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DEMONSTRATION OF PLATELET ACTIVATING FACTOR RECEPTOR IN GUINEA PIG KIDNEY

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Distribution of platelet activating factor (PAF) receptor was examined in the guinea pig kidney. Northern blot analysis showed a single band electrophoresed just below the 28S rRNA, and the mRNA was richest in the cortex with lesser amounts in the outer and then inner medulla. Scatchard analysis of membrane fraction using [^{3}H]WEB 2086, a specific PAF receptor antagonist, revealed a single binding site with Bmax of 522, 228, 58 fmol/mg protein for the cortex, outer medulla and inner medulla, respectively. Kd values were in the same order of magnitude (10^{-8} M). These results indicate the presence of a single class of PAF receptor in the guinea pig kidney which is most abundant in the cortex.

Platelet activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) has a wide range of biological activities (1, 2), including renal functions (3-5). Infusion of PAF into the renal artery induces renal hemodynamic changes (6). PAF contracts mesangial cells (7) and has direct effects on tubular cells (8-10). PAF also seems to play a role in the pathogenesis of nephrotoxic nephritis (11), nephrotic syndrome (12), endotoxemic acute renal failure (13), and cyclosporine-induced nephrotoxity (14). Although these data and others suggest that PAF exerts

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Abbreviations: PAF, platelet activating factor; PMSF, phenyl methylsulfonyl fluoride; and BSA, bovine serum albumin.

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biological activities through a specific membrane receptor (7, 11-14), information on the occurrence and distribution of PAF receptor in the kidney is limited. Binding parameters obtained using a radiolabeled ligand have apparently not been reported.

Using as a probe an isolated cDNA clone, we obtained direct evidence that PAF receptor mRNA is the most abundant in the cortex of the kidney, followed by outer medulla, then the inner medulla. The distribution of mRNA parallels the Bmax values of [^{3}H]WEB 2086 binding in these portions, suggesting the significant pathophysiological roles for PAF in the kidney.

MATERIALS AND METHODS

Poly $(A)^+$ RNA extraction from guinea pig kidney slices. Male guinea pigs weighing 350-450 g (Nippon Bio-supp. Center, Tokyo) were anesthetized by intraperitoneal injection of sodium phentobarbital (50 mg/kg). Both kidneys were perfused with 40-60 ml saline containing 1 mM EDTA from the bifurcation of the descending aorta. The inferior vena cava was cut for drainage. When the perfusate became transparent, both kidneys were removed. Sagittal slices of 1-2 mm width including the papilla was prepared using an autoclaved razer blade and further divided into three portions, namely, the cortex, outer medulla and inner medulla. The boundary between the cortex and outer medulla was determined by arcuate arteries, and the outer and inner medullae were distinguished by color (outer medulla, pale brown; inner medulla, white). Total RNA of each portion was obtained by the guanidium thiocyanate method (15), and poly $(A)^+$ RNA was obtained using an mRNA Purification Kit (Pharmacia).

Northern blot analysis. Poly $(A)^+$ RNA (20 μg each) was loaded on a 1 % agarose-formaldehyde gel. After electrophoresis, RNA was transferred onto a nylon filter (Biodyne, Pall). After baking for 2 hours at 80°C, the filter was prehybridized for 4 hours in a hybridization solution containing 0.2 mg/ml salmon sperm DNA, 10 x Denhardt's solution, 0.5 % SDS, 100 mM NaCl, 50 mM Tris-HCl (pH 8.0) and 10 mM EDTA. A *Sma* I-*Sma* I fragment of the coding region of guinea pig PAF receptor cDNA (16) was radiolabeled with [α-^{32}P]dCTP (111 TBq/mmol, Du Pont) using a Multiprime DNA Labeling System (Amersham) and used as a probe. The filter was hybridized with the probe in the hybridization solution overnight at 65°C. Then, the filter was washed sequentially with 3 x SSC, 0.1 % SDS for 2 min at room temperature, with 3 x SSC, 0.1 % SDS at 65°C for 30 min and finally twice with 0.2 x SSC, 0.1 % SDS for 20 min at 65°C. The dried filter was exposed to an X-ray film (Fuji, RX) at -70°C for 60 hours in a cassette with an intensifying screen. β-Actin was hybridized in essentially the same manner using a *Hinf* I-*Hinf* I fragment of human β-actin cDNA (17). The intensity of each band was quantified with a high speed TLC Scanner CS-920 (Shimadzu, Kyoto).

Preparation of membrane protein from guinea pig kidney. Three portions of guinea pig kidney slices were obtained as described above. All procedures were carried out at 0-4°C. The slices were minced in five volumes of 25 mM HEPES (pH 7.4)/0.25 M

sucrose/1 mM EDTA/0.2 mM PMSF and homogenized using a Polytron homogenizer for 1 min at the top speed. The homogenate was centrifuged at 10,000 x g for 10 min and the supernatant was further centrifuged at 45,000 x g for 15 min. The pellet was suspended in 25 mM HEPES (pH 7.4)/1 mM EDTA/0.2 mM PMSF and mixed gently using a Potter-Elvehjem homogenizer, followed by centrifugation at 45,000 x g for 15 min. The pellet was suspended in an appropriate volume of the same buffer and used as the membrane fraction. Protein concentration was determined by the method of Bradford (18) using BSA as standard.

[^{3}H]WEB 2086 binding assays. Binding reaction was initiated by adding 100 μg of guinea pig kidney membrane protein to a final volume of 200 μl of HEPES/BSA buffer (25 mM HEPES, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM PMSF, 0.1 % BSA, pH 7.4), containing [^{3}H]WEB 2086 (521.7 GBq/mmol) at the indicated concentrations. Nonspecific binding was determined in the presence of 10 μM unlabeled WEB 2086. The mixtures were incubated at 25^{o}C for 90 min, and bound and free radioligands were separated by rapid filtration through Whatman GF/C glass fibre filters, which were immediately washed with ice-cold washing buffer (HEPES 25 mM, $MgCl_2$ 10 mM, 0.1 % BSA, pH 7.4). After drying the filters at 80 ^{o}C, the bound radioactivity was determined by liquid scintillation counting with 4 ml of toluene scintillator (19). Binding experiments of the cortex and outer medulla were conducted twice in triplicate while that of inner medulla was done twice in duplicate. [^{3}H]WEB 2086 was purchased from Du Pont. Unlabeled WEB 2086 was kindly donated by Boehringer Ingelheim KG (Ingelheim).

RESULTS

Northern blot analysis. Northern blot analysis revealed a single band electrophoresed just below the 28S rRNA (Fig. 1). Size of the mRNA in three segments of the kidney was the same. The intensity of the band was highest in the cortex with a lesser amounts in the outer, then inner medulla. The ratios of intensity of the PAF receptor/β-actin in the cortex, outer medulla,

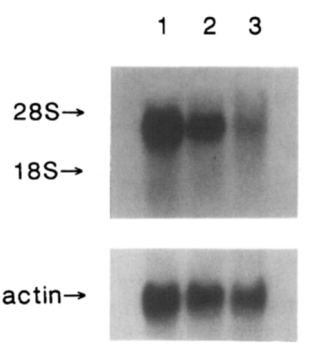

Fig.1. Northern blot analysis of the PAF receptor in guinea pig kidney slices. Lane 1, cortex; lane 2, outer medulla; and lane 3, inner medulla. Each lane contains 20 μg poly(A)$^+$ RNA.

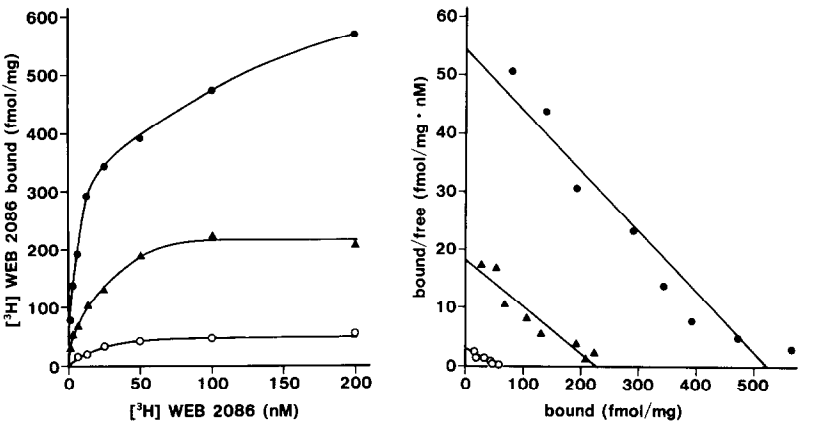

Fig.2. Equilibrium binding of [^{3}H]WEB 2086 to guinea pig kidney membranes. a. Specific binding isotherms. b. Scatchard plots. Data on cortex (●) and outer medulla (▲) are means of two experiments conducted in triplicate and data on the inner medulla (○) are means of two experiments done in duplicate.

inner medulla were 3.46, 1.22, 0.32, respectively (relative ratio, 10.8: 3.8: 1.0) (Table 1).

Binding Assay. The specific binding isotherm of [^{3}H]WEB 2086 to the guinea pig kidney slices are shown in Fig. 2a. Scatchard analysis of the saturation isotherm of each portion gave a straight line suggesting a single class of binding sites (Fig. 2b). Kd values of the cortex, outer medulla, and inner medulla were 9.6 (mean, n = 2), 12.6 , and 20.0 nM, respectively. Bmax values of these three portions were 522, 228, and 58 fmol/mg protein, respectively (relative ratio, 9.0: 3.9: 1.0). Comparisons of these binding parameters with the relative mRNA abundance are summarized in Table 1.

DISCUSSION

PAF appears to play various pathophysiological roles in renal function, and pharmacological evidence suggested the presence of PAF receptor in the kidney (7, 11-14). The present study was an attempt to obtain direct evidence for the PAF receptor in the kidney and to know its rough distribution. We have already cloned PAF receptor cDNA from the guinea pig lung as the

TABLE 1. Distribution of PAF receptor in guinea pig kidney slices

	mRNA (ratio)[b]	Bmax (ratio)[b]	Kd
		fmol/mg	nM
Cortex	3.46[a] (10.8)[b]	522 (9.0)[b]	9.6
Outer medulla	1.22[a] (3.8)[b]	228 (3.9)[b]	12.6
Inner medulla	0.32[a] (1.0)[b]	58 (1.0)[b]	20.0

a. The intensity ratio of PAF receptor/β-actin (Fig. 1) was determined by gel scanning.
b. The relative ratio was calculated, when values at inner medulla were defined as 1.0. Bmax and Kd values were obtained from Fig. 2.

first successful example of lipid autacoid receptor cloning (16). Using this cDNA as a probe, we demonstrated the presence of PAF receptor mRNA in guinea pig kidney slices. By Northern blot analysis, a single clear band was observed (Fig. 1) after intensive washing under stringent conditions. Size of the mRNA was comparable to that noted in the guinea pig lung (16). The relative amounts of mRNA paralleled the Bmax values obtained by Scatchard analysis (Fig. 2 and Table 1). These results, taken together, indicate that the band in Fig. 1 represents the PAF receptor mRNA. A similar size of mRNA and binding parameters suggests that the kidney contains a single class of PAF receptor.

This receptor was richest in the cortex of the kidney, in area where the blood flow is most abundant. The result reminds us that the various PAF actions in the kidney are through modulating renal hemodynamics including glomerular filtration (3-5). In this respect, as the physiological experiments suggested (7), the mesangial cells might be one of the main targets of PAF in the kidney. Other components such as proximal tubular cells (20) or cortical collecting tubules (10) may be sources of PAF receptor in the cortex. *In situ* hybridization and immunohistochemical analysis are ongoing to determine the exact cellular localization of the PAF receptor.

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