In the changing internal and external conditions, maintenance of a constant internal environment – homeostasis – plays a significant role in the proper functioning of the organism. Kidneys play a key role in the homeostasis of glucose, in which the sodium-dependent glucose co-transporters contribute to renal glucose reabsorption. Although the localisation of Na+-glucose co-transporters has been extensively covered in animals’ kidneys, the localisation of the transporters in birds’ kidneys is still understudied. The purpose of this study was to immunolocalise the sodium-dependent co-transporters SGLT1 and SGLT2 in kidneys of ostrich chickens of different ages. In the study, kidney material derived from fifteen ostriches was divided equally into three age groups – 1-, 7-, and 14-days-old ostrich chickens. The polyclonal antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) served as primary antibodies and were used together with the IHC kit (Abcam, UK). With the AxioCam HRc camera (Germany) connected to the microscope Zeiss Axioplan-2 Imaging (Germany), the photos were taken and saved to the computer. As the result of the study on ostrich chickens of different ages, SGLT1 was noted to be localised in the renal straight proximal tubules and SGLT2 in the proximal convoluted tubules of nephron. The immunohistochemical locations of sodium-dependent glucose co-transporters revealed to be similar in ostriches’ kidneys of all age groups. The staining for SGLT2 was noted to be more intensive compared to the staining for SGLT1. As avian kidneys have unique morphological and functional features compared to animals, it is recommended that further studies would be performed on the renal tissue of different avian species.

Keywords: SGLT1, SGLT2, chicken, immunohistochemistry, renal proximal tubules
INTRODUCTION

The central source of energy in the body is glucose (Hruby, 1997). Kidneys contribute to glucose homeostasis by filtering and reabsorbing glucose by Na(+)–coupled glucose transporters. The sodium-dependent glucose co-transporter 1 (SGLT1) and sodium-dependent glucose co-transporter 2 (SGLT2) are the members of the solute carrier 5 (SLC 5A) gene family (Mota et al., 2015; Wright, 2021). SGLT1, encoded by the SLC 5A1 gene (Liang et al., 2021), has been reported as the high affinity Na+-glucose co-transporter, while SGLT2 encoded by the SLC 5A2 gene — as the low affinity Na+-glucose co-transporter (Kanai et al., 1994). In humans, SLC 5A1 gene encodes the production of the SGLT1 protein to line the epithelial cells of the kidney tubules of the nephron for glucose uptake into cells (Lodish et al., 2016). SGLT2, found solely in kidney tubules, resorbs the glomerular filtrate in the proximal tubule (Wright, 2021; Ghezzi et al., 2015). The locations of both transporters are based on particular functional differences. SGLT1 and SGLT2 in the kidneys has been investigated by immunohistochemistry using antibodies to the cloned transporters (Cramer et al., 1992; Kim, 2019; Vrhovac et al., 2015). The locations of both transporters are well-established in mammals — SGLT2, responsible for reabsorption of 80-90% of the glucose filtered by the glomerulus, has been described to be located in the beginning parts of the proximal tubule (Wright, 2021; Bonora et al., 2020), while SGLT1, responsible for the remaining glucose absorption, has been localised in more distal sections of the proximal tubule of the kidney (Sędzikowska & Szablewski, 2021; Uehara-Watanabe et al., 2022). In greater details, the proximal tubule can be divided into two sections known as pars convoluta and pars recta based on particular functional differences. The convoluted part can be divided into two segments designated as S1 and S2. In case of such marking, the pars recta of the proximal tubule are marked as S3. While SGLT1 is only found in the apical membrane of the S3 segment, SGLT2 in mammals has been noted to localise in the proximal tubules of the S1 and S2 segments (Ghezzi et al., 2018). The membrane proteins of SGLT1 and SGLT2 have been detected in the apical membrane of the renal tubular cells where they are responsible for glucose reabsorption from the glomerular filtrate in the proximal tubule.

Despite punctual description of Na+-glucose cotransporters in mammals’ kidneys, there are still large knowledge gaps regarding the localisation of the SGLTs in birds’ kidneys. This is explained by the fact that, unlike mammals, the avian kidneys have several unique morphological and functional features (Yang & Nishimura, 2021; El-Bakary et al., 2015). Due to the scarce information available on glucose transportation in avian kidneys on the molecular level, the purpose of this study was the immunohistochemical localisation of SGLT1 and SGLT2 in the kidneys of ostrich chickens of various ages in their first post-hatching weeks.

MATERIAL AND METHODS

Fifteen female African Black ostriches (Struthio camelus var. Domesticus) reared at the ostrich farm Ozolini AB, located in Latvia’s Jēkabpils district took part in the current study. The ostriches were kept in boxes with a heated sand floor. Commercial ostrich chick feed Strus Premium-Strus 1 (Cargill, Poland) and water ad libitum was available during the entire experimental period. Feed ingredients (%): barley — 36.8; oats — 10; wheat — 18.2; wheat bran — 5; rapeseed oil — 3; chalk — 2; soy pellets — 22; Doslolos StrusMix PS-3; Calculated chemical analysis, (%): Protein — 17.6, Carbohydrates — 37.2; Fat — 9.7; Cellulose — 5.6; Calcium — 0.8. Ostriches were divided into three age groups: 1-7, and 14-days-old. Every experimental group comprised five birds. On day 1, 7, and 14, according to the experimental groups, ostriches were anaesthetised by intramuscular injection of 1 ml solution holding equal volumes of 10% ketamine and 2% xylazine to reduce the pain before euthanasia. Thereafter, euthanasia by intracardial injection with 0.5 ml of 20% pentobarbital was conducted.

Tissue sections 0.5–1.0 cm in diameter were removed from renal cortex and medulla, fixed in 10% neutral buffered formalin at room temperature for 48 h, dehydrated in a tissue processor (TISSUE-TEK II, Japan) and embedded into paraffin according to a standardised histological procedure for tissue processing (Carson, 1997). Thereafter, slices 6 μm thick were cut using the microtome Microm HM360 (USA), floated on Poly-L-Lysine coated slides (O. Kindler GmbH, Freiburg, Germany), dried at 44 ºC for 12 h, followed by deparaffinisation with xylene. The rehydration of the slices was carried out in a graded series of ethanol and the immunohistochemical staining using the Immunohistochemistry kit (IHC kit, Abcam, UK) according to the manufacturer’s guidelines. The slices were pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6) for 20 minutes and incubated with primary polyclonal antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) in 1/1,000 dilution for 20 min at 37 ºC. Biotinylated secondary antibody and streptavidin-conjugated peroxidase were used for detection using DAB (3.3’-diaminobenzidine tetrahydrochloride) as chromogen. Negative controls contained antibody diluent (Dako, S 3002, Denmark) instead of primary antibodies. Human kidney tissue sections for identifying SGLT-2 and SGLT-1 were used as positive controls available for comparison on Abcam antibody producer’s homepage as examples for the antibodies’ immunohistochemistry on paraffin-embedded tissues (IHC-P) (Official website of Abcam, 2022).

The Zeiss Axioplan-2 Imaging Microscope (Germany) was established for photography of the slices. The photos were saved to the computer and analysed visually using the camera AxioCam HRc (Germany) connected to the microscope.
The experiments were performed according to the guidelines laid down by the European Communities Council Directive of 22 September 2010 (2010/63/EU) and the Ethical Committee of Latvia University of Life Sciences and Technologies has approved the experiments (protocol number 2014/2).

RESULTS AND DISCUSSION

The immunohistochemical locations of SGLT1 and SGLT2 in kidneys of ostriches of different ages was performed. The immunohistochemical study revealed the localisation of both types of sodium-dependent glucose co-transporters in chicken of all age groups — 1-, 7-, and 14-days old ostriches. Compared visually, in different experimental age groups the staining intensities and localisation of SGLT1 and SGLT2 appeared to be similar in the kidneys of ostrich chickens.

Immunolocalisation of SGLT1

The staining for SGLT1 was noted to be poor in renal corpuscles and in most of the renal tubules, as demonstrated in Figures 1a, 2a.

**Figure 1.** The immunolocalisation of SGLT1 and SGLT2 in 1-day-old ostrich chickens’ kidneys: a) SGLT1 immunolocalised in renal proximal tubules (arrows). Note the poorly stained renal corpuscle (asterisk). Obj. 200x; b) Renal cortical proximal tubules strongly stained for SGLT2 (arrows) of the 1-day old ostrich chickens’ kidneys. Renal corpuscles poorly stained for SGLT2 (asterisk). Obj. 200x

**Figure 2.** The immunolocalisation of SGLT1 and SGLT2 in 7-days-old ostrich chickens’ kidneys: a) SGLT1 immunolocalised in renal proximal tubules in medullary rays (arrows). Obj. 200x; b) SGLT2 (arrows) immunolocalised strongly in renal cortical proximal tubules of the 7-days-old ostrich chickens’ kidneys. Obj. 200x.

**Figure 3.** The immunolocalisation of SGLT1 and SGLT2 in 14-days-old ostrich chickens’ kidneys: a) SGLT1 immunolocalised in epithelial cells of the tubules of the outer stripe of renal medulla (arrows). Obj. 200x; b) Strong staining for SGLT2 (arrows) in renal cortical proximal tubules of the 14 days old ostrich chickens’ kidneys. Obj. 200x.
Immunolocalisation of SGLT2

Strong staining for SGLT2 was detected to be in the renal cortical proximal convoluted tubules (Figs. 1b, 2b, 3b) in chicken of different ages. Compared the intensities of the staining for SGLT1 and SGLT2, the staining for SGLT2 was noted to be more intensive.

Based on the branching pattern of the ureter, the avian kidney can be divided into renal lobes and renal lobules (Liebich, 2019). The medullary region, which drains into the secondary branches of the ureter together with the region of the cortex drained by that medullary tissue, forms the renal lobes. Including both the medullary tissue and the cortical tissue that it drains, each of the renal lobes is composed of several renal lobules. The renal lobules drain into ureter’s tertiary branches, which combine and form the secondary ureteral branches. Enclosing blood vessels and loops of Henle of juxtamedullary nephrons, the renal lobule’s medullary parts comprise bundles of collecting tubules (tubuli collectantes medullares). As in histological sections of avian kidney’s the renal lobes and lobules are seen at various levels, the cortical and medullary regions appear like intermingled. Like in mammals, nephrons are the smallest functional units of the kidney consisting of renal corpuscles and tubular apparatus. However, whilst the renal corpuscles are considerably smaller than in mammals, there is a higher amount of corpuscles per volume unit of kidney tissue (Koenig et al., 2016). The avian kidneys, divided into two zones, the cortex and medulla, have two types of nephrons unlike mammals, who have one type. These “reptilian-type” nephrons are located in the cortex without Henle’s loop, and the “mammalian-type” nephrons are in the medulla and have a Henle’s loop (Casotti & Braun, 2000; Cazimir et al., 2008). In birds that have a high ability for water conservation, the medullary regions (and thus the loops of Henle) are particularly well-developed, with each medullary region draining only a relatively small area of cortex. This arrangement presumably allows for production of more concentrated urine. The ability to produce hyperosmotic urine in avians is limited compared to that of mammals (Sjaastad et al., 2016). While loop nephrons produce concentrated urine, their contribution to ureteral urine is made by nephrons without loops (Ghezzi et al., 2018).Histologically, large cortical areas with small medullary islands can be identified. The number of medullary islands varies in different breeds of birds (Nickel et al., 2004).

In the organism, the kidneys are the main excretory and osmoregulatory homeostatic organs (Imenez Silva & Mohebbi, 2022). Kidneys contribute to the organism’s homeostasis by removing the metabolic waste substances from blood and their excretion in the urine, which prevents toxic waste products from accumulating in the body, and by regulating the inorganic ion and water balance through filtering water and harmful substances from blood (Yang & Nishimura, 2021). In the healthy kidneys, the total amount of the filtered glucose in the glomerulus has to be reabsorbed along the nephron (Mota et al., 2015). Sudden decrease in renal excretion, e.g., during acute renal impairment, is accompanied by retention of uremic toxins and metabolic residues in plasma and impaired regulation of fluid and electrolyte homeostasis, causing high morbidity and mortality (Nespoux et al., 2019). SGLT1 and SGLT2 take part in the renal glucose reabsorption from the glomerular filtrate.

The earlier research has shown that in healthy mammals, glucose is filtered by the renal glomeruli and the filtered glucose is reabsorbed in the tubular system mainly by the proximal tubules (tubuli proximalis) (Nespoux et al., 2019). In the S1/S2 segments of the proximal tubules, apical low-affinity Na+-glucose co-transporter SGLT2 handles the major glucose uptake to the cytoplasm from the lumen (Vallon et al., 2011; Umino et al., 2018). The glucose that was not resorbed by SGLT2 reaching to the proximal tubule’s S2/S3 segment will be resorbed by SGLT1, GLUT1, and GLUT2 (Rieg et al., 2014). As the SGLT-mediated glucose transport depends on Na+ concentration gradient, SGLT1 serves as a minor active transporter. Together with SGLT1, SGLT2 resorbs glucose from blood-forming urine. It has been noted that of all tubular glucose reabsorption, SGLT1 accounts for about 10% (Koepsell & Vallon, 2020). In rats, SGLT1 has been identified on the apical side of the epithelial cells of the renal proximal tubules. In mammals’ kidneys, SGLT1 and SGLT2 have been localised in the S1/S2 and S3 segments of the proximal tubules, noted in the brush border membranes of the tubular epithelial cells. Comparing the results previously described for immunolocalisation of the Na+-glucose co-transporters SGLT1 and SGLT2 in mammalian kidney tissue with current data in ostrich kidneys, immunolocalisation was found to be similar (Sen & Heerspink, 2021; Vallon et al., 2011; Sano et al., 2020).

When comparing the intensity of SGLT1 and SGLT2 staining, it was noted that SGLT2 staining was more intense compared to the intensity of SGLT1 staining, which is due to greater reabsorption of glucose through SGLT2 in the nephron (Vallon et al., 2011).

CONCLUSIONS

In the current immunohistochemical study in ostrich chickens, localisation in the kidneys of SGLT1 and SGLT2 was revealed. The immunolocalisation of SGLT1 and SGLT2 in chickens of different ages was similar: SGLT1 was immunolocalised on the apical side of the epithelial cells of the direct proximal tubules in the brain rays (corresponding to the S3 segment), while SGLT2 was immunolocalised in the convoluted proximal tubules in the cortical region (corresponding to the S1/S2 segments). Due to the lack of knowledge about Na+-glucose co-transporters in avian kidneys, it would be highly informative to conduct additional comparative studies on the immunolocalisation of sodium-dependent glucose co-transporters in ostrich kidneys, as well as in the kidney tissue of other bird species, since avian kidneys have many unique morphological and functional features compared to animals.

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REFERENCES


Порівняльне дослідження натрій-залежних котранспортерів глюкози в нирках страусових куриць

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Анотація. У змінних внутрішніх та зовнішніх умовах підтримання постійної внутрішньої середовища — гомеостазу — грає головну роль правильному функціонуванні організму. В організмі нирки відіграють важливу роль у гомеостазі глюкози, при цьому натрійзалежні котранспортери сприяють реабсорбції глюкози у нирках. Хоча локалізація котранспортерів Na+-глюкози у нирках тварин широко охарактеризована, досі недостатньо інформації про локалізації транспортерів у нирках птахів. Метою цього дослідження була імунолокалізація натрійзалежних котранспортерів SGLT1 і SGLT2 у нирках курчат страусів різного віку. Під час дослідження нирковий матеріал отримано від 15 страусів, розділеного порівну на три вікові групи — 1-добові, 7-денні та 14-денні страусові курчата. Матеріал діаметром 0,5−1,0 см фіксували в 10 % забуференому нейтральному формаліні, зневоднювали, заливали в парафін; після цього вирізали і депарафінізували зрізи товщиною 6 мкм з подальшим імуногістохімічним забарвленням поліклональними первинними антитілами Rabbit anti- SGLT1 і Rabbit anti- SGLT2 (Abcam, Великобританія) відповідно до рекомендацій виробника (IHC kit, Abcam, UK). Фотографії препаратів були зроблені мікроскопом Zeiss Axioplan-2 Imaging (Німеччина) та збережені на комп'ютер для аналізу під візуальним контролем за допомогою камери (AxioCam HRc, Німеччина), підключеної до мікроскопа. У нашому дослідженні виявлена імуногістохімічна локалізація SGLT1 у епітеліальних клітинах прямих проксимальних канальців мозкових променів та SGLT2 у проксимальних звивистих канальцях нефрону. Виявлена подібність імуногістохімічної локалізації натрійзалежних котранспортерів глюкози у нирках страусів усіх вікових груп. Відзначено, що фарбування SGLT2 було більш інтенсивним, ніж фарбування SGLT1. Оскільки нирки птахів мають унікальні морфологічні та функціональні особливості в порівнянні з тваринами, рекомендується проводити подальші дослідження ниркової тканини різних видів птахів.

Ключові слова: SGLT1, SGLT2, курка, імуногістохімія, проксимальні канальці нирок