ELSEVIER

Comparative Biochemistry and Physiology, Part B 140 (2005) 487–495

CBP

www.elsevier.com/locate/cbpb

# Metabolism of sulfolipids in isolated renal tubules from rat

Ken-ichi Nagai[a], Keiko Tadano-Aritomi[a], Naoko Iida-Tanaka[a,b], Hideki Yoshizawa[c], Ineo Ishizuka[a,*]

[a]*Department of Biochemistry, Teikyo University School of Medicine, 2-11-1, Kaga, Itabashi, Tokyo 173-8605, Japan*
[b]*Department of Food Science, Otsuma Woman's University, 12, Sanbancho, Chiyoda, Tokyo 102–8357, Japan*
[c]*Department of Biology, Matsumoto Dental College, 1780, Gobara Hirooka, Shiojiri, Nagano 399-0871, Japan*

Received 27 August 2004; received in revised form 16 November 2004; accepted 17 November 2004

**Abstract**

Proximal-rich tubules were prepared from rat kidneys by using collagenase treatment. The isolated rat renal tubules were compared with the intact kidney on the following characteristics. (1) Composition of the sulfoglycolipid. (2) Sulfoglycolipid metabolism based on incorporation of [$^{35}$S]sulfate or some properties of sulfoglycolipid metabolism, including the activities of anabolic and catabolic enzymes. The results indicated following characteristics of the isolated renal tubules in comparison to the kidney in vivo. (1) The sulfoglycolipid compositions are qualitatively similar, except that the content of glucosyl sulfatide, $Gg_3Cer$ II$^3$-sulfate, and GM4 was slightly higher in the isolated tubules. (2) The apparent half-lives (15–55 min) of sulfoglycolipids in the isolated tubules could indicate the existence of a rapid turnover pool of these lipids. (3) The sulfotransferase and sulfatase activities related to sulfoamphiphiles in the renal tubule were similar to those reported for the whole kidney. Based on the above criteria, we conclude that the isolated rat renal tubule should be a useful metabolic system for clarification of the short-term physiological events, up to 90 min, of proximal tubular sulfoglycolipids. By using the present system, we showed that biosynthesis of the renal total sulfoglycolipid was significantly elevated in rats deprived of water for 24 h.


*Keywords:* Cholesterol sulfate; Collagenase; Dehydration; Half-life; LSIMS; Metabolism; Rat; Renal tubules; Sulfoglycolipids

## 1. Introduction

Sulfated amphiphiles, including sulfoglycolipids and cholesterol 3-sulfate ($HSO_3$-Chol), are components of the cell membrane of the animals of deuterostome lineage, echinoderms to vertebrates (Ishizuka, 1997). Among various tissues of mammals, the kidney is one of the organs most enriched in these sulfolipids. Previous studies, based on the whole animal (Karlsson, 1982), whole kidney (Umeda et al., 1976), or renal epithelial cell lines (Niimura and Ishizuka, 1991), suggested that sulfated amphiphiles play specific roles in the maintenance of renal ionic homeostasis, although the operating biochemical mechanisms remain to be clarified (see Rev. Ishizuka, 1997). Wirthensohn and Guder (1990) reported that tubular suspensions prepared from rat kidneys by treatment with collagenase could be one of the systems suitable to study the renal metabolism. This system is not only

*Abbreviations:* Chol, cholesterol; d18:1, 4-sphingenine; GalCer, galactosylceramide; GD3(NeuAc/NeuAc), II$^3$-α-NeuAcα8NeuAc-lactosylceramide; GM1a, II$^3$-α-NeuAc-gangliotetraosylceramide; GM3(NeuAc), II$^3$-α-NeuAc-lactosylceramide; GM4(NeuAc), I$^3$-α-NeuAc-GalCer; $HSO_3$-Chol, cholesterol 3-sulfate; Lyso-SM4g, lyso seminolipid; NeuAc, *N*-acetylneuraminic acid; PAPS, 3′-phosphoadenylyl sulfate; SB1a, gangliotetraosylceramide II$^3$,IV$^3$-*bis*-sulfate; SB2, gangliotriaosylceramide II$^3$,III$^3$-*bis*-sulfate; SM2a, gangliotriaosylceramide II$^3$-sulfate; SM3, lactosyl sulfatide, LacCer II$^3$-sulfate; SM4s, galactosyl sulfatide, GalCer I$^3$-sulfate; SM4s-Glc, glucosyl sulfatide, GlcCer I$^3$-sulfate; SM4s-h, SM4s with hydroxy fatty acid; SM4s-nh, SM4s with nonhydroxy fatty acid; t18:0, 4-hydroxysphinganine. Abbreviations for lipids follow those of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (IUPAC-IUB Joint Commission on Biochemical Nomenclature, 1999) and the symbols for sulfoglycolipids follow the system of Ishizuka (Ishizuka, 1997).

* Corresponding author. Tel.: +81 3 3964 3749; fax: +81 3 5375 6366.
*E-mail address:* ii@med.teikyo-u.ac.jp (I. Ishizuka).

1096-4959/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2004.11.013

convenient to process a large number of experiments simultaneously but also allows pretreatment of rats, in vivo, with hormones and drugs, or exposure to various environmental conditions, including salt-, water-load, and dehydration.

The purpose of our present study is to establish a useful organ culture system for clarification of the functions of sulfolipids in renal tubules. To establish the validity of the present system, we compared the composition and metabolism of sulfolipids of isolated tubules with those of the whole rat kidney. Using this system, we showed that the synthesis of the total sulfoglycolipid was significantly elevated in the tubules, when the rats had been deprived of water for 24 h.

## 2. Materials and methods

### 2.1. Materials

Male 6-week-old Wistar rats (*Rattus norvegicus*, 150–180 g body mass) were purchased from SEASCO (Saitama, Japan) and fed with standard rat chow and tap water ad libitum. For in vivo dehydration experiment, rats were water-deprived for 24 h. The present study was carried out in accordance with the Teikyo University Guide for the Care and Use of Laboratory Animals, accredited by the Japanese Ministry of Education, Culture, Sports, Science and Technology. Every effort was taken to minimize any pain or discomfort of animals used in experiments.

Reference gangliosides and sulfoglycolipids were prepared as previously described (Nagai et al., 1989). $HSO_3$-Chol, 2-hydroxypropyl-β-cyclodextrin, BSA essentially free of fatty acids, BSA-palmitic acid complex, 3′-phosphoadenylyl sulfate (PAPS), bovine brain galactosylceramide (GalCer), collagenase (type II from *Clostridium histolyticum*), 4-nitrocatechol, *p*-nitrocatechol sulfate (2-hydroxy-5-nitrophenyl sulfate), 4-methylumbelliferone, and 4-methylumbelliferyl sulfate were purchased from Sigma-Aldrich Japan, Tokyo, Japan. Carrier-free $H_2^{35}SO_4$ and [$^{35}$S]PAPS (59.2 GBq/mmol) were obtained from DuPont NEN Research Products, Wilmington, DE, USA. Sep-Pak Plus C18 cartridges (360 mg of sorbent, Waters, Tokyo, Japan) were prewashed sequentially with 10 mL portions of $CHCl_3$/$CH_3OH$/ $H_2O$ (30:60:8, by vol.), $CH_3OH$, water, 0.1 M KCl; and Sep-Pak Plus $NH_2$ (360 mg of sorbent) with 30 mL of water, 10 mL of $CH_3OH$, and 10 mL of $CHCl_3$/$CH_3OH$/$H_2O$ (30:60:8, by vol.).

### 2.2. Preparation of renal tubules from rat

Tubules were isolated from rat kidneys according to the method of Guder (Guder et al., 1971; Guder, 1979) with slight modifications. Briefly, kidneys (1.2–1.4 g/animal) were removed from rats sacrificed under light ether anesthesia and the renal cortices cut out and minced with scissors. The tissue "brei" (0.8–1.0 g), prepared by forcing the above tissue pieces through a piece of nylon mesh (aperture 512 μm), was freed from tissue debris by washing three times with cold sulfate-free Krebs–Henseleit medium in which $NaHCO_3$ and $MgSO_4$ were replaced with triethanolamine–HCl buffer (Guder et al., 1971) and $MgCl_2$, respectively. The pelleted "brei" was digested with collagenase (1700 U/g brei in 10 mL) in the above medium under pure oxygen gas phase with vigorous shaking (37 °C for 45 min). After the reaction was terminated by the addition of 2 vol. of the ice-cold medium, the supernatant transferred to another tube, and centrifuged at 10×*g* for 3 min to separate the tubules from subcellular particles and cell debris. Finally, the tubules were washed twice in the centrifuge and resuspended in a fresh medium (4 mL/g brei, corresponding to 15–20 mg protein). This preparation was used immediately for extraction of lipids, [$^{35}$S]sulfate incorporation experiments as below, or stored at −80 °C for enzyme assay.

### 2.3. Protein quantitation

The protein concentrations were determined using a modified method of Wang and Smith (1975). To eliminate the interference of triethanolamine in the Krebs–Henseleit medium, the tubules were washed three times with 0.9% NaCl before protein assay (Peterson, 1983). The renal tubules together with control BSA was solubilized in 1 M NaOH by heating (80 °C, 1 h) prior to protein assay.

### 2.4. Incorporation of [$^{35}$S]sulfate into renal tubular sulfolipids

The tubular suspension (0.1 mL, up to 0.5 mg protein) was transferred to a 15 mL polypropylene tube with a cap and incubated with the sulfate-free Krebs–Henseleit medium (final vol., 1 mL) containing 370 kBq of carrier-free $H_2^{35}SO_4$ and 5 mM glucose at 37 °C with gentle shaking under an $O_2$ gas phase. Incubations were stopped by chilling in ice water at the indicated time, and then 0.9 mL of the suspensions was transferred to a microcentrifuge tube and tubules collected by short centrifugation (10,000×*g* for 5 s; Microfuge B, Beckman, Palo Alto, CA, USA).

The extraction procedure for total lipids from the isolated tubules using mixtures of chloroform/methanol/water was similar to those described previously (Tadano and Ishizuka, 1982a; Nagai et al., 1984; Iida et al., 1989). In order to remove essentially all glycerophospholipids, the total lipid extract was treated with 0.2 M NaOH in methanol and neutralized. The fraction of total acidic lipids was prepared as follows. The crude alkali-resistant lipids were suspended in 1 mL of 0.1 M KCl by brief sonication and transferred to a C18 cartridge with the help of a reservoir. The residual lipids in the tube were washed with two 2 mL portions of 0.1 M KCl and applied to the cartridge (Nagai and Ishizuka, 1987). After eluting nonlipid compounds with 30 mL of