Kidney & Blood Pressure Research

Kidney Blood Press Res 2015;40:555-564
DOI: 10.1159/000368531
Published online: October 28, 2015
Accepted: September 30, 2015

1423-0143/15/0406-0555$39.50/0

Original Paper

Up-Regulation of Intestinal Phosphate Transporter NaPi-IIb (SLC34A2) by the Kinases SPAK and OSR1

Myriam Fezai[a,b] Bernat Elvira[a] Jamshed Warsi[a] Mossadok Ben-Attia[b] Zohreh Hosseinzadeh[a,c] Florian Lang[a]

[a]Department of Physiology I, University of Tübingen, Tübingen, Germany; [b]Environmental Biomonitoring Laboratory (LBE LR01/ES14), Faculty of Sciences of Bizerte, University of Carthage, Tunisia; [c]Experimental Retinal Prosthetics Group, Institute for Ophthalmic Research, University of Tübingen, Tübingen, Germany

Key Words
Phosphate • SLC34A2 • Oxidative stress-responsive kinase 1 • SPS1-related proline/alanine-rich kinase • WNK

Abstract
Background/Aims: SPAK (SPS1-related proline/alanine-rich kinase) and OSR1 (oxidative stress-responsive kinase 1), kinases controlled by WNK (with-no-K[Lys] kinase), are powerful regulators of cellular ion transport and blood pressure. Observations in gene-targeted mice disclosed an impact of SPAK/OSR1 on phosphate metabolism. The present study thus tested whether SPAK and/or OSR1 contributes to the regulation of the intestinal Na^+-coupled phosphate co-transporter NaPi-IIb (SLC34A2). ***Methods:*** cRNA encoding NaPi-IIb was injected into *Xenopus laevis* oocytes without or with additional injection of cRNA encoding wild-type SPAK, constitutively active ^{T233E}SPAK, WNK insensitive ^{T233A}SPAK, catalytically inactive ^{D212A}SPAK, wild-type OSR1, constitutively active ^{T185E}OSR1, WNK insensitive ^{T185A}OSR1 or catalytically inactive ^{D164A}OSR1. The phosphate (1 mM)-induced inward current (I_{Pi}) was taken as measure of phosphate transport. ***Results:*** I_{Pi} was observed in NaPi-IIb expressing oocytes but not in water injected oocytes, and was significantly increased by co-expression of SPAK, ^{T233E}SPAK, OSR1, ^{T185E}OSR1 or SPAK+OSR1, but not by co-expression of ^{T233A}SPAK, ^{D212A}SPAK, ^{T185A}OSR1, or ^{D164A}OSR1. SPAK and OSR1 both increased the maximal transport rate of the carrier. ***Conclusions:*** SPAK and OSR1 are powerful stimulators of the intestinal Na^+-coupled phosphate co-transporter NaPi-IIb.

Prof. Dr. Florian Lang
Department of Physiology, University of Tübingen, Gmelinstr. 5, D-72076 Tübingen (Germany), Tel. +49 7071/2972194, Fax +49 7071/295618
E-Mail florian.lang@uni-tuebingen.de

Kidney Blood Press Res 2015;40:555-564
DOI: 10.1159/000368531
Published online: October 28, 2015

www.karger.com/kbr

Introduction

SPAK (SPS1-related proline/alanine-rich kinase) [1-3] and OSR1 (oxidative stress-responsive kinase 1) [4, 5] are both powerful regulators of epithelial ion transport and blood pressure [6]. The kinases are phosphorylated by WNK (with-no-K[Lys] kinases) [1, 7-10], which similarly contribute to the regulation of ion transport and blood pressure [9-17]. SPAK and OSR1 stimulate NaCl (NCC) and Na^+,K^+,$2Cl^-$ (NKCC) co-transporters [4-8, 11, 18-26] as well as a number of ion channels [27-30]. SPAK/OSR1 are expressed in intestine [31]. Intestinal SPAK/OSR1 are both phosphorylated by treatment with forskolin, an activator of adenylate cyclase [31]. SPAK deficiency is followed by a decrease in paracellular permeability and partial resistance to inflammatory bowel disease [32]. SPAK transcription is stimulated by the transcription factors NF-kappaB and Sp1 [33]. Activators of SPAK include angiotensin II [34, 35], which upregulates the expression of the renal phosphate transporter NaPi-IIa [36]. OSR1 has similarly been shown to stimulate the renal phosphate transporter NaPi-IIa [37]. Along those lines gene targeted mice expressing WNK-resistant SPAK [38] or OSR1 [37] display subtle alterations of phosphate metabolism.

Nothing is known, however, on a putative influence of SPAK or OSR1 on the type II Na^+-coupled phosphate co-transporter NaPi-IIb (SLC34A2), the transporter accomplishing intestinal transport of inorganic phosphate [39]. NaPi-IIb is primarily expressed in small intestine [39]. Mutations in the SLC34A2 gene may be associated with accumulation of phosphate in lung with development of pulmonary alveolar microlithiasis [40]. SLC34A2 is further expressed in the epididymis and presumably participates in the fine tuning of luminal phosphate concentration [41]. Moreover, SLC34A2 expression was observed in ovarian, papillary thyroid and breast cancer [42-44].

The present study explored, whether SPAK and/or OSR1 participates in the regulation of NaPi-IIb. To this end, NaPi-IIb was expressed in *Xenopus* oocytes without or with additional expression of the kinases. The phosphate induced current was determined by dual electrode voltage clamp and taken as a measure of phosphate transport.

Materials and Methods

Ethical Statement

All experiments conform with the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No 123, Strasbourg 1985) and were conducted according to the German law for the welfare of animals and the surgical procedures on the adult *Xenopus laevis* frogs were reviewed and approved by the respective government authority of the state Baden-Württemberg (Regierungspräsidium) prior to the start of the study (Anzeige für Organentnahme nach §36).

Constructs

Constructs encoding human wild-type NaPi-IIb (SLC34A2) [45], human wild-type SPAK/pGHJ, constitutively active ^{T233E}SPAK/pGHJ, WNK insensitive ^{T233A}SPAK/pGHJ, catalytically inactive ^{D212A}SPAK/pGHJ [7], human wild-type OSR1/pGHJ, constitutively active ^{T185E}OSR1/pGHJ, WNK insensitive ^{T185A}OSR1/pGHJ and catalytically inactive ^{D164A}OSR1/pGHJ [37], were used to generate cRNA as described previously [46, 47]. The constructs were a kind gift from Dario Alessi (University of Dundee).

Voltage clamp in Xenopus laevis oocytes

Xenopus oocytes were prepared as previously described [48, 49]. 15 ng cRNA encoding NaPi-IIb and 10 ng of cRNA encoding wild-type, constitutively active or inactive kinase were injected on the same day after preparation of the oocytes. The oocytes were maintained at 17°C in ND96, a solution containing (in mM): 88.5 NaCl, 2 KCl, 1 $MgC1_2$, 1.8 $CaC1_2$, 2.5 NaOH, 5 HEPES, 5 sodium pyruvate ($C_3H_3NaO_3$), Gentamycin (100 mg/l), Tetracycline (50 mg/l), Ciprofloxacin (1.6 mg/l), Theophiline (90 mg/l) and pH 7.4 [50, 51]. The voltage clamp experiments were performed at room temperature 4 days after the first injection [47, 52]. Phosphate induced currents were taken as a measure of phosphate transport at a holding potential of

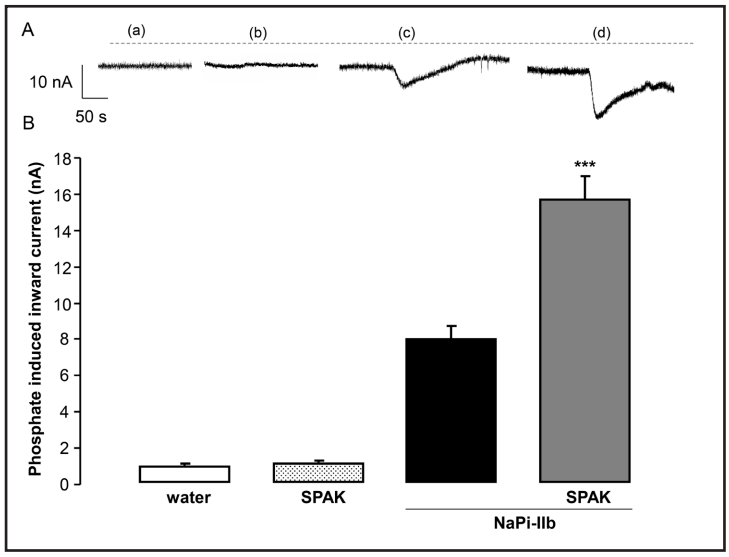

Fig. 1. Co-expression of SPAK increases electrogenic phosphate transport in NaPi-IIb expressing *Xenopus laevis* oocytes. A: Representative original tracings showing phosphate (1 mM)-induced current (I_{Pi}) in *Xenopus laevis* oocytes injected with water (a), expressing wild-type SPAK alone (b), or expressing NaPi-IIb without (c) or with (d) additional coexpression of wild-type SPAK (dashed line indicates zero current). B: Arithmetic means ± SEM (n = 9-16) of I_{Pi} in *Xenopus laevis* oocytes injected with water (white bar), SPAK alone (dotted bar), or expressing NaPi-IIb without (black bar) or with (grey bar) wild-type SPAK. ***(p<0.001) indicates statistically significant difference from the absence of SPAK.

-60mV [53, 54]. The data were filtered at 10 Hz and recorded with a Digidata A/D-D/A converter (1322A Axon Instruments) and Clampex 9.2 software for data acquisition and analysis (Axon Instruments) [55-57]. The control superfusate (ND96) contained (in mM): 93.5 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 2.5 NaOH and 5 HEPES, pH 7.4. The flow rate of the superfusion was approx. 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s [58-60]. For kinetic analysis the phosphate induced-current (I_{Pi}) was plotted against the respective phosphate concentration(s) and maximal current (I_{max}) as well as concentration required for halfmaximal current (k_m) calculated using the equation $I_{Pi} = I_{max} \cdot s/(k_m + s)$.

Statistical analysis

Data are provided as means ± SEM, n represents the number of oocytes investigated. As different batches of oocytes may yield different results, comparisons were always made within a given oocyte batch. All voltage clamp experiments were repeated with at least 3 batches of oocytes; in all repetitions qualitatively similar data were obtained. Data were tested for significance using ANOVA or the unpaired student's t-test, as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

The present study addressed whether the electrogenic phosphate transporter NaPi-IIb is regulated by the kinases SPAK (SPS1-related proline/alanine-rich kinase) and/or OSR1 (oxidative stress-responsive kinase 1). To this end, cRNA encoding NaPi-IIb was injected into *Xenopus laevis* oocytes with or without additional injection of cRNA encoding wild-type or mutant SPAK or OSR1. Dual electrode voltage clamp experiments were performed to quantify the phosphate-induced inward current (I_{Pi}) as a measure of electrogenic phosphate transport. As illustrated in Fig. 1, I_{Pi} was negligible in water-injected oocytes indicating that the oocytes did not express appreciable endogenous electrogenic phosphate transport. I_{Pi} was further negligible in oocytes expressing SPAK alone. In NaPi-IIb expressing *Xenopus laevis* oocytes, however, addition of phosphate (1 mM) to the bath solution was followed by appearance of a sizable I_{Pi}. The co-expression of wild-type SPAK in NaPi-IIb expressing *Xenopus laevis* oocytes was followed by a significant increase of I_{Pi}.

In order to test, whether SPAK co-expression modifies the maximal I_{Pi} and/or the affinity of the carrier, *Xenopus laevis* oocytes expressing NaPi-IIb without or with co-expression of

Kidney Blood Press Res 2015;40:555-564
DOI: 10.1159/000368531
Published online: October 28, 2015

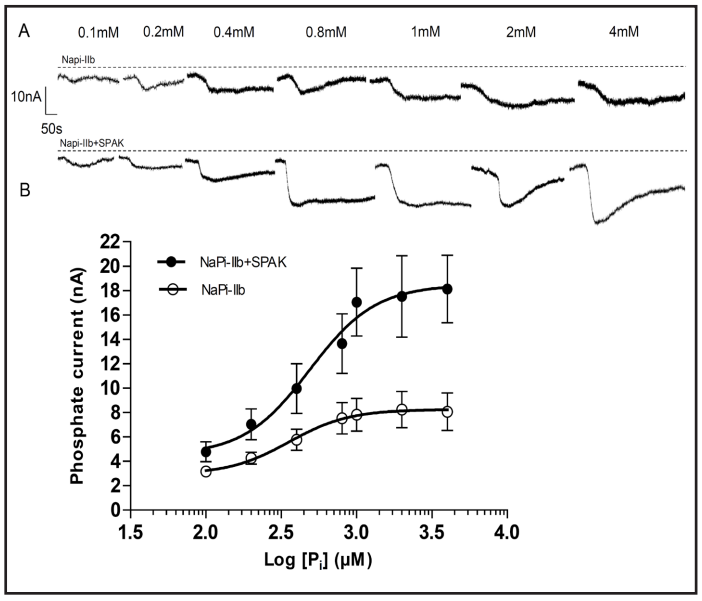

Fig. 2. Co-expression of SPAK increases maximal electrogenic phosphate transport in NaPi-IIb expressing *Xenopus laevis* oocytes. A: Representative original tracings showing the current induced by increasing concentrations of phosphate (from 0.1 mM to 4 mM) in *Xenopus laevis* oocytes expressing Napi-IIb without (upper panel) or with (lower panel) additional coexpression of wild-type SPAK (dashed line indicates zero current). B: Arithmetic means ± SEM (n = 6) of I_{Pi} as a function of Logarithmic phosphate concentrations in *Xenopus laevis* oocytes expressing NaPi-IIb without (white circles), or with (black circles) additional co-expression of wild-type SPAK.

SPAK were exposed to phosphate concentrations ranging from 0.1 mM to 4 mM. As shown in Fig. 2, I_{Pi} increased as a function of the extracellular phosphate concentration. Maximal I_{Pi} was significantly (p<0.001) higher in *Xenopus laevis* oocytes expressing NaPi-IIb together with SPAK (20.6 ± 1.1 nA, n = 6) than in *Xenopus laevis* oocytes expressing NaPi-IIb alone (8.9 ± 0.3 nA, n = 6). The concentration required for half maximal I_{Pi} (K_m) was significantly (p<0.05) higher in *Xenopus laevis* oocytes expressing NaPi-IIb together with SPAK (361 ± 66 µM, n = 6) than in *Xenopus laevis* oocytes expressing NaPi-IIb alone (193 ± 26 µM, n = 6).

Additional experiments were performed in *Xenopus laevis* oocytes expressing NaPi-IIb without or with SPAK mutants. As illustrated in Fig. 3, the effect of wild-type SPAK was mimicked by the constitutively active ^{T233E}SPAK but neither by the WNK insensitive ^{T233A}SPAK nor by the catalytically inactive ^{D212A}SPAK.

Similar experiments were performed to elucidate the effect of OSR1 on NaPi-IIb activity. As illustrated in Fig. 4, co-expression of wild-type OSR1 was followed by a significant increase of I_{Pi} in NaPi-IIb expressing *Xenopus* oocytes.

Further experiments again explored, whether OSR1 co-expression modifies the maximal I_{Pi} and/or the affinity of the carrier, As shown in Fig. 5, the maximal phosphate induced current was again significantly (p<0.001) higher in *Xenopus laevis* oocytes expressing NaPi-IIb together with OSR1 (14.9 ± 1.1 nA, n = 6) than in *Xenopus laevis* oocytes expressing NaPi-IIb alone (6.8 ± 0.1 nA, n = 6). The concentration required for half maximal I_{Pi} (K_m) was significantly (p<0.05) higher in *Xenopus laevis* oocytes expressing NaPi-IIb together with OSR1 (338 ± 92 µM, n = 6) than in *Xenopus laevis* oocytes expressing NaPi-IIb alone (119 ± 11 µM, n = 6).

Addtitional experiments were performed in *Xenopus laevis* oocytes expressing NaPi-IIb without or with OSR1 mutants. As a result, the effect of wild-type OSR1 was mimicked by constitutively active ^{T185E}OSR1 but neither by the WNK insensitive ^{T185A}OSR1 nor by the catalytically inactive ^{D164A}OSR1 (Fig. 6).

A final series of experiments explored the effect of simultaneous co-expression of SPAK and OSR1 on I_{Pi}. As a result, the electrogenic current in NaPi-IIb expressing *Xenopus laevis* oocytes tended to be slightly higher in oocytes co-expressing both, SPAK and OSR1 together than in oocytes co-expressing either SPAK or OSR1 alone. However, the difference of I_{Pi} between oocytes co-expressing both, SPAK and OSR1, and the co-expressing either SPAK or OSR1 alone did not reach statistical significance (Fig. 7).

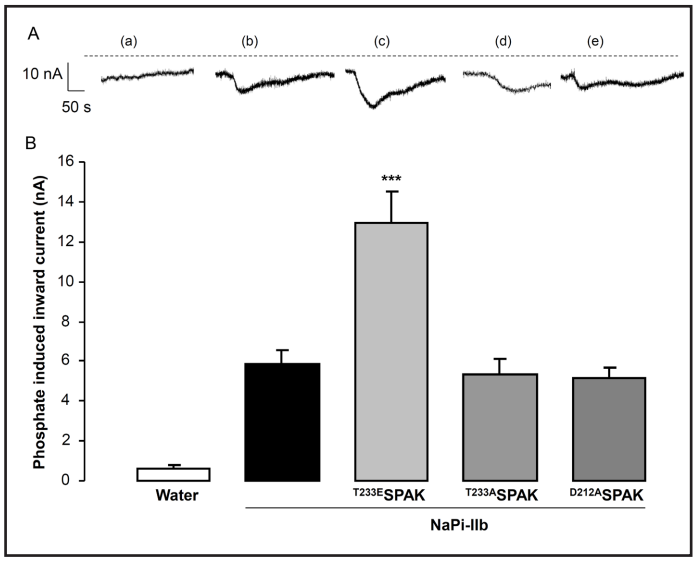

Fig. 3. The effect of SPAK is mimicked by active ^{T233E}SPAK but not by inactive mutants ^{T233A}SPAK or ^{D212A}SPAK. A: Representative original tracings showing phosphate (1 mM) induced current (I_{Pi}) in *Xenopus laevis* oocytes injected with water (a), expressing NaPi-IIb alone (b) or with additional co-expression of constitutively active ^{T233E}SPAK (c), WNK1 insensitive ^{T233A}SPAK (d), or catalytically inactive ^{D212A}SPAK (e) (dashed line indicates zero current). B: Arithmetic means ± SEM (n = 9–12) of I_{Pi} in *Xenopus laevis* oocytes injected with water (white bar), or expressing NaPi-IIb without (black bar) or with constitutively active ^{T233E}SPAK (light grey bar), WNK insensitive ^{T233A}SPAK (middle grey bar), or catalytically inactive ^{D212A}SPAK (dark grey bar). ***(p<0.001) indicates statistically significant difference from oocytes expressing NaPi-IIb alone.

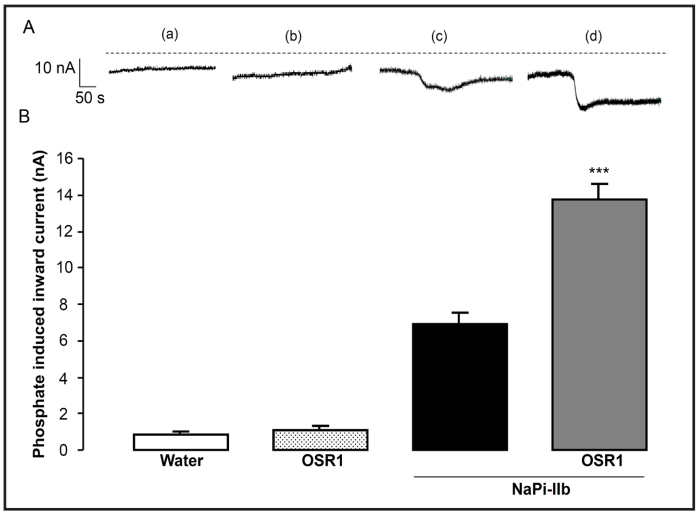

Fig. 4. Co-expression of OSR1 increases electrogenic phosphate transport in NaPi-IIb expressing *Xenopus laevis* oocytes. A: Representative original tracings showing phosphate (1 mM) induced current (I_{Pi}) in *Xenopus laevis* oocytes injected with water (a), expressing OSR1 alone (b), or expressing NaPi-IIb without (c) or with (d) additional co-expression of wild-type OSR1 (dashed line indicates zero current). B: Arithmetic means ± SEM (n = 9-17) of I_{Pi} in *Xenopus laevis* oocytes injected with water (white bar), expressing OSR1 alone (dotted bar), or expressing NaPi-IIb without (black bar) or with (grey bar) wild-type OSR1. *** (p<0.001) indicates statistically significant difference from the absence of OSR1.

Discussion

The present study reveals that SPAK (SPS1-related proline/alanine-rich kinase) and OSR1 (oxidative stress-responsive kinase 1) are both powerful positive regulators of the intestinal phosphate transporter NaPi-IIb. Co-expression of either SPAK or OSR1 increases the phosphate-induced inward current (I_{Pi}) in NaPi-IIb expressing oocytes. SPAK and OSR1 are both effective in large part by increase of the maximal transport rate. Co-expression of the kinases decreased the apparent affinity of NaPi-IIb. The simultaneous co-expression of both kinases SPAK and OSR1 does not show an additive effect. This outcome could be explained by the expression of SPAK and OSR1 in different cells relative to the same tissue or organ. Similar to wild type SPAK, constitutively active ^{T233E}SPAK increases the phoshpate induced current

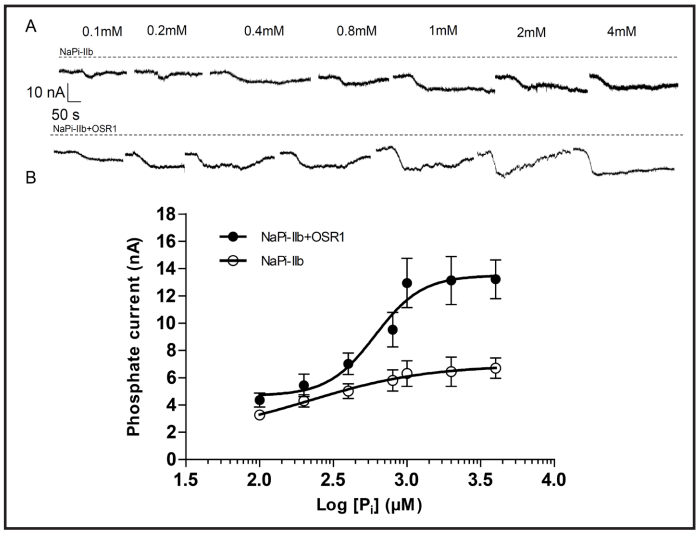

Fig. 5. Co-expression of OSR1 decreases maximal electrogenic phosphate transport in NaPi-IIb-expressing *Xenopus laevis* oocytes. A: Representative original tracings showing the current induced by increasing concentrations of phosphate (from 0.1 mM to 4 mM) in *Xenopus laevis* oocytes expressing Napi-IIb without (upper panel) or with (lower panel) additional co-expression of wild-type OSR1 (dashed line indicates zero current). B: Arithmetic means ± SEM (n = 6) of I_{Pi} as a function of logarithmic phosphate concentrations in *Xenopus laevis* oocytes expressing Napi-IIb without (white circles), or with (black circles) additional co-expression of wild-type OSR1.

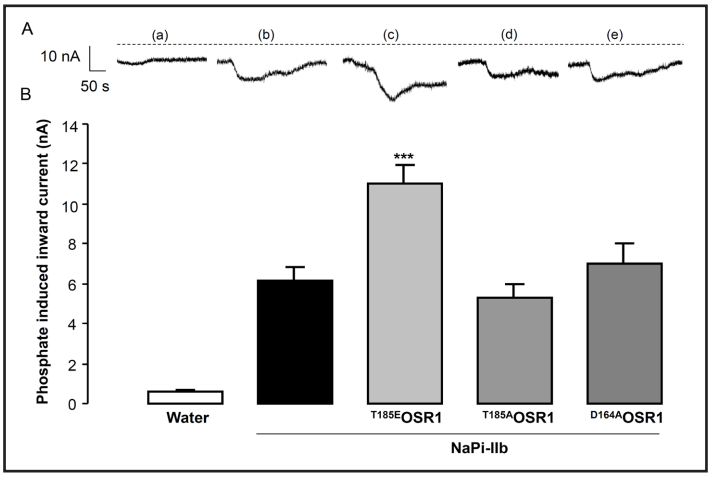

Fig. 6. The effect of OSR1 is mimicked by active ^{T185E}OSR1 but not by inactive mutants ^{T185A}OSR1 or ^{D164A}OSR1. A: Representative original tracings showing phosphate (1 mM) induced current (I_{Pi}) in *Xenopus laevis* oocytes injected with water (a), expressing NaPi-IIb alone (b) or with additional co-expression of constitutively active ^{T185E}OSR1 (c), WNK1 insensitive ^{T185A}OSR1 (d) or catalytically inactive ^{D164A}OSR1 (e) (dashed line indicates zero current). B: Arithmetic means ± SEM (n = 9-12) of I_{Pi} in *Xenopus laevis* oocytes injected with water (white bar) expressing NaPi-IIb without (black bar) or with constitutively active ^{T185E}OSR1 (light grey bar), WNK insensitive ^{T185A}OSR1 (middle grey bar), or catalytically inactive ^{D164A}OSR1 (dark grey bar). ***(p<0.001) indicates statistically significant difference from oocytes expressing NaPi-IIb alone.

in NaPi-IIb expressing *Xenopus* oocytes. In contrast, neither the WNK insensitive ^{T233A}SPAK nor the catalytically inactive ^{D212A}SPAK significantly modified the phosphate induced current. The effect of wild type OSR1 is similarly mimicked by the constitutively active ^{T185E}OSR1, but not by WNK insensitive ^{T185A}OSR1 or by the catalytically inactive ^{D164A}OSR1. These observations suggest that SPAK and OSR1 are activated by phosphorylation at the WNK phosphorylation site and that they are effective by phosphorylating target molecules. The observations do, however, not necessarily reflect direct phosphorylation of the NaPi-IIb carrier protein by SPAK and OSR1. Instead, the kinases might phosphorylate and thus modify the function of other NaPi-IIb regulating signaling molecules. Kinases previously shown to participate in the regulation of NaPi-IIb activity include AMP activated kinase (AMPK) [61], B-RAF [45], serum & glucocorticoid inducible kinase SGK1 [62], and mammalian target of rapamycin (mTOR) [63].

Kidney Blood Press Res 2015;40:555-564
DOI: 10.1159/000368531
Published online: October 28, 2015

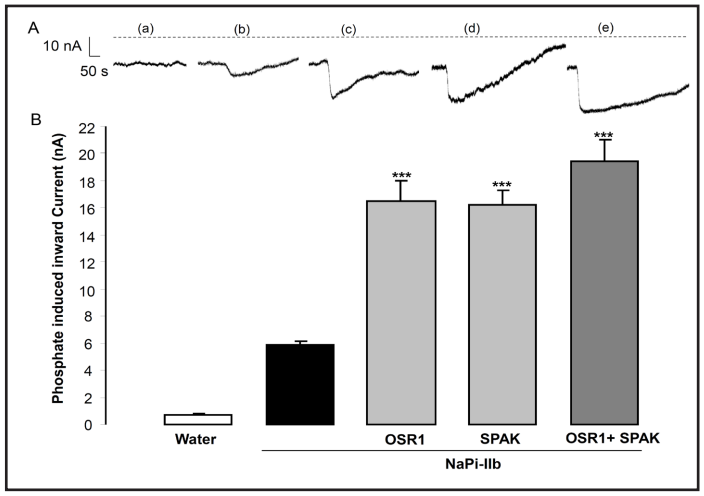

Fig. 7. Electrogenic phosphate transport following co-expression of both SPAK and OSR1 in NaPi-IIb expressing *Xenopus laevis* oocytes. A: Representative original tracings showing phosphate (1 mM) induced current (I_{Pi}) in *Xenopus laevis* oocytes injected with water (a), expressing NaPi-IIb alone (b) or with additional co-expression of wild type OSR1 (c), or wild type SPAK (d), or wild type OSR1 and SPAK (e) (dashed line indicates zero current). B: Arithmetic means ± SEM (n = 9-16) of I_{Pi} in *Xenopus laevis* oocytes injected with water (white bar), or expressing NaPi-IIb without (black bar) or with wild type OSR1 (light grey bar), or with wild type SPAK (light grey bar), or OSR1+SPAK (dark grey bar). ***(p<0.001) indicates statistically significant difference from oocytes expressing NaPi-IIb alone.

In view of the regulation of NaPi-IIb by SPAK and OSR1, the two kinases contribute to the orchestration of phosphate metabolism. Observations in gene targeted mice indeed reveal that SPAK [38] and OSR1 [37] affect phosphate metabolism and OSR1 modifies Napi-IIa activity expressed in the proximal tubule of the nephron [37]. However, the effects of SPAK [38] and OSR1 [37] on phosphate metabolism are not limited to regulation of NaPi-IIa and NaPi-IIb.

SPAK and OSR1 foster cellular KCl uptake by stimulating NaCl co-transporters and $Na^+,K^+,2Cl^-$ co-transporters and by inhibiting KCl co-transporters [4-8, 11, 18-26], effects leading to cell swelling [64-66]. Stimulation of Na^+ coupled phosphate transport is similarly expected to increase cell volume, as it leads to cellular uptake of Na^+ and phosphate as well as to depolarisation of the cell membrane, which favours entry of negatively charged chloride. Due to the low transport rate and the limited availability of phosphate in extracellular fluid, activation of NaPi-IIb is, however, not expected to rapidly swell cells.

Conclusion

NaPi-IIb is markedly up-regulated by the kinases SPAK and OSR1, an effect presumably contributing to the orchestration of phosphate metabolism.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

Acknowledgements

The authors acknowledge the meticulous preparation of the manuscript by Tanja Loch, as well as the technical support by Elfriede Faber. This study was supported by the Deutsche Forschungsgemeinschaft, GRK 1302, SFB 773 B4/A1, La 315/13-3.

References

1 Rafiqi FH, Zuber AM, Glover M, Richardson C, Fleming S, Jovanovic S, Jovanovic A, O'Shaughnessy KM, Alessi DR: Role of the WNK-activated SPAK kinase in regulating blood pressure. EMBO Mol Med 2010;2:63-75.

2 Castaneda-Bueno M, Gamba G: SPAKling insight into blood pressure regulation. EMBO Mol Med 2010;2:39-41.

3 Yang SS, Lo YF, Wu CC, Lin SW, Yeh CJ, Chu P, Sytwu HK, Uchida S, Sasaki S, Lin SH: SPAK-knockout mice manifest Gitelman syndrome and impaired vasoconstriction. J Am Soc Nephrol 2010;21:1868-1877.

4 Lin SH, Yu IS, Jiang ST, Lin SW, Chu P, Chen A, Sytwu HK, Sohara E, Uchida S, Sasaki S, Yang SS: Impaired phosphorylation of Na(+)-K(+)-2Cl(-) cotransporter by oxidative stress-responsive kinase-1 deficiency manifests hypotension and Bartter-like syndrome. Proc Natl Acad Sci USA 2011;108:17538-17543.

5 Villa F, Deak M, Alessi DR, van Aalten DM: Structure of the OSR1 kinase, a hypertension drug target. Proteins 2008;73:1082-1087.

6 Gagnon KB, Delpire E: Molecular physiology of SPAK and OSR1: two Ste20-related protein kinases regulating ion transport. Physiol Rev 2012;92:1577-1617.

7 Vitari AC, Deak M, Morrice NA, Alessi DR: The WNK1 and WNK4 protein kinases that are mutated in Gordon's hypertension syndrome phosphorylate and activate SPAK and OSR1 protein kinases. Biochem J 2005;391:17-24.

8 Vitari AC, Thastrup J, Rafiqi FH, Deak M, Morrice NA, Karlsson HK, Alessi DR: Functional interactions of the SPAK/OSR1 kinases with their upstream activator WNK1 and downstream substrate NKCC1. Biochem J 2006;397:223-231.

9 Glover M, Zuber AM, O'Shaughnessy KM: Hypertension, dietary salt intake, and the role of the thiazide-sensitive sodium chloride transporter NCCT. Cardiovasc Ther 2011;29:68-76.

10 O'Reilly M, Marshall E, Speirs HJ, Brown RW: WNK1, a gene within a novel blood pressure control pathway, tissue-specifically generates radically different isoforms with and without a kinase domain. J Am Soc Nephrol 2003;14:2447-2456.

11 Kahle KT, Rinehart J, Lifton RP: Phosphoregulation of the Na-K-2Cl and K-Cl cotransporters by the WNK kinases. Biochim Biophys Acta 2010;1802:1150-1158.

12 Flatman PW: Cotransporters, WNKs and hypertension: an update. Curr Opin Nephrol Hypertens 2008;17:186-192.

13 Furgeson SB, Linas S: Mechanisms of type I and type II pseudohypoaldosteronism. J Am Soc Nephrol 2010;21:1842-1845.

14 Uchida S: Pathophysiological roles of WNK kinases in the kidney. Pflugers Arch 2010;460:695-702.

15 Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP: Human hypertension caused by mutations in WNK kinases. Science 2001;293:1107-1112.

16 Achard JM, Disse-Nicodeme S, Fiquet-Kempf B, Jeunemaitre X: Phenotypic and genetic heterogeneity of familial hyperkalaemic hypertension (Gordon syndrome). Clin Exp Pharmacol Physiol 2001;28:1048-1052.

17 Capasso G, Cantone A, Evangelista C, Zacchia M, Trepiccione F, Acone D, Rizzo M: Channels, carriers, and pumps in the pathogenesis of sodium-sensitive hypertension. Semin Nephrol 2005;25:419-424.

18 Delpire E, Gagnon KB: SPAK and OSR1, key kinases involved in the regulation of chloride transport. Acta Physiol (Oxf) 2006;187:103-113.

19 Delpire E, Gagnon KB: SPAK and OSR1: STE20 kinases involved in the regulation of ion homoeostasis and volume control in mammalian cells. Biochem J 2008;409:321-331.

20 Gimenez I: Molecular mechanisms and regulation of furosemide-sensitive Na-K-Cl cotransporters. Curr Opin Nephrol Hypertens 2006;15:517-523.

21 Richardson C, Sakamoto K, de los HP, Deak M, Campbell DG, Prescott AR, Alessi DR: Regulation of the NKCC2 ion cotransporter by SPAK-OSR1-dependent and -independent pathways. J Cell Sci 2011;124:789-800.

22 Gagnon KB, Delpire E: On the substrate recognition and negative regulation of SPAK, a kinase modulating Na+-K+-2Cl- cotransport activity. Am J Physiol Cell Physiol 2010;299:C614-C620.

23 Glover M, O'Shaughnessy KM: SPAK and WNK kinases: a new target for blood pressure treatment? Curr Opin Nephrol Hypertens 2011;20:16-22.

24 Huang CL, Yang SS, Lin SH: Mechanism of regulation of renal ion transport by WNK kinases. Curr Opin Nephrol Hypertens 2008;17:519-525.
25 Mercier-Zuber A, O'Shaughnessy KM: Role of SPAK and OSR1 signalling in the regulation of NaCl cotransporters. Curr Opin Nephrol Hypertens 2011;20:534-540.
26 Richardson C, Alessi DR: The regulation of salt transport and blood pressure by the WNK-SPAK/OSR1 signalling pathway. J Cell Sci 2008;121:3293-3304.
27 Falin RA, Miyazaki H, Strange K: C. elegans STK39/SPAK ortholog-mediated inhibition of ClC anion channel activity is regulated by WNK-independent ERK kinase signaling. Am J Physiol Cell Physiol 2011;300:C624-635.
28 Falin RA, Morrison R, Ham AJ, Strange K: Identification of regulatory phosphorylation sites in a cell volume- and Ste20 kinase-dependent ClC anion channel. J Gen Physiol 2009;133:29-42.
29 Miyazaki H, Strange K: Differential regulation of a CLC anion channel by SPAK kinase ortholog-mediated multisite phosphorylation. Am J Physiol Cell Physiol 2012;302:C1702-1712.
30 Park HW, Nam JH, Kim JY, Namkung W, Yoon JS, Lee JS, Kim KS, Venglovecz V, Gray MA, Kim KH, Lee MG: Dynamic regulation of CFTR bicarbonate permeability by [Cl-]i and its role in pancreatic bicarbonate secretion. Gastroenterology 2010;139:620-631.
31 Xiao F, Li J, Singh AK, Riederer B, Wang J, Sultan A, Park H, Lee MG, Lamprecht G, Scholte BJ, De Jonge HR, Seidler U: Rescue of epithelial HCO3- secretion in murine intestine by apical membrane expression of the cystic fibrosis transmembrane conductance regulator mutant F508del. J Physiol 2012;590:5317-5334.
32 Zhang Y, Viennois E, Xiao B, Baker MT, Yang S, Okoro I, Yan Y: Knockout of Ste20-like proline/alanine-rich kinase (SPAK) attenuates intestinal inflammation in mice. Am J Pathol 2013;182:1617-1628.
33 Yan Y, Dalmasso G, Nguyen HT, Obertone TS, Sitaraman SV, Merlin D: Ste20-related proline/alanine-rich kinase (SPAK) regulated transcriptionally by hyperosmolarity is involved in intestinal barrier function. PLoS One 2009;4:e5049.
34 Kamat NV, Thabet SR, Xiao L, Saleh MA, Kirabo A, Madhur MS, Delpire E, Harrison DG, McDonough AA: Renal transporter activation during angiotensin-II hypertension is blunted in interferon-gamma-/- and interleukin-17A-/- mice. Hypertension 2015;65:569-576.
35 van der Lubbe N, Zietse R, Hoorn EJ: Effects of angiotensin II on kinase-mediated sodium and potassium transport in the distal nephron. Curr Opin Nephrol Hypertens 2013;22:120-126.
36 Xu L, Dixit MP, Chen R, Dixit NM, Collins JF, Ghishan FK: Effects of angiotensin II on NaPi-IIa co-transporter expression and activity in rat renal cortex. Biochim Biophys Acta 2004;1667:114-121.
37 Pathare G, Foller M, Daryadel A, Mutig K, Bogatikov E, Fajol A, Almilaji A, Michael D, Stange G, Voelkl J, Wagner CA, Bachmann S, Lang F: OSR1-Sensitive Renal Tubular Phosphate Reabsorption. Kidney Blood Press Res 2012;36:149-161.
38 Pathare G, Foller M, Michael D, Walker B, Hierlmeier M, Mannheim JG, Pichler BJ, Lang F: Enhanced FGF23 serum concentrations and phosphaturia in gene targeted mice expressing WNK-resistant SPAK. Kidney Blood Press Res 2012;36:355-364.
39 Marks J, Debnam ES, Unwin RJ: Phosphate homeostasis and the renal-gastrointestinal axis. Am J Physiol Renal Physiol 2010;299:F285-296.
40 Ferreira Francisco FA, Pereira e Silva JL, Hochhegger B, Zanetti G, Marchiori E: Pulmonary alveolar microlithiasis. State-of-the-art review. Respir Med 2013;107:1-9.
41 Xu Y, Yeung CH, Setiawan I, Avram C, Biber J, Wagenfeld A, Lang F, Cooper TG: Sodium-inorganic phosphate cotransporter NaPi-IIb in the epididymis and its potential role in male fertility studied in a transgenic mouse model. Biol Reprod 2003;69:1135-1141.
42 Chen DR, Chien SY, Kuo SJ, Teng YH, Tsai HT, Kuo JH, Chung JG: SLC34A2 as a novel marker for diagnosis and targeted therapy of breast cancer. Anticancer Res 2010;30:4135-4140.
43 Jarzab B, Wiench M, Fujarewicz K, Simek K, Jarzab M, Oczko-Wojciechowska M, Wloch J, Czarniecka A, Chmielik E, Lange D, Pawlaczek A, Szpak S, Gubala E, Swierniak A: Gene expression profile of papillary thyroid cancer: sources of variability and diagnostic implications. Cancer Res 2005;65:1587-1597.
44 Rangel LB, Sherman-Baust CA, Wernyj RP, Schwartz DR, Cho KR, Morin PJ: Characterization of novel human ovarian cancer-specific transcripts (HOSTs) identified by serial analysis of gene expression. Oncogene 2003;22:7225-7232.

45 Pakladok T, Hosseinzadeh Z, Lebedeva A, Alesutan I, Lang F: Upregulation of the Na(+)-coupled phosphate cotransporters NaPi-IIa and NaPi-IIb by B-RAF. J Membr Biol 2014;247:137-145.
46 Almilaji A, Munoz C, Hosseinzadeh Z, Lang F: Upregulation of Na+,Cl(-)-coupled betaine/gamma-amino-butyric acid transporter BGT1 by Tau tubulin kinase 2. Cell Physiol Biochem 2013;32:334-343.
47 Almilaji A, Sopjani M, Elvira B, Borras J, Dermaku-Sopjani M, Munoz C, Warsi J, Lang UE, Lang F: Upregulation of the creatine transporter Slc6A8 by Klotho. Kidney Blood Press Res 2014;39:516-525.
48 Hosseinzadeh Z, Dong L, Bhavsar SK, Warsi J, Almilaji A, Lang F: Upregulation of peptide transporters PEPT1 and PEPT2 by Janus kinase JAK2. Cell Physiol Biochem 2013;31:673-682.
49 Pakladok T, Almilaji A, Munoz C, Alesutan I, Lang F: PIKfyve sensitivity of hERG channels. Cell Physiol Biochem 2013;31:785-794.
50 Almilaji A, Honisch S, Liu G, Elvira B, Ajay SS, Hosseinzadeh Z, Ahmed M, Munoz C, Sopjani M, Lang F: Regulation of the voltage gated K channel Kv1.3 by recombinant human klotho protein. Kidney Blood Press Res 2014;39:609-622.
51 Almilaji A, Szteyn K, Fein E, Pakladok T, Munoz C, Elvira B, Towhid ST, Alesutan I, Shumilina E, Bock CT, Kandolf R, Lang F: Down-regulation of Na/K+ atpase activity by human parvovirus B19 capsid protein VP1. Cell Physiol Biochem 2013;31:638-648.
52 Fezai M, Elvira B, Borras J, Ben-Attia M, Hoseinzadeh Z, Lang F: Negative regulation of the creatine transporter SLC6A8 by SPAK and OSR1. Kidney Blood Press Res 2014;39:546-554.
53 Dermaku-Sopjani M, Almilaji A, Pakladok T, Munoz C, Hosseinzadeh Z, Blecua M, Sopjani M, Lang F: Down-regulation of the Na+-coupled phosphate transporter NaPi-IIa by AMP-activated protein kinase. Kidney Blood Press Res 2013;37:547-556.
54 Elvira B, Munoz C, Borras J, Chen H, Warsi J, Ajay SS, Shumilina E, Lang F: SPAK and OSR1 dependent down-regulation of murine renal outer medullary K channel ROMK1. Kidney Blood Press Res 2014;39:353-360.
55 Hosseinzadeh Z, Luo D, Sopjani M, Bhavsar SK, Lang F: Down-regulation of the epithelial Na(+) channel ENaC by Janus kinase 2. J Membr Biol 2014;247:331-338.
56 Hosseinzadeh Z, Sopjani M, Pakladok T, Bhavsar SK, Lang F: Downregulation of KCNQ4 by Janus kinase 2. J Membr Biol 2013;246:335-341.
57 Munoz C, Almilaji A, Setiawan I, Foller M, Lang F: Up-regulation of the inwardly rectifying K(+) channel Kir2.1 (KCNJ2) by protein kinase B (PKB/Akt) and PIKfyve. J Membr Biol 2013;246:189-197.
58 Warsi J, Dong L, Elvira B, Salker MS, Shumilina E, Hosseinzadeh Z, Lang F: SPAK dependent regulation of peptide transporters PEPT1 and PEPT2. Kidney Blood Press Res 2014;39:388-398.
59 Warsi J, Elvira B, Bissinger R, Shumilina E, Hosseinzadeh Z, Lang F: Downregulation of peptide transporters PEPT1 and PEPT2 by oxidative stress responsive kinase OSR1. Kidney Blood Press Res 2014;39:591-599.
60 Warsi J, Hosseinzadeh Z, Elvira B, Bissinger R, Shumilina E, Lang F: Regulation of ClC-2 activity by SPAK and OSR1. Kidney Blood Press Res 2014;39:378-387.
61 Kirchner S, Muduli A, Casirola D, Prum K, Douard V, Ferraris RP: Luminal fructose inhibits rat intestinal sodium-phosphate cotransporter gene expression and phosphate uptake. Am J Clin Nutr 2008;87:1028-1038.
62 Palmada M, Dieter M, Speil A, Bohmer C, Mack AF, Wagner HJ, Klingel K, Kandolf R, Murer H, Biber J, Closs EI, Lang F: Regulation of intestinal phosphate cotransporter NaPi IIb by ubiquitin ligase Nedd4-2 and by serum- and glucocorticoid-dependent kinase 1. Am J Physiol Gastrointest Liver Physiol 2004;287:G143-150.
63 Shojaiefard M, Lang F: Stimulation of the intestinal phosphate transporter SLC34A2 by the protein kinase mTOR. Biochem Biophys Res Commun 2006;345:1611-1614.
64 Hoffmann EK: Ion channels involved in cell volume regulation: effects on migration, proliferation, and programmed cell death in non adherent EAT cells and adherent ELA cells. Cell Physiol Biochem 2011;28:1061-1078.
65 Hoffmann EK, Lambert IH, Pedersen SF: Physiology of cell volume regulation in vertebrates. Physiol Rev 2009;89:193-277.
66 Lang F: Mechanisms and significance of cell volume regulation. J Am Coll Nutr 2007;26:613S-623S.