital, and 9 volumes (ml) of blood from the inferior vena cava were removed and mixed with 1 volume of 3.8% citrate disodium.

To assay the release of TXA\textsubscript{2} from platelets of all the rats, the citrated blood was centrifuged at 94 g at 25°C for 10 minutes and the platelet-rich plasma (PRP) was obtained. The citrated whole blood of the acutely salt-loaded rats or the PRP of the chronically salt-loaded rats was incubated separately with thrombin, and the released TXA\textsubscript{2} was radioimmunoassayed. Thromboxane A\textsubscript{2} released from thrombin-activated platelets was also expressed as picograms per 10\textsuperscript{6} platelets.

Experiment 2

In Experiment 2, PIF was extracted from the pooled plasma of the rats in Group 3. Plasma constituents with molecular weights of 10\textsuperscript{3} to 10\textsuperscript{4} daltons were fractionated using an ultrafiltration system (Pericon Lab Cassette System, Japan Millipore, Tokyo, Japan). The substances were loaded into a high performance liquid chromatograph equipped with a reverse-phase (C\textsubscript{18}) column (Model A-312 ODS, Yamamura Chemical Laboratories, Kyoto, Japan). The platelet microsomes (1.6 g) were preincubated with PIF in 50 mM Tris-Cl, including 0.1 M NaCl, at pH 7.3. The assay was started by adding 2.5 nmol of PGH\textsubscript{2} as substrate and terminated with a 25-mM FeCl\textsubscript{2} solution. The reaction was initiated by adding 3 \times 10\textsuperscript{9} dpm of [\textsuperscript{14}C]arachidonate (50–60 mCi/mmol) and terminated using Folch’s solvent one hour after the incubation. The products were extracted with ethyl acetate, and the [\textsuperscript{14}C]thromboxane B\textsubscript{2} (TXB\textsubscript{2}) was separated using silicic acid thin-layer chromatography. The radioactivity of [\textsuperscript{14}C]TXB\textsubscript{2} was counted by a liquid scintillation counter.

The activity of TXA\textsubscript{2} synthase was measured according to the method of H. H. Tai. The platelet microsomes (3.5 g) were preincubated with PIF in 50 mM Tris-Cl, including 0.1 M NaCl at pH 7.3. The assay was started by adding 2.5 nmol of PGH\textsubscript{2} as substrate and terminated with a 25-mM FeCl\textsubscript{2} solution. The conversion rate of TXA\textsubscript{2} synthase was determined using the specific radioimmunoassay for TXB\textsubscript{2}.

Phospholipase A\textsubscript{2} activity was measured with a modification of the method of Billah et al. In our experiment, 0.2 µg of platelet microsomes and PIF were preincubated at 37°C for 30 minutes in 200 µl of 0.1 M glycine buffer (pH 8.0) containing 0.2 M NaCl, 2 mM CaCl\textsubscript{2}, and 1.25 mM deoxycholic acid sodium. The assay was initiated by adding 3 \times 10\textsuperscript{9} dpm of 1-\textsuperscript{14}C(arachidonyl)-3-phosphatidylethanolamine (50–60 mCi/mmol) and was terminated using Folch’s solvent after 30 minutes of incubation. The

Two hundred microliters of the PRP was preincubated for 15 minutes with 25 µl of Ca\textsuperscript{2+} - and Mg\textsuperscript{2+}-free Dulbecco’s physiological solution containing PIF. The aggregation was initiated by adding either 25 µl of 2.5 \times 10\textsuperscript{3} M adenosine 5’-diphosphate (ADP), 10 µg/ml collagen, 2.5 U/ml thrombin, or 2 mM arachidonate. Patterns of aggregation were recorded by an aggregometer.

Experiment 4

To assay the effects of PIF on cyclooxygenase, hydroperoxidase and TXA\textsubscript{2} synthase activities, pooled PRP was prepared from 40 eight-week-old Wistar rats. The platelets were separated from the PRP by a centrifuge at 550 g and washed twice with Ca\textsuperscript{2+} ,Mg\textsuperscript{2+}-free Dulbecco’s solution containing 1 mM EDTA. The platelets were homogenized with a Polytron blender (Kinematica, Lucerne, Switzerland) and spun under refrigeration at 1 \times 10\textsuperscript{5} g for 1 hour. Cytosol from the platelets provided the enzyme for measuring phospholipase C activity. Pellets enriched with platelet microsomes were used as the enzyme for measuring TXA\textsubscript{2} synthase, cyclooxygenase, hydroperoxidase, and phospholipase A\textsubscript{2} activity.

In order to investigate the effects of PIF on a set of enzymes involved in prostaglandin H\textsubscript{2} (PGH\textsubscript{2}) formation from arachidonate, we examined the conversion rate of [\textsuperscript{14}C]arachidonate to [\textsuperscript{14}C]TXA\textsubscript{2} by platelet microsomes. Since TXA\textsubscript{2} synthase is involved in the conversion of PGH\textsubscript{2} to TXA\textsubscript{2}, the production rate of [\textsuperscript{14}C]TXA\textsubscript{2} reflects not only the enzymatic activities of cyclooxygenase and hydroperoxidase, but also that of TXA\textsubscript{2} synthase. The platelet microsomes (1.6 g) were preincubated with PIF at 37°C for 15 minutes. The reaction was initiated by adding 3 \times 10\textsuperscript{9} dpm of [\textsuperscript{14}C]arachidonate (50–60 mCi/mmol) and terminated using Folch’s solvent one hour after the incubation. The products were extracted with ethyl acetate, and the [\textsuperscript{14}C]thromboxane B\textsubscript{2} (TXB\textsubscript{2}) was separated using silicic acid thin-layer chromatography. The radioactivity of [\textsuperscript{14}C]TXB\textsubscript{2} was counted by a liquid scintillation counter.
[\textsuperscript{14}C]arachidonate cleaved from phosphatidylcholine was extracted using Folch's solvent and separated with silicic acid thin-layer chromatography. The radioactivity of [\textsuperscript{14}C]arachidonate was determined.

Based on a method modified from that of Rittenhouse,\textsuperscript{10} the effects of PIF on phospholipase C of platelets were examined. Fifty micrograms of protein of the platelet cytosol and PIF were preincubated at 37°C for 30 minutes in 200 \( \mu \)l of 50 mM HEPES buffer (pH 7.0), which contained 0.1 M NaCl, 5 mM CaCl\(_2\), and 1.25 mM deoxycholic acid sodium. The reaction was started by adding 3 \( \times \) 10\(^4\) dpm of 1-\textit{stearyl}-2-[\textsuperscript{14}C]arachidonyl L-3-phosphatidylinositol (50–60 mCi/mmol) and terminated with Folch's solvent. The product of [\textsuperscript{14}C]diacylglycerol was extracted with Folch's solvent and separated with [\textsuperscript{14}C]phosphatidylglycerol using silicic acid thin-layer chromatography.\textsuperscript{11} The radioactivity of [\textsuperscript{14}C]diacylglycerol was measured.

### Experiment 5

To determine the effects of PIF on the production of PGE\(_2\) in cultured renal cells, kidneys were obtained from 2-week-old male Wistar rats under anesthesia (diethoxysulfonyl ether). The kidneys were minced and treated with Hanks' saline solution containing 0.1% collagenase and 0.05% hyaluronidase (pH 7.35) at 37°C under moistened 95% O\(_2\) and 5% CO\(_2\) for 1 hour.\textsuperscript{12} The isolated tubuloglomeruli were resuspended in a Coon's modified Ham's F-12 solution (pH 7.35) supplemented with heat-inactivated 10% fetal bovine serum, 15 mM HEPES-Na, 2 \( \mu \)g/ml of fibronectin, 100 U/ml of penicillin and 100 \( \mu \)g/ml of streptomycin. The renal cells that reached confluency 3 days after inoculation were incubated with PIF for 3 hours and the released PGE\(_2\) was radioimmunoassayed.

To extract substances with PIF-like activity from the kidneys of salt-loaded rats, 20 uninephrectomized 8-week-old Wistar rats were first challenged with 2% saline for 7 days. The kidneys were isolated under pentobarbital anesthesia and perfused with 10\(^4\) to 10\(^5\) daltons, using reverse-phase (C\(_{18}\)) HPLC. Inhibition of TXA\(_2\) release was found in the later fractions of the peak having a retention time of 7.19 minutes. The lower chromatogram (Figure 2A) shows the separation pattern of the plasma constituents with molecular weights of 10\(^4\) to 10\(^5\) daltons, using reverse-phase (C\(_{18}\)) HPLC. Inhibition of TXA\(_2\) release from platelets was confined to a peak with a retention time of 2.84 minutes. Figure 2B shows the chromatogram of the active fraction obtained using reverse-phase (C\(_{18}\)) HPLC by straight-phase (Diol) HPLC. Inhibition of TXA\(_2\) release was found in the later fractions of the peak having a retention time of 7.19 minutes. The lower chromatogram (Figure 2C) represents the GP-mode separation pattern for the active fraction shown in Figure 2B. Inhibition of TXA\(_2\) corresponded to the single peak with a retention time of 10.02 minutes. When the active fraction

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**Results**

**Thromboxane A\(_2\) Release from Platelets in Salt-Loaded Rats**

The 1-hour intravenous infusion of physiological saline (see Experiment 1) decreased the hematocrit from 48 ± 0.4 to 42 ± 0.5%, corresponding to an approximately 14% increase in intravascular blood volume. However, there was no significant change in the amount of TXA\(_2\) released from thrombin-activated platelets (38 ± 12 before infusion, 32 ± 8 pg/10\(^6\) platelets after infusion; NS).

When TXA\(_2\) release from thrombin-activated platelets was examined in rats subjected to chronic salt loading, the TXA\(_2\) release in Group 3 was significantly reduced compared to that in Groups 1 and 2 (Figure 1). There was no significant difference in TXA\(_2\) release between Groups 1 and 2.

**Characterization of Platelet Inhibitory Factor**

Because the results of Experiment 1 indicated that PIF appears in the circulating blood of rats subjected to chronic salt loading (i.e., rats in Group 3), extraction and purification of PIF was attempted using plasma pooled from these rats (see Experiment 2). Figure 2 depicts three chromatograms of PIF. The upper chromatogram (Figure 2A) shows the separation pattern of the plasma constituents with molecular weights of 10\(^4\) to 10\(^5\) daltons, using reverse-phase (C\(_{18}\)) HPLC. Inhibition of TXA\(_2\) release from platelets was confined to a peak with a retention time of 2.84 minutes. Figure 2B shows the chromatogram of the active fraction obtained using reverse-phase (C\(_{18}\)) HPLC by straight-phase (Diol) HPLC. Inhibition of TXA\(_2\) release was found in the later fractions of the peak having a retention time of 7.19 minutes. The lower chromatogram (Figure 2C) represents the GP-mode separation pattern for the active fraction shown in Figure 2B. Inhibition of TXA\(_2\) corresponded to the single peak with a retention time of 10.02 minutes. When the active fraction

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**FIGURE 1.** Thromboxane A\(_2\) (TXA\(_2\)) release from thrombin-activated platelets in sham-operated rats given tap water (open bar), uninephrectomized rats given tap water (shaded bar), and uninephrectomized rats given 2% saline (solid bar). Uni-NX = uninephrectomy.
FIGURE 2. Chromatograms made by HPLC for platelet inhibitory factor (PIF) and a calibration curve for molecular weight (MW) estimation made by gel-permeation (GP-mode) HPLC. 
A. Reverse-phase (C18) chromatogram showing separation pattern of the plasma constituents having molecular weights of $10^3$ to $10^6$ daltons. B. Subsequent Diol straight-phase chromatogram of the active fraction of C18 chromatogram. C. Separation pattern illustrated by a GP-mode chromatogram for the active fraction of the Diol chromatogram. The solid bars under the base lines show the sites with PIF activity. The graph in chromatogram C shows the relationship between retention time and molecular weight depicted on the GP-mode chromatogram. Points on the graph represent 1) human serum albumin (66,000); 2) soybean trypsin inhibitor (20,100); 3) aprotinin (6512); 4) parathyroid hormone (human 69-84, 1717). The open circle between 3 and 4 represents PIF.

obtained by GP-mode HPLC was superimposed on the standard curve of the same chromatogram, the molecular weight of the PIF was estimated to be about 4300 daltons. The eluate corresponding to the single peak of the GP-mode chromatogram was lyophilized and stored at $-70^\circ$C. Two hundred milligrams of PIF were obtained from about 400 ml of plasma. The partially purified PIF was dissolved in Dulbecco’s saline solution for assay.

When 30 mg of the lyophilized PIF was incubated with 10 mg of trypsin at 37°C for 30 minutes, PIF activity was reduced to equal that of 16 mg of PIF (a 46% reduction).

As shown in Figure 3, PIF that was finally purified by GP-mode HPLC decreased dose-dependently the TXA$_2$ released from thrombin-activated platelets of normal Wistar rats. Furthermore, the PIF fraction obtained by reverse-phase (C18) HPLC significantly decreased the release of prostaglandins E$_2$ and D$_2$ (PGE$_2$ and PGD$_2$) from the platelets (Table 1).

The effects of PIF on the aggregation of human platelets were also examined (see Experiment 3). The PIF significantly decreased platelet aggregation induced by 25 $\mu$M ADP, 10 $\mu$g/ml collagen, and 2.5 U/ml thrombin, while not affecting aggregation induced by 5 mM arachidonate (Table 2).

**Effects of Platelet Inhibitory Factor on Enzymes**

To clarify the inhibitory mechanisms of PIF affecting TXA$_2$ release from platelets, the effects of PIF on various enzymes involved in the metabolism of arachidonate in platelets were examined (see Experiment 4). First, the effects of PIF on the liberation of arachidonate were explored. As shown in Figure 4, PIF at concentrations of 2 to 50 mg/ml reduced the activities of phospholipase A$_2$ and phospholipase C in a dose-dependent manner. Second, because the conversion of arachidonate to TXA$_2$ is modulated by the enzymatic system that includes cyclooxygenase, hydroperoxidase, and TXA$_2$ synthase, the effects of PIF on the conversions of arachidonate and PGH$_2$ were examined separately. As shown in Table 3, PIF at concentrations of 2 to 50 mg/ml did not affect the conversions of [1$^4$C] arachidonate or PGH$_2$ to TXA$_2$.

**TABLE 1. The Effects of Platelet Inhibitory Factor on the Release of PGE$_2$ and PGD$_2$ from Thrombin-Activated Platelets**

<table>
<thead>
<tr>
<th>Platelet inhibitory factor</th>
<th>PGE$_2$ (pg/10$^6$ platelets)</th>
<th>PGD$_2$ (pg/10$^6$ platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/ml</td>
<td>17.7 ± 0.09 (5)</td>
<td>0.71 ± 0.08 (5)</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>5.4 ± 1.2* (8)</td>
<td>0.02 ± 0.17* (8)</td>
</tr>
</tbody>
</table>

PGE$_2$ and PGD$_2$ were measured by radioimmunoassay. Values are means ± SE. Numbers in parentheses represent the number of studies.

*<p<0.001 vs control.
TABLE 2. The Effects of Platelet Inhibitory Factor on Human Platelet Aggregation

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Control</th>
<th>Platelet inhibitory factor (50 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP, 25 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{50}$ (min)</td>
<td>0.40 ± 0.01 (5)</td>
<td>0.56 ± 0.04* (5)</td>
</tr>
<tr>
<td>Max (%)</td>
<td>69 ± 3.6</td>
<td>21 ± 4.2*</td>
</tr>
<tr>
<td>Collagen, 10 µg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{50}$ (min)</td>
<td>0.62 ± 0.2 (4)</td>
<td>— (4)</td>
</tr>
<tr>
<td>Max (%)</td>
<td>66 ± 0</td>
<td>—</td>
</tr>
<tr>
<td>Thrombin, 2.5 U/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{50}$ (min)</td>
<td>0.62 ± 0.06 (3)</td>
<td>— (3)</td>
</tr>
<tr>
<td>Max (%)</td>
<td>74 ± 4.0</td>
<td>—</td>
</tr>
<tr>
<td>Arachidonate, 2 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{50}$ (min)</td>
<td>0.66 ± 0.1 (3)</td>
<td>0.65 ± 0.04 (3)</td>
</tr>
<tr>
<td>Max (%)</td>
<td>63 ± 2.3</td>
<td>74 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses represent the number of studies. $T_{50}$ = time required to reach 50% of maximum aggregation. Max = the ratio of optical absorbance at the maximum aggregation to the optical absorbance of platelet-poor plasma. *p < 0.01 versus control.

TABLE 3. Effects of Platelet Inhibitory Factor on Cyclooxygenase, Hydroperoxidase, and Thromboxane A2 Synthase of Platelets

<table>
<thead>
<tr>
<th>Platelet inhibitory factor (mg/ml)</th>
<th>Generation rate $[^14]C$TXA2 $(10^{-9} \text{ dpm/g protein/hr})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiled microsomes + $[^14]C$AA</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.57 ± 0.07 (5)</td>
</tr>
<tr>
<td>2</td>
<td>15.7 ± 2.3 (5)</td>
</tr>
<tr>
<td>10</td>
<td>17.6 ± 1.3 (5)</td>
</tr>
<tr>
<td>50</td>
<td>13.7 ± 2.0 (5)</td>
</tr>
<tr>
<td>50</td>
<td>15.8 ± 1.9 (5)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses represent the number of studies. $[^14]C$AA = $[^14]C$arachidonate; TXA2 = thromboxane A2.

Figure 4. The effects of platelet inhibitory factor (PIF) on the cleavages of $[^14]C$arachidonate from $[^14]C$phosphatidylcholine (phospholipase A2 activity [PLA2]) and $[^14]C$diacylglycerol from $[^14]C$phosphatidylinositol (phospholipase C activity [PLC]).

Effects of Platelet Inhibitory Factor on PGE2 Production in Renal Tissue

To assess the possible action of PIF on tissues other than platelets, we explored the effects of PIF on the production of PGE2 in renal cells in primary culture (see Experiment 5). The release of PGE2 from the cells was significantly reduced by adding PIF to the incubation medium (Figure 5).

Demonstration of Platelet Inhibitory Factor–Like Activity in Renal Extracts

Since our previous study indicated that the kidneys of Dahl S rats were related to PIF activity in plasma, we attempted to extract PIF from the kidneys of rats that had been uninephrectomized and given 2% saline for a week (see Experiment 5). Renal extracts having molecular weights of $10^3$ to $10^6$ daltons were obtained by ultrafiltration through membrane sieves. These extracts were found to reduce the release of TXA2 from thrombin-activated platelets (Figure 6).

Discussion

The present study demonstrated that a combination of chronic excessive salt intake and a maneuver that diminishes the renal capacity to excrete sodium might be necessary to produce a humoral factor that inhibits the release of TXA2 from platelets. The release of TXA2 from thrombin-activated platelets was reduced in rats uninephrectomized and given 2% saline for 7 days as compared with rats uninephrectomized and given tap water. Acute salt-loading did not produce a significant rise in amounts of inhibitory factor.

We attempted to extract the plasma factor, which was tentatively termed PIF, from plasma pooled from uninephrectomized rats that had been subjected to chronic salt loading. The factor was present in the plasma fraction having molecular weights from $10^3$ to
In order to clarify the inhibitory mechanisms of PIF, the effects of the partially purified PIF on the enzymes involved in the generation of TXA$_2$ in platelets were examined. The PIF decreased the activities of phospholipases A$_2$ and C in a dose-dependent manner, but it did not seem to affect a set of enzymes related to the late steps of arachidonate metabolism. That phospholipases A$_2$ and C may be targets for PIF activity was supported by findings that 1) PIF diminished the release of PGE$_2$ and PGD$_2$, and TXA$_2$ from thrombin-activated platelets and 2) it reduced the aggregation of human platelets induced by ADP, collagen, and thrombin, but not that induced by arachidonate. Arachidonate induces platelet aggregation by directly stimulating TXA$_2$ synthesis, while ADP, collagen, and thrombin accelerate the aggregation by activating phospholipases to make arachidonate available. Thus, PIF is considered to have a unique ability to inhibit phospholipases.

It has been reported that endogenous inhibitors for phospholipases occur in rabbit neutrophils, guinea pig lung, and rat renal medulla. However, the inhibitors in these tissues are produced and released in response to glucocorticoid treatment and have molecular weights of 1.5 x 10$^6$ to 4 x 10$^6$ daltons, which are more than four times the molecular weight of PIF. Accordingly, it seemed quite possible that the PIF extracted from uninephrectomized, salt-loaded rats is distinct from these endogenous inhibitors and is a new substance that has not yet been described.

The present study indicated that PIF obtained from the circulating blood of uninephrectomized rats subjected to chronic salt loading diminished the production of prostaglandins in platelets and in renal tissue by affecting phospholipases. Thus, PIF probably has various effects on many tissues and organs, just as prostaglandins do. Its effects on platelets and kidney cells may be just one aspect of a broad range of actions, in which case the factor will have to be renamed. Further studies are needed to clarify and assess fully the biological roles of PIF.

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