Agraarteadus
2 • XXXI • 2020 147–150

Journal of Agricultural Science
2 • XXXI • 2020 147–150

SHORT COMMUNICATION: IMMUNOHISTOCHEMICAL STUDY OF SODIUM-DEPENDENT GLUCOSE CO-TRANSPORTERS IN OSTRICHES KIDNEYS

Piret Hussar[1], Ilmārs Dūrītis[2], Florina Popovska-Percinic[3], Tõnu Järveots[4]

[1]Faculty of Medicine, University of Tartu, Tartu 50411, Estonia
[2]Faculty of Veterinary Medicine, Latvian University of Agriculture, Jelgava, Latvia
[3]Faculty of Veterinary Medicine, Ss.Cyril & Methodius University in Skopje, 1000 Skopje, R. Macedonia
[4]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

Saabunud: Received: 02.10.2020
Aktsepteeritud: Accepted: 20.11.2020
Avaldatud veebis: Published online: 21.11.2020

Vastutav autor: Corresponding author: Piret Hussar
E-mail: piretut@gmail.com

Keywords: glucose transporters, SGLT1, SGLT2, ostriches, kidneys, immunohistochemistry.

DOI: 10.15159/jas.20.23

ABSTRACT. Out of the two families of glucose transporters identified, the sodium-dependent glucose co-transporters contribute to renal glucose reabsorption. Due to the lack of knowledges of the localization of SGLTs in bird's kidneys, the present study aimed to immunolocalize Na+-glucose co-transporters SGLT1 and SGLT2 in ostrich's kidneys. In the study kidney material derived from five 14 days old female ostriches. Material 0.5–1.0 cm in diameter was fixed in 10% formalin, dehydrated, embedded into paraffin; thereafter slices 7 μm in thickness were cut and deparaffinized, followed by immunohistochemical staining with polyclonal primary antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) according to the manufacturers' guidelines (IHC kit, Abcam, UK). Our study revealed the immunohistochemical localization of SGLT1 and SGLT2 in the proximal tubules of the renal cortex. The immunohistochemical locations of sodium-dependent glucose transporters resembled those in mammals.

Introduction

In the body, glucose is the central source of energy and the glucose homeostasis is of critical importance to health (Hruby, 1997). Kidneys play a role in glucose homeostasis in the body by ensuring that glucose is not lost in the urine (Ghezzi *et al.* 2018). Two families of glucose transporters have been identified: the facilitated-diffusion glucose transporter family (GLUT family), and the NA(+)-dependent glucose co-transporters (or sodium-glucose linked transporters, SGLT family) (Takata, 1996). Sodium-dependent glucose co-transporters are a family of glucose transporters found in the intestinal mucosa of the small intestine and the proximal tubule of the nephron (Miller, Bihler, 1961; Wright 2001). Membrane proteins which are responsible for glucose reabsorption from the kidney's glomerular filtrate are sodium-glucose co-transporters SGLT1 and SGLT2 (Vallon, Thomson 2012; Haas *et al.*, 2014).

SGLT2, the major co-transporter involved in glucose reabsorption in the kidney, is located in the beginning part of the proximal tubule and is responsible for reabsorption of 80–90% of the glucose filtered by the glomerulus (You *et al.*, 1995; Bonora *et al.*, 2020). Most of the remaining glucose absorption is carried out by SGLT1 in more distal sections of the proximal tubule (Horiba *et al.*, 2003; Vallon, Thomson, 2012) of the kidney. Failures of these transporters in kidneys result in the excretion of filtered glucose in the urine (Wright *et al.*, 2007).

As most of the experiments on kidney's SGLTs have been carried out in rats and mice, the renal locations of both transporters are well established in animals (Takata, 1996; Hussar *et al.*, 2004; Vrhovac *et al.*, 2015). However, due to the scarce information available on the molecular basis of glucose transport in bird's kidneys, the aim of the present study was the immunohistochemical localization of SGLT1 and SGLT2 in the kidneys of ostriches chicken.

Material and methods

The study was carried out on five 14 days old female ostriches *(Struthio camelus var. Domesticus)*. The commercial ostrich chicken's feed – Strus Premium-Strus 1 and water were available *ad libitum*. Tissue sections 0.5–1.0 cm in diameter were removed from renal cortex

and medulla, fixed in 10% formalin, dehydrated in a tissue processor and embedded into paraffin according to the standardized tissue histological procedure (Carson, 1997). Slices 7µm thick were cut (microtome Microm HM360), floated on Poly-L-Lysine coated slides, deparaffinized with xylene and rehydrated in a graded series of ethanol followed by immunohistochemical staining with polyclonal primary antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) in 1/1000 dilution, for 30 min at 37° C according to the manufacturer's guidelines (IHC kit, Abcam, UK). Biotinylated secondary antibody and streptavidin-conjugated peroxidase were used for detection using DAB as a chromogen. Negative controls contained antibody diluent (Dako, S0809) instead of primary antibodies. Photos of the slides were taken by the microscope Zeiss Axioplan-2 Imaging (Germany) and saved to the computer for analyzing by visual control using a camera (AxioCam HRc, Germany) connected to the microscope.

The experiments were carried out following the guidelines laid down by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Ethical Committee of Latvian University of Agriculture has approved the experiments (protocol number 2014/2).

Results

The immunolocalization of SGLT1 and SGLT2 in 14 days old ostrich's kidneys was performed. SGLT1 was detected in the epithelial cells of the straight proximal tubules in medullary rays and outer stripe of the renal medulla (Figure 1a). SGLT2 was noted on the apical side of the epithelial cells in the renal cortical proximal tubules (Figure 1b). The epithelial cells of the collecting tubules as well as in the thin segment of the loop of Henle remained unstained for both antibodies.

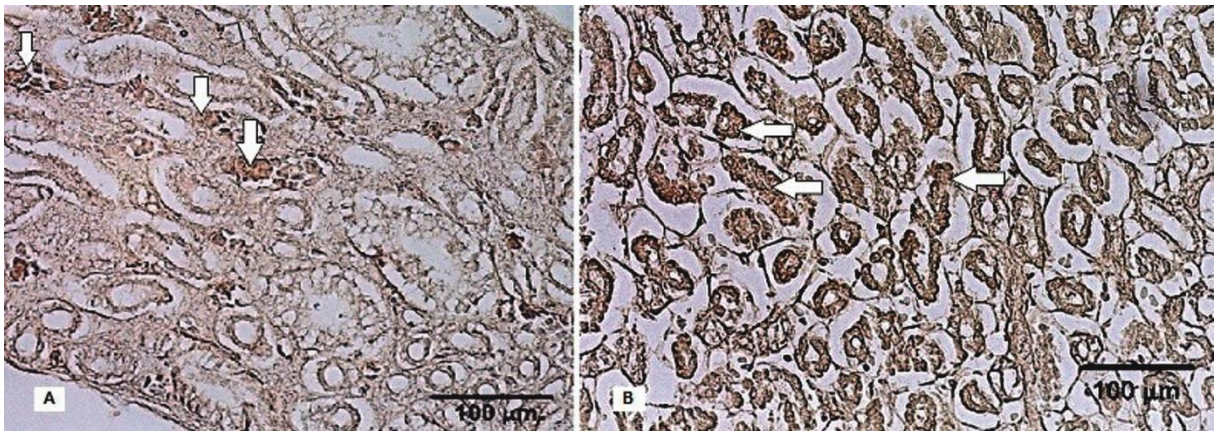

Figure 1. The immunolocalization of SGLT1 and SGLT2 in 14 days old ostriches kidneys: a) SGLT1 immunolocalized in straight proximal tubules in medullary rays (arrows). Bar:100 µm; b) SGLT2 in the renal cortical proximal tubules (arrows) of the 14 days old ostriches chicken. IHC, bar:100 µm.

Discussion

Kidneys contribute to glucose homeostasis by filtering and reabsorbing glucose (Mota *et al.,* 2015). In the kidneys, 100% of the filtered glucose in the glomerulus has to be reabsorbed along the nephron. Glucose is never secreted by a healthy nephron. If the plasma glucose concentration is too high (hyperglycemia), glucose is excreted in urine (glycosuria) because sodium-glucose co-transporters are saturated with the filtered glucose.

Among sodium-glucose co-transporters, SGLT1 and SGLT2 are responsible for glucose reabsorption from the glomerular filtrate. 'Knockout' of these transporters in mice and men results in the excretion of filtered glucose in the urine. SGLT1 is responsible for about 10% of the tubular glucose reabsorption (Wright *et al.*, 2007) and has been detected on the apical (urine) side of the proximal tubule where it facilitates the reabsorption of urinary glucose from the glomerular filtrate (Castaneda-Sceppa, Castaneda, 2011). In our study, SGLT1 was immunolocalized also in the proximal tubules in medullary rays and outer stripe in the kidneys of ostriches chicken.

Another member of the sodium-glucose linked transporters, SGLT2, detected in the early proximal tubules, is the primary renal glucose transporter. SGLT2 in conjunction with SGLT1 resorbs glucose into the blood from the forming urine. By inhibiting SGLT2, and not targeting SGLT1, glucose is excreted which in turn lowers blood glucose levels. Thus SGLT2 inhibitors, called *gliflozins*, are used in the treatment of type-2 diabetes (Song *et al.*, 2016). The selective SGLT1 inhibitor KGA 2727 has been developed as an antidiabetic agent, and it efficiently blocks the transporter function in cells overexpressing SGLT1 (Shibazaki *et al.*, 2012). LX4211, a dual SGLT1/SGLT2 inhibitor, improved glycemic control in patients with type-2 diabetes in a randomized, placebo-controlled trial (Zambrowicz *et al.*, 2012). LX4211 enhanced urinary glucose excretion by inhibiting SGLT2-mediated renal glucose reabsorption improving multiple measures of glycemic control, including fasting plasma glucose, oral glucose tolerance and significantly lowered serum triglycerides. In mammals, SGLT1 and SGLT2 have been noted in the brush border membrane of proximal tubule S1/S2 and S3 segments, respectively

(Aschenbach *et al.*, 2009). Different from rodents, the renal expression of SGLT1 has been noted to be absent in thick ascending limb of Henle (TALH) and macula densa in human (Vrhovac *et al.*, 2015). Our study revealed the renal locations of both transporters in 14 days old ostriches chicken resembled those in rats and mice – SGLT1 and SGLT2 were immunolocalized on the apical side of the cortical proximal tubules. By the previous studies, SGLT1 localized in straight proximal tubules in medullary rays corresponding to the S3 segment and SGLT2 in renal cortical proximal tubules corresponding to S1/S2 and S1 segments.

Conclusions

The current immunohistochemical study showed the SGLT1 and SGLT2 similar locations in the renal proximal tubules as previously detected in mammals. For a better understanding of the localization of the sodium-dependent glucose transporters in bird's kidneys, immunohistochemical studies on chicken in different ages should be carried out in future.

Acknowledgements
The authors wish to thank Mrs Mare Tamm for her laboratory assistance.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

PH, ID – conceptualization and planning;
ID – data collection;
PH, FP-P, TJ, ID – analysis and interpretation;
PH – writing – original draft preparation;
PH, FP-P, TJ, ID – writing – review and editing.
All authors have read and agreed to the final version of the manuscript.

References

Aschenbach, J. R., Steglich, K., Gäbel, G., Honscha, K.U. 2009. Exression of mRNA for glucose transport proteins in jejunum, liver, kidney and skeletal muscle in pigs. – Journal of Physiology and Biochemistry, 65(3):251–266. DOI: 10.1007/BF03180578

Bonora, B.M., Avogaro, A., Fadini, G.P. 2020. Extraglycemic Effects of SGLT2 Inhibitors: A Review of the Evidence. – Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 13:161–174. DOI: 10.2147/DMSO.S233538

Carson, F.L. 1997. Histotechnology: A Self-Instructional Text. (2nd ed.). – American Society for Clinical Pathology Press, Chicago, USA. 304 p.

Castaneda-Sceppa, C., Castaneda, F. 2011. Sodium-dependent glucose transporter protein as a potential therapeutic target for improving glycemic control in diabetes. – Nutrition Reviews, 69(12):720–729. DOI: 10.1111/j.1753-4887.2011.00423.x

Ghezzi, C., Loo, D.D.F., Wright, E.M. 2018. Physiology of renal glucose handling via SGLT1, SGLT2 and GLUT2. – Diabetologia, 61(10):2087–2097. DOI: 10.1007/s00125-018-4656-5

Haas, B., Eckstein, N., Pfeifer, V., Mayer, P., Hass, M.D. 2014. Efficacy, safety and regulatory status of SGLT2 inhibitors: focus on canagliflozin. – Nutrition & Diabetes, 4(11):e143. DOI: 10.1038/nutd.2014.40

Horiba, N., Masuda, S., Takeuchi, A., Takeuchi, D., Okuda, M., Inui, K. 2003. Cloning and characterization of a novel Na+-dependent glucose transporter (NaGLT1) in rat kidney. – Journal of Biological Chemistry, 278(17):14669–14676. DOI: 10.1074/jbc.M212240200

Hruby, V.J. 1997. Glucagon: Molecular Biology and Structure-Activity. Principles of Medical Biology. – In Molecular and Cellular Endocrinology. Bittar, E., Bittar, N. (Eds.). – JAI Press Inc., Greenwich, USA, Vol. 10, pp. 387–401.

Hussar, P., Suuroja, T., Hussar, Ü., Haviko, T. 2004. Transport proteins in rats' renal corpuscle and tubules. – Medicina (Kaunas), 40(7):650–656.

Miller, D., Bihler, I. 1961. The restrictions on possible mechanisms of intestinal transport of sugars. – In Proceedings of a Symposium Membrane Transport and Metabolism. Prague. Czechoslovakia. August 22–27, 1960 (Eds. A. Kleinzeller, A. Kotyk). Czech Academy of Sciences & Academic Press, Prague. Czechoslovakia. pp. 439–449.

Mota, M., Mota, E., Dinu, I.-R. 2015. The Role of the Kidney in Glucose Homeostasis. – In Treatment of Type 2 Diabetes. (Ed. C. Croniger). – IntechOpen, Rijeka, Croatia, pp. 13–17.

Shibazaki, T., Tomae, M., Ishikawa-Takemura, Y., Fushimi, N., Itoh, F., Yamada, M., Isaji, M. 2012. KGA-2727, a novel selective inhibitor of a high-affinity sodium glucose cotransporter (SGLT1), exhibits antidiabetic efficacy in rodent models. – Journal of Pharmacology and Experimental, 342(2):288–296. DOI: 10.1124/jpet.112.193045

Song, P., Onishi, A., Koepsell, H., Vallon, V. 2016. Sodium glucose cotransporter SGLT1 as a therapeutic target in diabetes mellitus. – Expert Opinion on Therapeutic Targets, 20(9):1109–1125. DOI: 10.1517/14728222.2016.1168808

Takata, K. 1996. Glucose transporters in the transepithelial transport of glucose. – Journal of Electron Microscopy, 45(4):275–284. DOI: 10.1093/oxfordjournals.jmicro.a023443

Vallon, V., Thomson, S.C. 2012. Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. – Annual Review of Physiology, 74:351–375. DOI: 10.1146/annurev-physiol-020911-153333

Vrhovac, I., Balen Eror, D., Klessen, D., Burger, C., Breljak, D., Kraus, O., Radović, N., Jadrijević, S., Aleksic, I., Walles, T., Sauvant, C., Sabolić, I., Koepsell, H. 2015. Localizations of Na(+)-D-glucose cotransporters SGLT1 and SGLT2 in human kidney and of SGLT1 in human small intestine, liver, lung, and heart. – Pflügers Archiv - European Journal of

Physiology, 467(9):1881–1898. DOI: 10.1007/s00424-014-1619-7

Wright, E.M. 2001. Renal Na(+)-glucose cotransporters. – American Journal of Physiology-Renal Physiology, 280(1):F10–F18. DOI: 10.1152/ajprenal.2001.280.1.F10

Wright, E.M., Hirayama, B.A., Loo, D.F. 2007. Active sugar transport in health and disease. – Journal of Internal Medicine, 261(1):32–43. DOI: 10.1111/j.1365-2796.2006.01746.x

You, G., Lee, W.S., Barros, E.J., Kanai, Y., Huo, T.L., Khawaja, S., Wells, R.G., Nigam, S.K., Hediger, M.A. 1995. Molecular characteristics of Na(+)-coupled glucose transporters in adult and embryonic rat kidney. – Journal of Biological Chemistry, 270(49):29365–29371. DOI: 10.1074/jbc.270.49.29365

Zambrowicz, B., Freiman, J., Brown, P.M., Frazier, K.S., Turnage, A., Bronner, J., Ruff, D., Shadoan, M., Banks, P., Mseeh, F., Rawlins, D.B., Goodwin, N.C., Mabon, R., Harrison, B.A., Wilson, A., Sands, A., Powell, D.R. 2012. LX4211, a dual SGLT1/SGLT2 inhibitor, improved glycemic control in patients with type 2 diabetes in a randomized, placebo-controlled trial. – Clinical Pharmacology & Therapeutics, 2:158–169. DOI: 10.1038/clpt.2012.58