SULFATIDE IN THE KIDNEY: HOW IS THIS LIPID INVOLVED IN SODIUM CHLORIDE TRANSPORT?

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1. Introduction

In vertebrates, both sulfatide (galactosyl-3-sulfate ceramide) and the ouabain-sensitive ($Na^+ + K^+$)-dependent ATPase (EC 3.6.1.3) have a highly localized distribution. Concentrated in brain tissues, they are also found in organs specialized in active sodium transport, e.g. salt glands of cartilaginous fish and marine birds or mammalian kidney medulla [1]. A direct link between the amount of sulfatide and of the ($Na^+ + K^+$)-dependent ATPase has been elegantly demonstrated by Karlsson who observed that in domestic ducks adapted to hypertonic saline, both the concentration of sulfatide and the ATPase activity, in their hypertrophied supraorbital gland, increased by 200% within a week [2]. A similar correlation was established by Umeda et al. [3] in the mouse during the compensatory renal hypertrophy consecutive to unilateral nephrectomy. The enzymatic activity of the ATPase has been shown to reflect closely the transport of sodium across the plasma membrane [4]. The strong and unique correlation between sulfatide concentration and metabolism and ($Na^+ + K^+$)-dependent ATPase activity led these authors to suggest that sulfatide may be a part of the sodium-potassium translocation unit [1–3].

We wish to demonstrate, by means of immunohistochemistry, that the specific location of sulfatide is topologically different from that of the ($Na^+ + K^+$)-ATPase. Furthermore, the enzymatic activity is unmodified by the presence of antisulfatide antibodies.

2. Experimental

2.1. *($Na^+ + K^+$)-ATPase assay*

(Na^+–K^+) dependent ATPase was determined by a modification of the radiometric method of Blostein [5]. Rabbit kidneys were separated in cortex and medulla. The cortex was further fractionated in glomeruli and tubules as previously described [6,7]. Each fraction was preincubated for 15 min at 37°C with the antiserum to sulfatide diluted 1/10. Controls were run by substituting, for the antiserum, either Tris–HCl buffer pH 7.4 or normal rabbit serum diluted 1/10. Each sample was assayed in the presence or absence of ouabain 0.3 mM (Sigma). The incubation mixture contained: Tris–HCl buffer pH 7.4 50 mM, KCl 20 mM, NaCl 150 mM and $MgCl_2$ 5 mM in a final volume of 0.5 ml. The substrate (3.6 mM ATP containing 0.05–0.1 μCi (γ–^{32}P) ATP) was then added and the incubation proceeded for 15 min at 37°C. The reaction was stopped by adding 0.5 ml of charcoal suspension (2.5 g in 20 ml 0.8% sodium dodecyl sulfate (SDS)). In order to avoid hydrolysis of ATP at acid pH, SDS was employed rather than the commonly used trichloracetic acid. Thus, in one step, the reaction was stopped and the nucleotides adsorbed on charcoal. The suspension was shaken and then centrifuged (4000 rev/min × 5 min). The supernatant was removed and the sedimented charcoal was resuspended in 1 ml of water and centrifuged. The radioactivity of an aliquot (1 ml) of the pooled supernatants (2 ml) was

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measured in 10 ml of Instagel (Packard) in an Intertechnique SL 30 scintillation counter. The blank values, obtained by incubating boiled homogenates, were substracted. The (Na^+–K^+) dependent ATPase activity was obtained by the difference in between the radioactivity measured in the absence and in the presence of ouabain.

2.2. *Sulfatide determination*

Sulfatide was assayed by the method of Kean [8], after saponification [9] and separation on TLC, in the solvent system: chloroform–acetone–methanol acetic acid–water (50:20:10:10:5, by vol.), of the glycolipid fraction, following Vance and Sweeley [10]. General conditions have been described before [11].

2.3. *Antiserum to sulfatide*

Antisulfatide antiserum was obtained by repeated intravenous injections of a mixture of sulfatide–lecithin–cholesterol–methylated bovine serum albumin in the respective ratio 0.1:0.4:1:1 (mg/ml) [12]. Assayed in the complement fixation test, with sulfatide, the titer of this antiserum was 256. Antiserum to galactosyl and glucosyl ceramide was prepared similarly by injecting a mixture of hapten–cholesterol–human serum albumin (0.2:1:4, mg/ml). Pure antibodies were prepared, by immunoadsorbtion following Coulon-Morelec's procedure [13]. Pure anti glucuronosyl diglyceride antibodies were a generous gift of Dr M. J. Coulon-Morelec (Pasteur Institute).

2.4. *Indirect immunofluorescence*

For indirect immunofluorescence, the use of antibodies purified on an immunoadsorbant was required in order to circumvent non specific staining [14]. The cortex, outer medulla and inner medulla were dissected from the kidney of a rat. The frozen sections of each block, cut at 8 μm in a cryostat, were fixed in cold 10% buffered formaldehyde. After fixation, the sections were washed 4 × 5 min in phosphate buffered saline pH 7.2 (PBS), incubated 60 min with pure anti-sulfatide antibodies (diluted 1 : 2), washed 4 × 5 min in PBS, incubated 30 min in the dark, with a solution of goat fluorescein isothiocyanate conjugated globulin–anti-rabbit IgG (Pasteur Institute) diluted 1 : 50 (containing Evans blue diluted 1/10 000) washed 4 × 5 min in PBS and mounted in buffered glycerol. Sections were then examined on a Leitz Orthomat fluorescent microscope with a Ploem^R system for epi-illumination. Control of the specificity of the staining was performed by incubating adjacent sections with either pure anti-glucuronosyl diglyceride antibodies, or by omitting anti-sulfatide antibodies.

3. Results

3.1. *Influence of antisulfatide antiserum on ($Na^+ + K^+$)-ATPase activity*

We first determined the sulfatide content on the ATPase activity in the four renal subfractions studied [7]. As shown in table 1, the sulfatide concentration

Table 1

Effect of antisulfatide antiserum on the activity of the ($Na^+ + K^+$)-dependent ATPase of rabbit kidney

	Sulfatide concentration[a] (nmol per 100 mg of dry weight)	($Na^+ + K^+$)-dependent ATPase specific activity[b] (μmol of $^{32}P_i \cdot h^{-1} \cdot$mg or protein $^{-1}$)		
		Control	Normal rabbit serum	Antisulfatide antiserum
Glomeruli	8 ± 2 (3)	1.6	1.9	1.3
Cortex	13 ± 5 (4)	8.6	8.7	9.2
Tubules	27 ± 8 (3)	8.8	10.3	10.9
Medulla	104 ± 41 (5)	10.4	10.1	10.7

[a] Figures in brackets are the number of independent experiments. Values are means ± SD
[b] Values are means of triplicate experiments

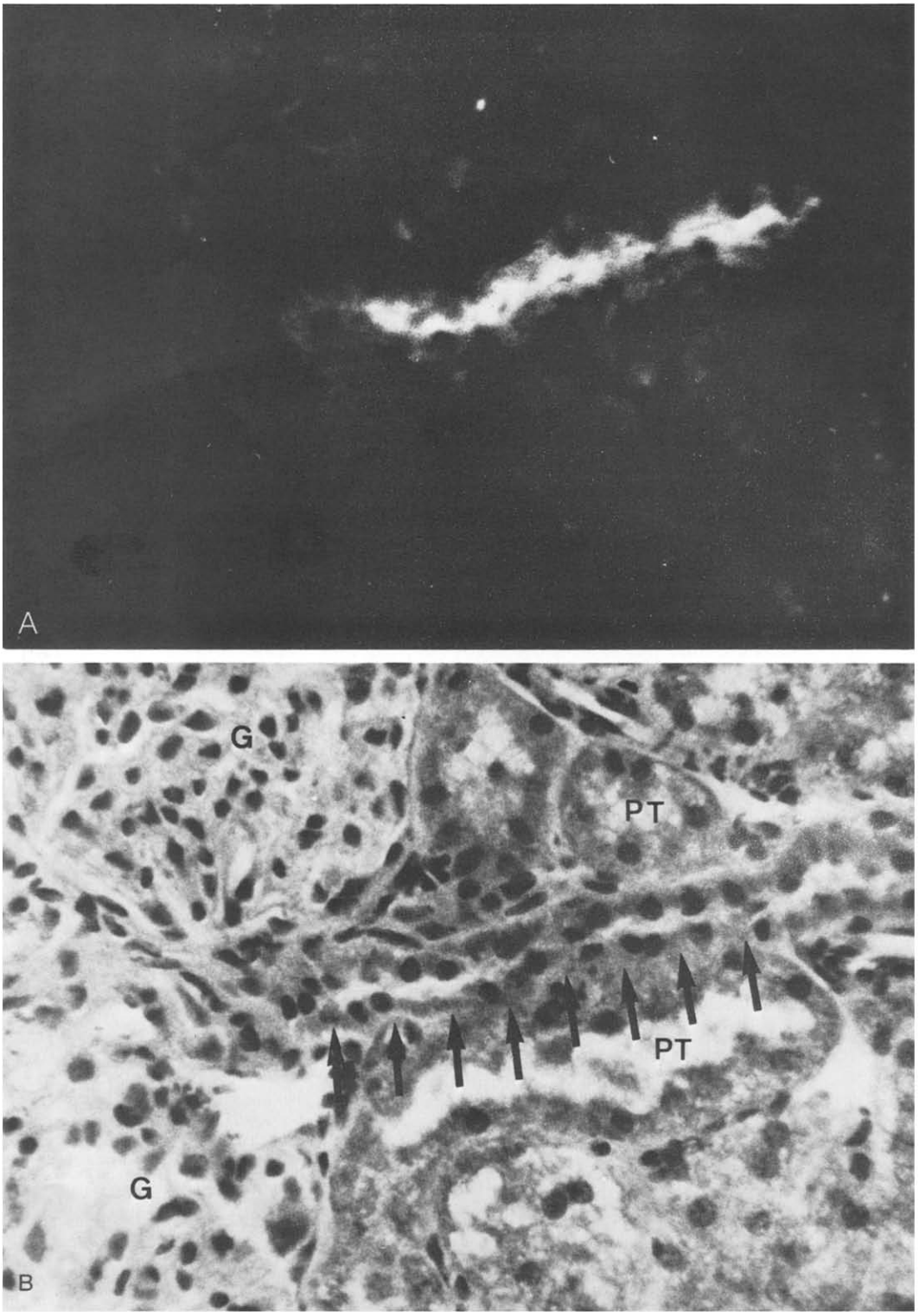
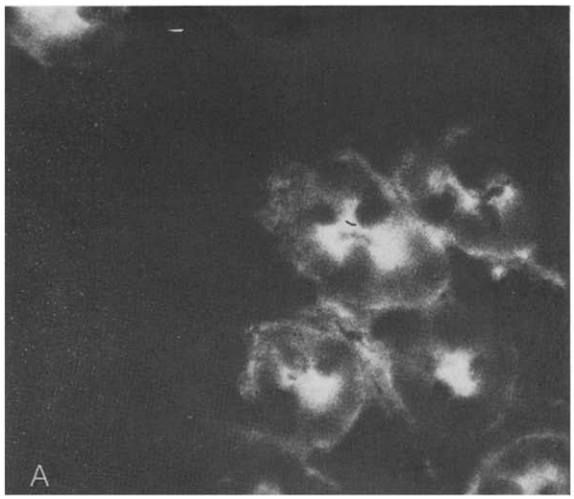


Fig. 1

increases steeply in these fractions, i.e. from the glomeruli to the cortex, the tubules and the medulla. A far less significant modification of the $(Na^+ + K^+)$-dependent ATPase activity is evident; however, there is a striking elevation in specific activity in the cortex, the tubules and the medulla, relative to that in the glomeruli. Pre-incubation of each four renal subfraction with antisulfatide antiserum (or with normal rabbit serum as control) did not modify the specific activity values of the ouabain sensitive $(Na^+ + K^+)$-ATPase (table 1). In order to eliminate the possibility that, under these conditions, kidney sulfatide was not accessible to the antibodies, we have determined, by complement fixation, the percentage of antibodies bound to each fraction in our ATPase assay medium: 18% were bound to the glomeruli, 30% to the cortex, 51% to the tubules and 59% to the medulla.



3.2. *Localization of sulfatide*

Figure 1 shows that in the cortex fluorescence was located only on narrow diameter, rectilineal structures in contact with the glomerulus. These can be either the very initial part of distal tubules or the cortical thick ascending limb of the loops of Henle. In any case, both are near-identical: i.e. possess the same ultrastructure (as reviewed by Oswaldo-Delima [15] and an identical sensitivity to hormones [16]. Neither the proximal tubules nor the glomeruli were stained, as objectivized by the adjacent section stained with haematoxylin eosin (fig.1B). In the outer medulla (fig.2A), the thick ascending limbs of the loops of Henle were fluorescent, but the collecting tubules were negative (fig.2B). No fluorencence could be seen in the inner medulla. Furthermore, in the stained tubules the fluorescence was selectively localized on the luminal membrane of the cells. This contrasts with the uniform staining observed, when identical sections were incubated with either pure antigalactocerebroside or antiglucocerebroside antibodies. The control sections incubated with fluorescent globulins alone were negative. Those incubated with pure antibodies against glucuronosyl diglyceride (a synthetic

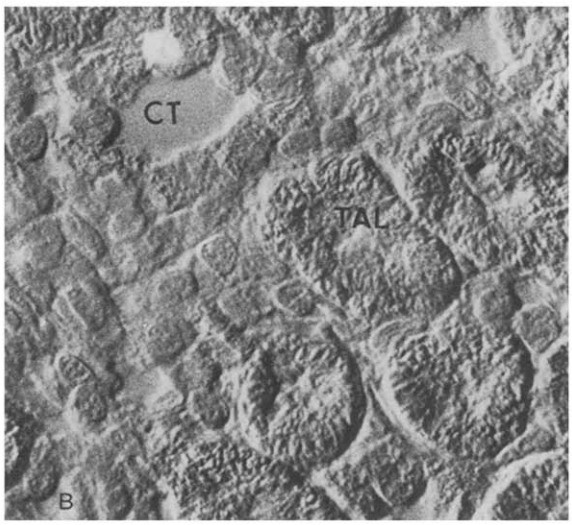


Fig.2. Localization of sulfatide on cryostat sections of rat renal outer medulla. (A) Indirect immunofluorescent staining by pure antibodies against sulfatide. In (B) the same section has been photographed with an achromatic light using a Nomarski interference contrast optic, set on the same microscope [21]. CT, collecting tubule. TAL, thick ascending limb of the loop of Henle (× 450).

Fig.1. Localization of sulfatide on cryostat sections of rat renal cortex. (A) indirect immunofluorescent staining by pure antibodies against sulfatide. (B) haematoxylin eosin staining of the adjacent section. G, glomerulus; PT, proximal tubule; arrows indicate the fluorescent tubule, cut longitudinally (× 500).

lipid absent from renal tissue) showed only faint fluorescence of the basal membrane.

4. Discussion

The results of Karlsson et al. [2] on the salt glands or the one by Umeda et al. [3] on the kidney implicated the sulfatide in sodium chloride transport. The absence of inhibition of the $(Na^+ + K^+)$-ATPase by the antisulfatide antibodies, together with a specific localization of sulfatide, on the luminal membrane of the thick ascending limb of the loop of Henle, rule out the possibility that this lipid might be an element of the sodium pump, since it is now well established that this enzymatic complex is part of the antiluminal membrane [17]. This is in accordance with the recent finding that the purified $(Na^+ + K^+)$-ATPase reconstituted in vesicles made of lecithin, as single lipid, catalyses fully the ouabainsensitive active Na^+ transport coupled to active K^+ transport [18,19]. Physiological studies on isolated renal tubules have shown that, in the thick ascending limb of the loop of Henle, the primary overall ion movement is net NaCl reabsorption, from the lumen of the tubules into the interstitial space, rather than the active exchange of Na^+ and K^+ [20]. We propose that sulfatide, in this structure of the nephron, is implicated in the passive diffusion of sodium chloride from the lumen of the tubule into the interstitial space.

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