

Effect of nutritional substrate on sulfolipids metabolic turnover in isolated renal tubules from rat

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Abstract: Effects of a glycolytic (glucose) and a gluconeogenic renal nutritional substrate (glutamine) on metabolic turnover of sulfolipids, determined as [³⁵S]sulfate incorporation, were compared in renal tubules prepared from well-fed rats. The results showed that the effects of glucose and glutamine, at nearly physiological serum concentration, are quite contrary to each other. Glucose increased the turnover rates of relatively long chain ganglio-series sulfoglycolipids (Gg₃Cer II³-sulfate and Gg₄Cer II³,IV³-bis-sulfate) (1.7 to 2.4-fold), but not of cholesterol 3-sulfate (0.9-fold). In contrast, glutamine accelerated the turnover rates of relatively short chain sulfoglycolipids (glucosyl sulfatide, galactosyl sulfatide and lactosyl sulfatide) (1.3 to 2.7-fold), as well as cholesterol 3-sulfate (2.4-fold). The possible mechanism which causes these marked differences is also discussed.

Keywords: cholesterol 3-sulfate, gluconeogenic and glycolytic substrates, proximal tubules, sulfoglycolipids, well-fed rats

Introduction

Sulfoglycolipids²⁾ and cholesterol 3-sulfate (HSO₃-Chol)^{2)–4)} are components of the cell membrane of the animals of deuterostome lineage, echinoderms to vertebrates. However, little is known about the biological function of these acidic lipids *in vivo*. We reported recently that isolated renal tubules prepared by collagenase treatment

from rat kidney could serve as a tool for clarification of the physiological role of the proximal tubular sulfated amphiphiles.⁵⁾ In this previous study, we observed that addition of gluconeogenic substrates to the medium significantly increased sulfate incorporation into HSO₃-Chol, whereas that markedly suppressed the incorporation into longer chain sulfoglycolipids.⁵⁾ These findings aroused our concern to investigate the effects of nutritional substrates in the medium on metabolism of sulfolipids in isolated renal tubules.

The purpose of the present study is to compare the effects of a glycolytic (glucose) and a gluconeogenic substrate (glutamine) on metabolic turnover of sulfoamphiphiles in renal tubules prepared from rats fed *ad libitum*.

Materials and methods

Materials. Male, 6-week-old Wistar rats (150–180 g body weight) were purchased from SEASCO (Saitama, Japan) and fed with standard rat chow and tap water *ad libitum*. 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

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‡ deceased. We dedicate this article to the memory of Prof. Ishizuka.

Abbreviations: GalCer-h: galactosylceramide with hydroxy fatty acid; HSO₃-Chol: cholesterol 3-sulfate; PAPS: 3'-phosphoadenylyl sulfate; SB1a: gangliotetraosylceramide II³,IV³-bis-sulfate; SB2: gangliotriaosylceramide II³,III³-bis-sulfate; SM2a: gangliotriaosylceramide II³-sulfate; SM3: lactosyl sulfatide; SM4s: galactosyl sulfatide; SM4s-Glc: glucosyl sulfatide; SM4s-h: SM4s with hydroxy fatty acid; SM4s-nh: SM4s with non-hydroxy fatty acid. Abbreviations for lipids follow those of the IUPAC-IUB Joint Commission on Biochemical Nomenclature¹⁾ and the symbols for sulfoglycolipids follow the system of Ishizuka.²⁾

and Technology. Every effort was taken to minimize any pain or discomfort of animals used in experiments.

Collagenase (type II from *Clostridium histolyticum*) was purchased from Sigma Aldrich Japan, Tokyo, Japan. Carrier-free $\text{H}_2^{35}\text{SO}_4$ was obtained from DuPont NEN Research Products, Wilmington, DE, USA. BondElut C18 cartridges (100 mg of sorbent, Varian, Harbor City, USA) were prewashed sequentially with 1 ml portions of CH_3OH , H_2O , 0.1 M KCl and BondElut DEA (100 mg of sorbent) with 1 ml of H_2O and CH_3OH . Sulfate-free modified Krebs-Henseleit medium was made by replacing NaHCO_3 and MgSO_4 with triethanolamine-HCl buffer and MgCl_2 respectively.⁵⁾

Preparation of renal tubules from rat. Proximal-rich tubules were isolated from rat kidneys according to the method of Guder^{6,7)} with slight modifications as described.⁵⁾ Briefly, kidneys were removed from rats sacrificed under light ether anesthesia and the renal cortices cut out and minced with scissors. The minced tissue was digested with collagenase (1,700 U/g minced tissue in 10 ml) in the sulfate-free Krebs-Henseleit medium under pure oxygen gas phase with vigorous shaking (37°C for 45 min). The dispersed tubules were washed in centrifuge and resuspended in a fresh medium. This preparation was used immediately for [^{35}S]sulfate incorporation experiments as below.

Protein quantitation. Protein was determined by the modified method⁸⁾ of Bradford⁹⁾ using bovine serum albumin as the standard. The renal tubules together with control BSA was solubilized in 1 M NaOH by heating (80°C, 1 h) prior to protein assay.

Incorporation of [^{35}S]sulfate into renal tubular sulfolipids. The tubular suspension (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 $\text{H}_2^{35}\text{SO}_4$ and the renal substrate (glucose or glutamine) at 37°C for 90 min with gentle shaking under O_2 gas phase. Incubations were stopped by chilling in ice water, and then 0.9 ml of the suspensions were transferred to a microcentrifuge tube and tubules collected by short centrifugation.⁵⁾

The extraction procedure for total lipids from the isolated tubules using mixtures of CHCl_3 /

$\text{CH}_3\text{OH}/\text{H}_2\text{O}$ was similar to those described.¹⁰⁻¹²⁾ In order to remove essentially all glycerophospholipids, the total lipid extract was treated with 0.2 M NaOH in CH_3OH 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. The residual lipids in the tube were washed with two 2 ml portions of 0.1 M KCl, and applied to the cartridge.⁵⁾ After eluting non-lipid compounds with 3 ml of H_2O , the outlet of the C18 cartridge was connected tandem to the inlet of a DEA cartridge. The lipids adsorbed on the C18 cartridge were eluted with 2 ml of CH_3OH . Finally, the acidic lipids retained on the DEA cartridge were procured by elution with 1 ml of 3% ammonia water in CH_3OH and dried under nitrogen flow. An aliquot of the alkali-resistant total acidic lipids was used to determine the radioactivity by a liquid scintillation counter. The rest was separated by high-performance TLC (Merck, Darmstadt, Germany) in a solvent system, $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{CH}_3\text{COCH}_3/\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ (7:2:4:2:1, by vol.) and the distribution of incorporated radioactivities analyzed using BAS-1500 Bioimaging Analyzer (Fuji Film, Tokyo, Japan) (Fig. 1). The incorporation of [^{35}S]sulfate into the individual sulfoamphiphiles increased linearly up to approximately 0.7 mg of tubular protein and 120 min of incubation.⁵⁾

By addition of the ^{35}S -sulfated lipid mixture of known composition and radioactivity to the total lipid extract, the overall recovery (%) of individual sulfated lipids in this assay system was calculated as follows (mean \pm SD, $n = 6$): $\text{HSO}_3\text{-Chol}$ (80.9 ± 7.2); glucosyl sulfatide (SM4s-Glc) (73.9 ± 5.2); galactosyl sulfatide with non-hydroxy fatty acid (SM4s-nh) (73.7 ± 7.1) and with hydroxy fatty acid (SM4s-h) (67.4 ± 6.8); lactosyl sulfatide (SM3) (85.5 ± 6.6); gangliotriaosylceramide II^3 -sulfate (SM2a) (69.6 ± 7.5); gangliotriaosylceramide $\text{II}^3, \text{III}^3$ -bis-sulfate (SB2) (75.3 ± 8.5); gangliotetraosylceramide II^3, IV^3 -bis-sulfate (SB1a) (58.1 ± 7.4). Compared with our previous study,⁵⁾ the recovery of sulfolipids, especially long-chain sulfoglycolipids, was greatly improved by using BondElut (58.1–85.5%) instead of SepPAK (17.5–56.8%). This improvement could be attributed to the difference in amounts of sorbent in these cartridges, which are 100 mg in BondElut and 360 mg in SepPAK.

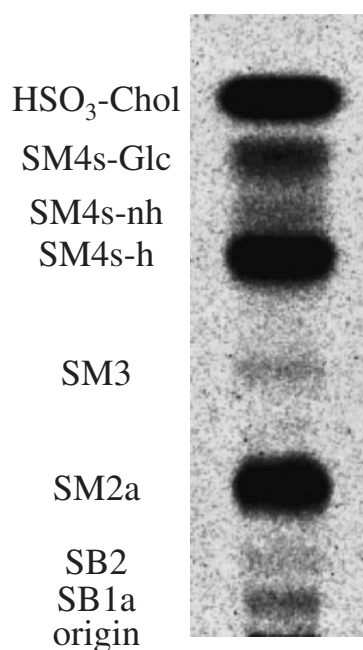


Fig. 1. TLC of renal tubular ^{35}S -sulfolipids. Renal tubular sulfolipids were labeled by ^{35}S -sulfate in the presence of 5 mM glucose as a renal substrate. The incubation condition and purification procedures of alkali-stable acidic lipids were described in Materials and methods. Sulfolipids were separated on TLC with the solvent system $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{CH}_3\text{COCH}_3/\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ (7:2:4:2:1, by vol.) and imaged using BAS-1500 Bioimaging Analyzer.

Incorporation of ^{35}S -sulfate into individual sulfolipids was corrected for the above recovery.

For all figures, means and standard deviations were calculated. Statistical comparison of the two means was performed using unpaired Student's *t*-test.

Results

Effect of a glycolytic renal substrate, glucose, on incorporation of ^{35}S -sulfate into tubular sulfolipids. Fig. 2A shows dose dependency of glucose-induced sulfate incorporation into tubular sulfolipids. At subphysiological serum concentrations, 1.25 to 2.5 mM, the curves could be classified into two groups, a high-glucose-sensitive and a low-glucose-sensitive. The former was characterized by two ganglio-series sulfolipids, SM2a and SB1a, and a major renal sulfolipid, SM4s-h. The latter consisted of relatively short sugar chain lipids, SM4s-nh, SM3, and a sulfated endproduct of SM2a, i.e. SB2. These stimulative effects were saturated at the concentrations higher

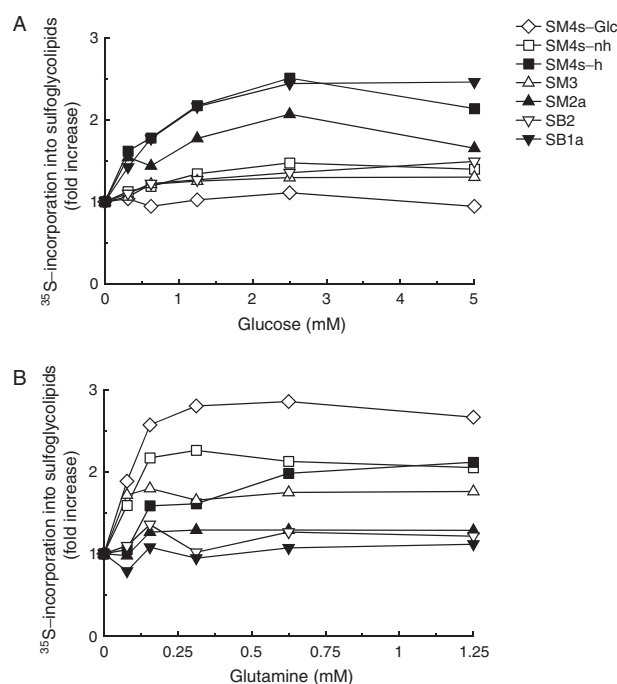


Fig. 2. A glycolytic (glucose, A) and a gluconeogenic (glutamine, B) renal substrate concentration dependencies of the incorporation of ^{35}S -sulfate into tubular sulfolipids. The assay procedures were described in Materials and methods. Each point represents the means of more than four experiments.

than 2.5 mM. On the other hand, glucose had little effect on sulfate incorporation into SM4s-Glc at all concentrations determined. These results were confirmed in another set of our experiment where effect of glucose (5 mM) on sulfate incorporation into sulfolipids was examined (Table 1, ratio 1). Glucose at the nearly physiological serum concentration (5 mM) significantly stimulated sulfate incorporation into all sulfolipids except SM4s-Glc, among which the stimulatory effect was much more prominent in SM4s-h, SM2a and SB1a (Table 1, ratio 1).

Effect of a gluconeogenic renal substrate, glutamine, on incorporation of ^{35}S -sulfate into tubular sulfolipids. As shown in Fig. 2B, effect of a gluconeogenic substrate, glutamine, on sulfolipids turnover was a striking contrast to that of glucose (Fig. 2A). Sulfate incorporation into SM4s-Glc, which had not been affected by glucose, was stimulated most prominently by glutamine at the concentrations higher than 0.156 mM. Incorporation into SM4s-nh, which classified to the low-

Table 1. Effects of renal substrates at nearly physiological serum concentration on ^{35}S -incorporation into tubular sulfolipids

Sulfolipids	Cont ^{a)} (no substrate) (n = 26)	Glc ^{a)} (5 mM) (n = 6)	Gln ^{a)} (0.625 mM) (n = 12)	Glc/Cont (ratio 1)	Gln/Cont (ratio 2)	Gln/Glc (ratio 3)
HSO ₃ -Chol	9,080 ± 1,810	7,880 ± 2,130	21,300 ± 6,180	0.87	2.35*** ^{c)}	2.70*** ^{d)}
SM4s-Glc	910 ± 198	854 ± 132	2,480 ± 812	0.94	2.73***	2.90***
SM4s-nh	311 ± 57	431 ± 59	607 ± 181	1.39** ^{b)}	1.95***	1.41**
SM4s-h	988 ± 263	1,860 ± 327	1,810 ± 475	1.88***	1.83***	0.97
SM3	90 ± 31	135 ± 18	119 ± 46	1.50***	1.32	0.88
SM2a	436 ± 114	777 ± 209	502 ± 154	1.78**	1.15	0.65*
SB2	74 ± 21	123 ± 26	80 ± 21	1.66**	1.08	0.65**
SB1a	199 ± 100	467 ± 192	178 ± 44	2.35*	0.89	0.38*
Total sulfoglycolipids	3,010 ± 667	4,650 ± 859	5,780 ± 1,510	1.54**	1.92***	1.24
Total sulfolipids	12,100 ± 2,280	12,500 ± 2,970	27,100 ± 7,400	1.03	2.24***	2.17***

^{a)}Cont, control; Glc, glucose; Gln, glutamine. Values (dpm/mg/90 min) are mean ± SD. Concentrations of glucose and glutamine were nearly physiological levels of well-fed rats.²³⁾

^{b)}Glc is significantly different from Cont (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

^{c)}Gln is significantly different from Cont (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

^{d)}Gln is significantly different from Glc (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

glucose-sensitive group (Fig. 2A), also markedly increased in the presence of glutamine as a renal substrate. On the other hand, little effect was observed for SM2a and SB1a which belong to the high-glucose-sensitive group. These results were confirmed in Table 1 (ratio 2 and ratio 3).

Effect of glucose and glutamine on incorporation of [^{35}S]sulfate into tubular cholesterol 3-sulfate. Similar to the sulfoglycolipids, the effect of gluconeogenic substrate (glutamine) on incorporation of sulfate into HSO₃-Chol was quite different from that of the glycolytic substrate (glucose) (Fig. 3). Glutamine (up to 0.625 mM) dramatically increased the sulfate incorporation into HSO₃-Chol, whereas glucose had little effect at all concentrations examined (Fig. 3 and Table 1).

Discussion

In the present study, we compared for the first time the effects of glycolytic (glucose) and gluconeogenic (glutamine) renal nutritional substrates, at nearly physiological concentrations, on the metabolic turnover of sulfolipids in the isolated rat renal proximal tubules. The results clearly indicated that the effect of glucose and glutamine is fundamentally different. Scheme 1 summarizes our working hypothesis, explanations for which are described below (1) to 3)).

1) It has been reported that anaerobic glycolytic activity of renal cortical slices from well-fed

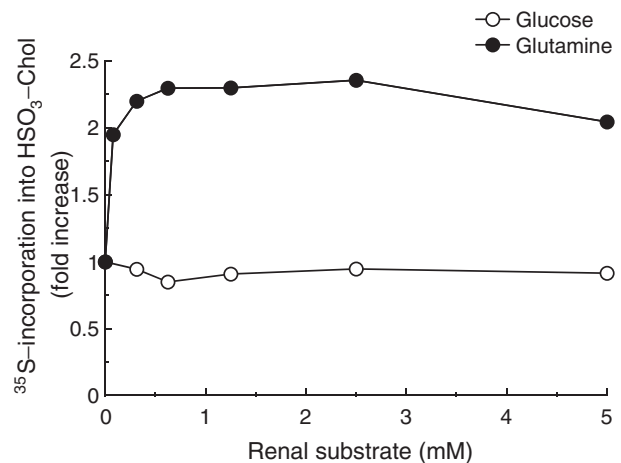


Fig. 3. A glycolytic (glucose) and a gluconeogenic (glutamine) renal substrate concentration dependencies of the incorporation of [^{35}S]sulfate into tubular cholesterol 3-sulfate (HSO₃-Chol). The assay procedures were described in Materials and methods. Each point represents the means of more than four experiments.

animals is relatively low,^{13),14)} whereas aerobic metabolism of glucose in the cortices is high (see Rev.¹⁵⁾). According to their results, it is speculated that glucose could hardly be converted to cholesterol by anaerobic glycolysis via acetyl-CoA and mevalonic acid. The remaining glucose, which was not utilized by aerobic metabolism or not converted to the other intermediates, could change into UDP-sugars via glucose 6-p, glucose 1-p and

It should be also noted that whether a single enzyme, GalCer sulfotransferase (CST), catalyzes the synthesis of various sulfoglycolipids. Honke *et al.*^{21,22)} demonstrated that the experimentally produced CST-deficient mouse generates no sulfated glycolipids in the brain and testis. This conclusively suggests that a single enzyme, CST, is responsible for sulfation of all glycolipids in rat renal tubules.

In the present assay system, the incubation time (90 min) was too short to up-regulate the expression of enzyme proteins, i.e. tubular glycosyltransferases and/or sulfotransferases. Therefore, metabolic turnover of renal tubular sulfoamphiphiles in this system should principally be affected by metabolic fate of nutritional substrates.

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