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Effect of acetazolamide on lithium reabsorption and lithium-induced GSK3β phosphorylation in rat kidney

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Lithium promotes the phosphorylation of glycogen synthase kinase-3β (GSK3β), and this reaction protects against acute kidney injury mediated by renal apoptosis. Lithium is considered to be reabsorbed by sodium-phosphate cotransporters and sodium-proton exchanger NHE3. This study evaluated the relation between the lithium reabsorption and the phosphorylation of GSK3β, by using acetazolamide, an NHE3 inhibitor. In rats infused with lithium chloride, the plasma concentration of lithium was 4.77 mEq/l, and the renal clearance of lithium and its fractional excretion were calculated to be 2.29 ml/min/kg and 0.405, respectively. Coadministration of acetazolamide decreased creatinine clearance and the reabsorption rate of lithium, increased the fractional excretion of lithium, and did not affect its plasma concentration. Western blot analysis exhibited the facilitation of GSK3β phosphorylation in the kidney cortex by lithium infusion, and acetazolamide inhibited the lithium-induced phosphorylation of GSK3β. Lithium did not affect GSK3β phosphorylation in the liver and did not affect Akt in the kidney cortex and liver. These data show that lithium reabsorption contributes to GSK3β phosphorylation in the kidney cortex.

1. Introduction

Glycogen synthase kinase-3β (GSK3β), a serine/threonine protein kinase, has a role in many cellular processes including gene transcription, translocation, cytoskeletal organization, and cell cycle progression (Doble and Woodgett 2003). Lithium, used for the treatment of bipolar disorder, inhibits GSK3β *via* two modes (Jope 2003). Lithium is a competitive inhibitor of magnesium in GSK3β (Ryves and Harwood 2001). In addition, the phosphorylation of serine in its N-terminal region, Ser9 of GSK3β, reduces its activity (McManus et al. 2005), and the amount of phosphorylated GSK3β (p-GSK3β) is increased by lithium (Jope 2003). GSK3β is responsible for apoptosis in the kidney (Havasi and Borkan 2011). Renal proximal tubule-specific GSK3β knockout mice showed resistance to mercuric chloride-induced renal injury (Howard et al. 2012). Inhibitors for GSK3β including lithium exhibited renoprotective effects on acute kidney injury caused by diclofenac, cisplatin, ischemia, and lipopolysaccharide in rats and mice (Bao et al. 2012, 2014; Talab et al. 2012; Wang et al. 2009).

The kidney plays an important role in the elimination of lithium from plasma. The renal clearance of lithium (Li CL_r) is approximately 25 % of creatinine clearance (C_{cr}), which suggests that 75 % of lithium filtered through the glomeruli is reabsorbed (Finley et al. 1995). The proximal tubule is the main site for the lithium reabsorption (Thomsen et al. 1968). Previously, we showed that foscarnet and parathyroid hormone, inactivators for sodium-phosphate cotransporters, increased the fractional excretion of lithium (a ratio of Li CL_r to C_{cr}) in rats intravenously injected with lithium chloride at 3 mg/kg or 2.5 mg/kg as a bolus, suggesting that sodium-phosphate cotransporters contribute to the reabsorption of lithium (Uwai et al. 2014, 2018). And, we showed no response of the lithium reabsorption to foscarnet in rats injected with lithium chloride at 25 mg/kg (Uwai et al. 2018). These findings imply that lithium is reabsorbed by another transporter as well as sodium-phosphate cotransporters in rats. It has been considered that sodium-proton exchanger NHE3 is responsible for lithium reabsorption (Timmer et al. 1999).

In this study, we evaluated the effect of acetazolamide, known as a NHE3 inhibitor (Krishnan et al. 2015), on the renal excretion of lithium in rats, and examined GSK3β phosphorylation in the kidney cortex of the rats infused with lithium chloride and acetazolamide by Western blotting. And, the effects of lithium administration on the amount of p-GSK3β in the liver and the phosphorylation of Akt were assessed.

2. Investigations, results and discussion

Rats were randomly divided into four groups: rats infused with saline, rats infused with acetazolamide, rats infused with lithium chloride, and rats infused with lithium chloride and acetazolamide. The pharmacokinetic parameters of lithium are summarized in the Table. In rats administered lithium chloride alone, the plasma concentration of lithium (Li C_p) was 4.77±0.12 mEq/l, and Li CL_r and the fractional excretion of lithium were calculated to be 2.29±0.16 ml/min/kg and 0.405±0.040, respectively. No effect of acetazolamide was recognized on Li C_p and Li CL_r, and its coadministration decreased C_{cr} and the rate of the lithium reabsorption. Acetazolamide elevated the fractional excretion of lithium to 0.872±0.046, indicating that acetazolamide inhibited the reabsorption of lithium. Protein binding of lithium is negligible in plasma (Price et al. 1994), and tubular secretion of lithium has not been reported. Therefore, the complete inhibition of the lithium reabsorption increases the fractional excretion of lithium up to one. This study showed a potent inhibition of lithium reabsorption by acetazolamide, and this report is the first to show the almost maximum increase in the fractional excretion of lithium, to our knowledge.

Figure 1 represents the results of Western blot analysis for p-GSK3β and GSK3β using protein prepared from the kidney cortex of rats infused with lithium chloride and acetazolamide. The infusion of lithium chloride increased the amount of p-GSK3β, and simultaneous administration of acetazolamide reduced the band intensity of p-GSK3β. Statistical analysis showed no difference between saline group and acetazolamide group. Acetazolamide decreased the reabsorption rate of lithium without affecting Li C_p (Table), suggesting that lithium reabsorption stimulates the phosphorylation of GSK3β in the kidney cortex.

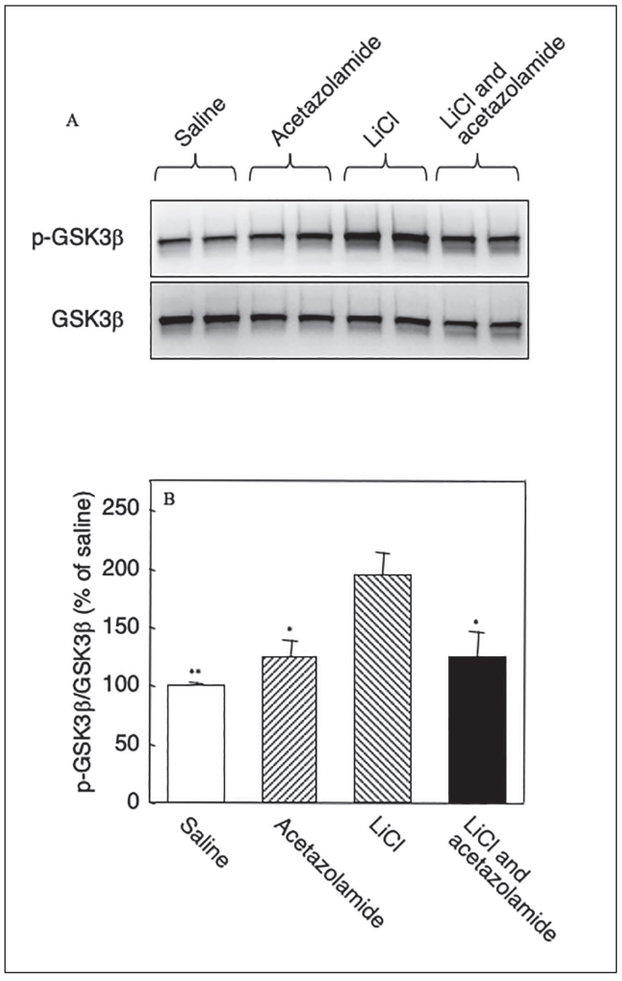

Fig. 1: Phosphorylation of GSK3β in the kidney cortex of rats infused with lithium chloride and acetazolamide. Rats were infused with saline in the absence or presence of 1 % lithium chloride or 1.1 % acetazolamide sodium at 0.03 ml/min for 2 h. (A) Protein (20 μg) from the kidney cortex of each group was separated by SDS-PAGE and blotted onto a PVDF membrane. p-GSK3β and GSK3β were identified by their respective antibodies. (B) The ratio of the density of p-GSK3β to that of GSK3β was determined in each rat and calculated as a % of the saline group. Each column represents the mean ± SE of 6 rats. * $P < 0.05$; ** $P < 0.01$; vs the LiCl group.

Table: Pharmacokinetic parameters of lithium in rats infused with lithium chloride and the effects of acetazolamide

Parameter	LiCl	LiCl and acetazolamide
N	6	6
Weight (g)	205 ± 4	204 ± 4
P_{cr} (mg/dl)	0.625 ± 0.031	0.733 ± 0.024*
C_{cr} (ml/min/kg)	5.76 ± 0.34	3.07 ± 0.26***
Li C_p (mEq/l)	4.77 ± 0.12	5.15 ± 0.27
Li CL_r (ml/min/kg)	2.29 ± 0.16	2.66 ± 0.23
Fractional excretion of Li	0.405 ± 0.040	0.872 ± 0.046***
Reabsorption rate of Li (μEq/min)	3.43 ± 0.47	0.430 ± 0.172***

Each parameter represents the mean ± SE. *, $P < 0.05$; ***, $P < 0.001$; vs the LiCl group.

Phosphorylation of GSK3β in the liver was examined of other rats infused with lithium chloride by Western blotting. As shown in Fig. 2, lithium chloride increased the amount of p-GSK3β in the kidney cortex, but no increase was observed in the liver. This supports our previous finding that lithium was hardly excreted into bile in rats (Uwai et al. 2015).

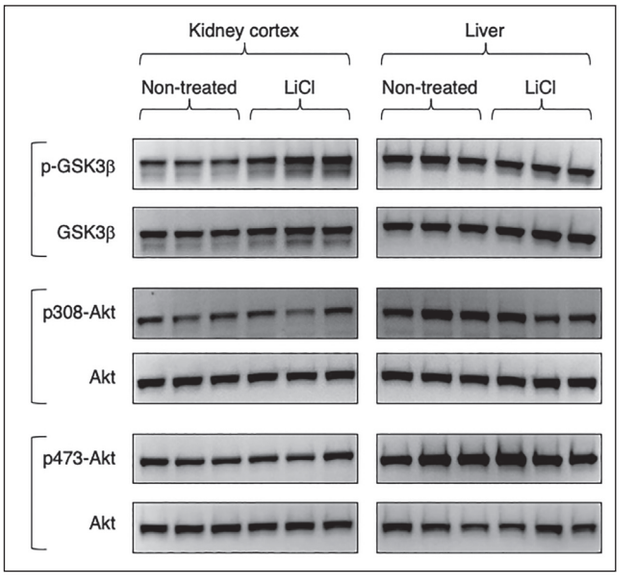

Fig. 2: Phosphorylation of GSK3β and Akt in the kidney cortex and liver of rats infused with lithium chloride. Rats were infused with 1 % lithium chloride at 0.03 ml/min for 2 h. Protein (15 μg) from the kidney cortex or liver of each group of rats was separated by SDS-PAGE and blotted onto a PVDF membrane. p-GSK3β, GSK3β, p308-Akt, p473-Akt, and Akt were identified by their respective antibodies.

It is accepted that activated Akt, which is phosphorylated at Thr308 or Ser473, mediates GSK3β phosphorylation (Downward 1998). However, the mechanisms by which lithium induces GSK3β phosphorylation remain unclear. In this study, the activated forms of Akt, p308-Akt and p473-Akt, were investigated. But lithium had no effect (Fig. 2), which suggests that Akt is not involved in lithium-induced phosphorylation of GSK3β in the rat kidney cortex. Lithium facilitated GSK3β phosphorylation without increasing p473-Akt in human neuroblastoma SH-SY5Y cells (De Sarno et al. 2002). Meanwhile, increases in p308-Akt, p473-Akt, and p-GSK3β occurred in the inner medullary collecting duct after 1 or 2 weeks of lithium treatment in rats (Nielsen et al. 2008). It is not clear whether activated Akt mediated the phosphorylation of GSK3β in the rats. Lithium administration may initially facilitate GSK3β phosphorylation by an unknown mechanism, and long-term administration of lithium may accelerate Akt phosphorylation, which results in an increase in p-GSK3β.

In conclusion, this study explains the effect of acetazolamide on the renal handling of lithium in rats. And, the reabsorption of lithium was shown to promote the phosphorylation of GSK3β in the rat kidney cortex. These findings provide useful information in developing a protection strategy for acute kidney injury based on GSK3β.

3. Experimental

3.1. Materials

Lithium chloride and mannitol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Acetazolamide sodium (Diamox®) was from Sanwa Kagaku Kenkyusho (Nagoya, Japan).

3.2. Pharmacokinetic experiments in rats

Animals were treated in accordance with the regulations of the Institutional Animal Use and Care Committee of the School of Pharmacy, Aichi Gakuin University. Seven-week-old male Wistar/ST rats were from Chubu Kagaku Shizai (Nagoya, Japan). Under ethyl carbamate and a-chloralose anesthesia, catheters were inserted into the femoral artery and femoral vein with polyethylene tubes (SP-31; Natsume Seisakusho, Tokyo, Japan) filled with heparin solution (50 IU/ml) for blood sampling and drug administration, respectively. Urine was collected from the urinary bladder catheterized with SP-31 polyethylene tubes. Rats were infused with 1 % lithium chloride or 1.1 % acetazolamide sodium in saline at 0.03 ml/min for 2 h. Urine samples were collected from the urinary bladder catheterized with SP-31 polyethylene tubes during the last 20 min of infusion, and blood collection was conducted at the midpoint.

After dilution with 0.1 % nitric acid, the concentrations of lithium in plasma and urine were determined using atomic absorption spectrometry Agilent 280Z AA (Agilent Technologies, Santa Clara, CA, USA). Li CL_r was calculated by dividing the urinary excretion rate of lithium by its plasma concentration. Creatinine concentrations in the final bladder urine or plasma samples 60 min after lithium injection were measured using the assay kit from Wako Pure Chemical Industries (Osaka, Japan). C_{cr} was calculated by dividing its urinary excretion rate by plasma concentrations of creatinine (P_{cr}). The fractional excretion of lithium was determined by dividing Li CL_r by C_{cr}. The reabsorption rate of lithium was calculated using C_{cr}, the plasma concentration of lithium, and its urinary excretion rate.

3.3. Western blotting for p-GSK3β, GSK3β, p308-Akt, p473-Akt, and Akt

After drug infusion, protein was prepared from the rat kidney cortex or liver using PRO-PREP™ protein extraction solution (iNtRON Biotechnology, Korea). Samples were separated using Mini-PROTEAN® TGX™ Gels (10 %; Bio-Rad, Hercules, CA, USA) and transferred onto an Immoblion®-P PVDF membrane (Millipore, Billerica, MA, USA) using an electroblotter. After blocking with 5 % nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris, 137 mM NaCl, pH7.5) containing 0.5 % Tween 20 at room temperature for 1 h, the membrane was incubated with rabbit anti-p-GSK3β antibody, rabbit anti-p308-Akt antibody, or rabbit anti-p473-Akt antibody (Cell Signaling Technology, Danvers, MA, USA; 1:2000 dilution) at room temperature for 1 h or at 4 °C overnight. The membrane was then treated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:2000 dilution; Bio-Rad). The binding of HRP-conjugated IgG to each protein band was visualized by ImageQuant LAS 4000 (GE Healthcare, Little Chalfont, UK), and the density of bands was determined using the ImageQuant TL Analysis Toolbox (GE Healthcare). After the analysis of p-GSK3β, p308-Akt, or p473-Akt, the antibody was stripped from the membrane, and the membrane was blocked with 5 % nonfat dry milk in TBS containing 0.5 % Tween 20 at room temperature for 1 h. The membrane was incubated with a rabbit anti-GSK3β antibody (Cell Signaling Technology; 1:2000 dilution) or a rabbit anti-Akt antibody (Cell Signaling Technology; 1:2000 dilution) at room temperature for 1 h, and the same procedure was performed for the detection of GSK3β and Akt.

3.4. Statistical analysis

Data were expressed as the mean±SE and analyzed by an unpaired *t*-test or one-way analysis of variance followed by Scheffé's test using KaleidaGraph (Synergy Software, Reading, PA, USA). Differences were considered significant at $P < 0.05$.

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