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The Role of Uroguanylin in Regulation of Ion Transport in Salivary Glands

Uloga urogvanilina u regulaciji prijenosa iona u žlijezdama slinovnicama

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Abstract

Objective of work: Guanylin peptides are considered to be the only intrinsic regulators of salivary glands secretion. Therefore, the aim of this study is to determine the effects of systemic uroguanylin (UGN) of the salivary flow and ion composition as well as if those effects include activation of guanylate cyclase C (GC-C). **Materials and Methods:** This study was conducted on 7 months old C57Bl6NCrl (wild type, WT) and GC-C knockout (KO) mice. Salivary flow rate and ion composition were determined after pilocarpine stimulation with UGN (30 µg/animal) or saline i.p. application. The expression of mRNA for AQPs, NHEs, NBCn1, Slc26a3/a6 and CFTR were determined by qPCR in submandibular salivary glands. **Results:** When applied i.p., UGN decreases the pilocarpine stimulated saliva flow rate and increased concentration of Na^+, H^+ and Cl^-. In GC-C KO mice, UGN shows no effect on saliva flow rate, while the concentrations of Na^+, H^+ and Cl^- are the same in GC-C KO littermates when compared to WT mice. UGN increased expression of Slc26a6 while in GC-C KO mice Slc26a6 had a higher expression when compared to WT mice, suggesting involvement of GC-C independent signalling pathway for UGN. The difference in Slc26a6 in GC-C KO mice is not unique for salivary glands because it was found also in duodenum and kidney cortex. **Conclusions:** The effects of UGN via basolateral membrane of salivary glands cells have not been considered up to date. In our study, UGN, when applied i.p., decreased salivary flow rate, pH, and changed composition of other ions. Therefore, plasma UGN an hour after a meal could have physiological and pathological importance (development of cavities, inflammations or demineralisations) and inhibition of systemic UGN effects could be consider as a new approach in treatment of those conditions.

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Introduction

Uroguanylin (UGN) is a member of the guanylin peptides family which belongs to the family of naiuretic peptides. UGN is a small peptide composed of 16 amino acids and has two disulfide bonds (1). Gene for UGN (GUCA2B) is located at the 1st chromosome in humans and 4th chromosome in mice (2). UGN is expressed in the intestine and after a meal is secreted in gut lumen and blood (3). In the intestine lumen, UGN binds to guanylate cyclase C (GC-C) receptor located at the luminal membrane of enterocytes, which leads to an increase in intracellular cGMP concentration (4, 5). Finally, UGN stimulates Cl^- and HCO_3^- secretion via the Cystic

Uvod

Urogvanilin (UGN) je član obitelji gvanilinskih peptida koja pripada obitelji natrijuretskih peptida. UGN je mali peptid sastavljen od 16 aminokiselina i ima dvije disulfidne veze (1). Gen za UGN (GUCA2B) nalazi se na 1. kromosomu kod ljudi i 4. kromosomu kod miševa (2). UGN je izražen u crijevima, a nakon obroka izlučuje se u lumen crijeva i krv (3). U lumenu crijeva UGN se veže na receptor gvanilat ciklazu C (GC-C) koji se nalazi na luminalnoj membrani enterocita, što dovodi do povećanja unutarstanične koncentracije cGMP (4, 5). Konačno, UGN stimulira lučenje Cl^- i HCO_3^- putem transmembranskog regulatora provodljivosti

fibrosis transmembrane conductance regulator (CFTR) and Slc26a3/a6 and inhibition of Na^+ reabsorption by Na^+/H^+ exchanger (4-10).

UGN and its GC-C dependent signalling pathway are also expressed in the brain, kidneys, heart, pancreas, lungs, reproductive system, spleen, lymph nodes, lungs and airways as well as in salivary glands (parotid and submandibular glands) (6-8, 11-21).

The existence of guanylin peptides and the GC-C signalling pathway in salivary glands has been known for more than two decades. Kulaksiz et al. showed expression of guanylin peptides, GC-C, cGMP dependent protein kinase II, and CFTR in the human parotid and submandibular glands. Due to importance of CFTR in salivary glands physiology, detection of differences in saliva composition can serve as diagnostic tools for cystic fibrosis. Saliva as diagnostic tool can be also used in verity of other systemic diseases (Sjogren's syndrome, cardiovascular diseases, diabetes or idiopathic infertility) (22, 23). GC-C is located at the apical membrane of the ducts of salivary glands. Its agonists, guanylin and UGN, are found in the cells of the intralobular and interlobular ducts in small vesicles at the apical part of the secretory epithelial cells (12, 24). The physiological function of guanylin peptides and regulation of their expression is unknown.

In addition to the GC-C dependent signalling pathway, guanylin peptides activate yet another signalling pathway which is located at the basolateral membrane of the target epithelial cells allowing hormones from the blood to exert their function (25). The existence of this, cGMP independent signalling pathway, has been found in the kidneys, intestine, and brain. Activation of this signalling pathway leads to an increase in intracellular Ca^{2+} concentration but also a decrease in intracellular cAMP concentration (26-31).

Since guanylin peptides are considered only as potential intrinsic regulators of salivary glands secretion (12), the aim of this study is to determine the effects of systemic UGN of the salivary flow and ion composition. Performing experiments on GC-C KO mice we will clarify if the UGN function via GC-C or other signalling pathway.

Material and methods

Ethical approval

Experimental procedures used in this study were approved by the National Ethical Committee Ministry of Agriculture (UP/I-322-01/22-01/36) and the School of Medicine University of Zagreb (641-01/22-02/01). All experiments were performed in accordance with ARRIVE guidelines and followed the Ethical Codex of Croatian Society for Laboratory Animal Science. Special efforts were done to minimize animal suffering and reduce the number of animals used.

Animals

Experiments were performed on the C57Bl6NCrl (wild type, WT) male mice and littermates derived from GC-C knock out mice (GC-C KO) 28.7 ± 1.3 weeks old. GC-C KO mice were a donation from Dr. K. A. Steinbrecher

cistične fibroze (eng. *Cystic fibrosis transmembrane conductance regulator*, CFTR) i Slc26a3/a6 te inhibira reapsorpciju Na^+ putem Na^+/H^+ izmjenjivača (4-10).

UGN i njegov signalni put ovisan o GC-C-u također se nalazi u mozgu, bubrezima, srcu, gušterači, plućima, reproduktivnom sustavu, slezeni, limfnim čvorovima, plućima i dišnim putovima kao i u žlijezdama slinovnicama (parotidne i submandibularne žlijezde) (6 – 8, 11 – 21).

Postojanje gvanilinskih peptida i GC-C signalnog puta u žlijezdama slinovnicama poznato je više od dva desetljeća. Kulaksiz i sur. pokazali su izražaj gvanilinskih peptida, GC-C-a, cGMP ovisne protein kinaze II i CFTR-a u ljudskim parotidnim i submandibularnim žlijezdama. Zbog važnosti CFTR-a u fiziologiji žlijezda slinovnica, otkrivanje razlika u sastavu sline može poslužiti kao dijagnostički alat za cističnu fibrozu. Slina kao dijagnostičko sredstvo može se koristiti i kod većine drugih sistemskih bolesti (Sjogrenov sindrom, kardiovaskularne bolesti, dijabetes ili idiopatska neplodnost) (22, 23). GC-C je smješten na apikalnoj membrani izvodnih kanala žlijezda slinovnica. Njegovi agonisti, gvanilin i UGN, smješteni su u malim vezikulama na apikalnom dijelu sekretornih epitelnih stanica intralobularnih i interlobularnih duktusa (12, 24). Nepoznata je fiziološka uloga gvanilinskih peptida te regulacija njihova izražaja.

Uz signalni put ovisan o GC-C, gvanilinski peptidi aktiviraju još jedan signalni put koji se nalazi na bazolateralnoj membrani ciljnih epitelnih stanica omogućujući djelovanje hormona (25). Postojanje ovog signalnog puta neovisnog o cGMP-u pronađeno je u bubrezima, crijevima i mozgu. Njegova aktivacija uzrokuje povećanje unutarstanične koncentracije Ca^{2+}, ali i smanjenje unutarstanične koncentracije cAMP (26-31).

Budući da se gvanilinski peptidi smatraju samo potencijalnim unutrašnjim regulatorima lučenja žlijezda slinovnica (12), cilj ovog istraživanja je utvrditi učinke sistemskog UGN-a na ionski sastav i protok sline. Izvođenjem istraživanja na miševima GC-C KO razjasnit ćemo funkcionira li UGN preko GC-C ili nekoga drugog signalnog puta.

Materijal i metode

Etičko odobrenje

Metode korištene u ovoj studiji odobrilo je Etičko povjerenstvo za zaštitu životinja koje se koriste u znanstvene svrhe Ministarstva poljoprivrede (UP/I-322-01/22-01/36) i Medicinskog fakulteta Sveučilišta u Zagrebu (641-01/22-02/01). Svi pokusi provedeni su u skladu s ARRIVE smjernicama i prema Etičkom kodeksu Hrvatskog društva za znanost o laboratorijskim životinjama. Posebni napori uloženi su kako bi se smanjila patnja životinja te smanjio broj korištenih životinja.

Životinje

Studija je provedena na C57Bl6NCrl (divlji tip, WT) mužjacima miševa i potomcima iz legala dobivenim od miševa kojima nedostaje GC-C (GC-C KO) starih 28,7 ± 1,3 tjedna. GC-C KO miševi bili su donacija dr. K. A. Ste-

(Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA) and were crossbred with the C57Bl6NCrl strain. Obtained heterozygotes were crossbred with each other, after which their offspring were genotyped and knockout (GC-C KO) and wild type (GC-C WT) littermates were used in this study. The animals were housed in our animal facilities (HR-POK-006) under controlled environmental conditions and under the 12-hour light-dark cycle. All animals were fed *ad libitum* with standard rodent chow and had free access to water.

Sialometry

Before sialometry (measurement of stimulated saliva flow rate), mice were fasted for 2 hours, with free access to water to exclude saliva production due to feeding. Animals were first anesthetized with an i.p. injection of ketamine and xylazine (80-100 mg/kg, 5-10 mg/kg, respectively) (IACUC Guidelines: Anaesthesia) and immobilized on a heated pad to prevent anaesthesia-related hypothermia. After induction of anesthesia, to experimental group of WT mice (n = 8) human UGN (30 µg/animal in 250 µl of saline) (PeptaNova GmbH, Sandhausen, Germany) were administered i.p., while the control group of WT mice (n = 8) received i.p. 250 µl of saline (0.9% NaCl; Croatian Institute for Transfusion Medicine, Zagreb, Croatia). Both GC-C WT (n = 7) and GC-C KO (n = 6) mice were administered the same amount of UGN as the experimental group of WT mice. After fifteen minutes, to stimulate salivation, the cholinergic agonist, pilocarpine hydrochloride (0.001mg/g; Fagron Hrvatska d.o.o., Donja Zelina, Croatia), was administered i.p. (32).

After fifteen minutes, the animals were positioned so that the animal's head was tilted lower than the tail, to reduce the possibility of deglutition and aspiration of saliva. Saliva was collected with filter paper (Medical Intertrade, Sveta Nedelja, Croatia) cut into strips positioned behind the upper and lower incisors. Saliva was collected for 15 min, after which the strips were inserted in test tubes (ThermoFisher Scientific, Waltham, Massachusetts, USA) and centrifuged (15 000 g, 24°C). Supernatant was collected and the amount of saliva was determined gravimetrically (Kern & Sohn GmbH, Balingen, Germany). The collected samples were stored at -20°C until further analysis. The saliva flow rate was calculated by g of animal weight in 15 minutes.

Still in anaesthesia, both experimental groups of WT mice were sacrificed by cervical dislocation 1h after UGN or saline application while GC-C WT and GC-C KO animals 3h after UGN application to determine the effect of the GC-C independent signalling pathway. Submandibular salivary glands, duodenum and kidneys were isolated. The kidney cortex was carefully dissected and tissue was flash frozen in liquid nitrogen and stored at -80°C until use.

pH and ion measurements in saliva: pH was measured potentiometrically using dedicated pH-sensors on the blood gas analyzer GEM Premier 4000 (Instrumentation Laboratory, Bedfor, USA). Concentrations of Na^+, K^+ and Cl^- were determined by indirect potentiometry using original manufacturer's reagents and protocols on the fully-automated analyzer Alinity c (Abbott Laboratories, Chicago, USA).

inbrechera (Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, SAD) i križani su sa sojem C57Bl6NCrl. Dobiveni heterozigoti međusobno su križani, nakon čega su njihovi potomci genotipizirani te su u ovom istraživanju korišteni GC-C KO miševi i divlji tip (GC-C WT) miševa iz istog legla. Životinje su bile smještene u našoj životinjskoj nastambi (HR-POK-006) u kontroliranim uvjetima okoline i pod 12-satnim ciklusom svjetlo-tama. Sve su životinje hranjene *ad libitum* standardnom hranom za glodavce i imale su slobodan pristup vodi.

Sijalometrija

Dva sata prije izvođenja sijalometrije (mjerenje stimuliranog protoka sline), miševi su stavljeni na gladovanje uz slobodnim pristup vodi kako bi se isključila proizvodnja sline zbog hranjenja. Životinje su prvo anestezirane i.p. injekcijom ketamina i ksilazina (80-100 mg/kg, odnosno 5-10 mg/kg) (smjernice IACUC-a: Anestezija) i imobilizirani na grijanoj podlozi kako bi se spriječila anestezijom izazvana hipotermija. Nakon uvoda u anesteziju, pokusnoj skupini WT miševa (n = 8) primijenjen je ljudski UGN i.p. (30 µg/životinji u 250 µl fiziološke otopine) (PeptaNova GmbH, Sandhausen, Njemačka), dok je kontrolnoj skupini WT miševa (n = 8) primijenjen i.p. 250 µl fiziološke otopine (0,9% NaCl; Hrvatski zavod za transfuzijsku medicinu, Zagreb, Hrvatska). I GC-C WT (n = 7) i GC-C KO (n = 6) miševi primili su istu količinu UGN kao pokusna skupina WT miševa. Nakon petnaest minuta, za poticanje salivacije, i.p. je primijenjen kolinergički agonist pilokarpin hidroklorid (0,001 mg/g; Fagron Hrvatska doo, Donja Zelina, Hrvatska) (32).

Nakon petnaest minuta, životinje su postavljene tako da im je glava bila nagnuta niže od repa, kako bi se smanjila mogućnost gutanja i aspiracije sline. Slina je sakupljena filtar- papirom (Medical Intertrade, Sveta Nedelja, Hrvatska) izrezanim na vrpce koje su bile postavljene iza gornjih i donjih sjekutića. Slina je sakupljana 15 minuta, nakon čega su vrpce umetnute u epruvete (ThermoFisher Scientific, Waltham, Massachusetts, SAD) i centrifugirane (15 000 g, 24 °C). Nadtalog je sakupljen i količina sline određena je gravimetrijski (Kern & Sohn GmbH, Balingen, Njemačka). Za dodatnu analizu, prikupljeni uzorci pohranjeni su na -20 °C. Brzina protoka sline izračunata je prema g težine životinje u 15 minuta.

Još uvijek u anesteziji, obje pokusne skupine WT miševa žrtvovane su dislokacijom vrata 1 sat nakon primjene UGN-a ili fiziološke otopine, a životinje GC-C WT i GC-C KO 3 sata nakon primjene UGN-a kako bi se odredio učinak GC-C neovisnog signalnog puta. Izolirane su submandibularne žlijezde slinovnice, duodenum i bubrezi. Korteks bubrega pažljivo je odvojen, a tkivo je brzo zamrznuto u tekućem dušiku i pohranjeno na -80 °C za kasnije korištenje.

Mjerenja pH i iona u slini: pH mjeren je potenciometrijski korištenjem namjenskih pH-senzora na plinskom analizatoru krvi GEM Premier 4000 (Instrumentation Laboratory, Bedfor, SAD). Koncentracije Na^+, K^+ i Cl^- određene su neizravnom potenciometrijom korištenjem originalnih reagensa i protokola proizvođača na potpuno automatiziranom analizatoru Alinity c (Abbott Laboratories, Chicago, SAD).

Expression of ion transporters and channels for water mRNA

Total RNA was isolated in the following manner. Tissue samples (100 mg) were homogenized in 1 mL Trizol solution (ThermoFisher Scientific) with an ultrasonicator (QSonica CL188, Newtown, Connecticut, USA). After the addition of 0.2 mL of chloroform (Kemika, Zagreb. Croatia), tissue samples were incubated for 5 min at room temperature and centrifuged (15 min, +4°C, 12 000 g). Supernatant containing RNA was transferred to a new test tube and 0.5 mL of isopropyl alcohol (Carl Roth, Karlsruhe, Germany) was added. After centrifugation (15 min, +4°C, 12 000 g), the supernatant was removed and the precipitate was washed with 1 mL of ethanol (75%; Claro-Prom, Zagreb, Croatia). Samples were briefly centrifuged (5 min, +4°C, 8 000 g), ethanol was removed and the samples were left to air-dry. Isolated RNA was dissolved in sterile dH_2O and quantified with the NanoDrop ND-1000 spectrophotometer (Marshall Scientific LLC, Hampton, New Hampshire, USA).

RNA (1 µg) was transcribed into complementary DNA (cDNA) by using the GoScriptTM Reverse Transcription System (Madison, Wisconsin, USA) in the following way: 5 µL RNA (1 µg), Oligo(dt)primers (1 µL) and H_2O were incubated at 70°C for 5 min and subsequently cooled to +4°C. Afterwards, GoScript reaction buffer (4 µL), $MgCl_2$ (2 µL), nucleotide mixture (1 µL), ribonuclease inhibitors (0.5 µL), GoScript reverse transcriptase (1 µL) and H_2O (6.5 µL) were added. The reaction mixture was then incubated at 25°C (5 min), 42°C (60 min) and 70°C (15 min).

TaqMan Real-Time PCR Assay (ThermoFisher Scientific) was used for quantitative expression of ion transporters and channels for water. Probes specific to aquaporins 1 (AQP1; Mm00431834_m1), 3 (AQP3; Mm01208559_m1) and 5 (AQP5; Mm00437578_m1), sodium-hydrogen exchanger 1 (NHE1; Mm00444270_m1) and 3 (NHE3; Mm01352473_m1), electroneutral sodium-bicarbonate cotransporter 1 (NBCn1; Mm01310972_m1), solute carrier family 26 members 3 (Slc26a3; Mm00445313_m1) solute carrier family 26 members 6 (Slc26a6; Mm00506742_m1), and cystic fibrosis transmembrane conductance regulator (CFTR; Mm00445197_m1) were used to quantify gene expression. As a housekeeping gene, we used beta-actin (ActB; Mm00607939_s1). A reaction mixture, containing TaqMan Master Mix (10 µL), labelled primers (1 µL), cDNA (1 µL) and H_2O (8 µL) was added to a 96-well plate, briefly centrifuged (5 min, 250 g) and placed in the 7500 Real-Time PCR System (ThermoFisher Scientific). Obtained results were normalized according to the tissue expression of ActB.

Statistical analysis

The Kolmogorov-Smirnov test was used to test the normal distribution. Student's unpaired *t* tests were used with each effect compared with its own control. The data was presented as mean ± SEM. $p < 0.05$ was considered statistically significant. A correlation was calculated using Pearson's correlation test. The GraphPad Instat statistical software (GraphPad Software, Boston, MA, USA) was used for statistical analyses.

Izražaj mRNA za ionske transportere i kanale za vodu

Ukupna RNA je izolirana na sljedeći način. Uzorci tkiva (100 mg) homogenizirani su u 1 mL otopine Trizol (ThermoFisher Scientific) koristeći ultrasonifikator (QSonica CL188, Newtown, Connecticut, SAD). Nakon dodatka 0,2 mL kloroforma (Kemika, Zagreb. Hrvatska), uzorci tkiva su inkubirani 5 min na sobnoj temperaturi i centrifugirani (15 min, +4°C, 12 000 g). Nadtalog, koji je sadržavao RNA, prebačen je u novu epruvetu u koji je dodano je 0,5 mL izopropilnog alkohola (Carl Roth, Karlsruhe, Njemačka). Nakon centrifugiranja (15 min, +4°C, 12 000 g), nadtalog je uklonjen, a talog ispran s 1 mL etanola (75%; Claro-Prom, Zagreb, Hrvatska). Uzorci su kratko centrifugirani (5 min, +4°C, 8 000 g), etanol je uklonjen, a uzorci su ostavljeni da se osuše na zraku. Izolirana RNA je otopljena u sterilnoj dH_2O i kvantificirana spektrofotometrom NanoDrop ND-1000 (Marshall Scientific LLC, Hampton, New Hampshire, SAD).

RNA (1 µg) prepisana je u komplementarnu DNA (cDNA) korištenjem GoScriptTM Reverse Transcription System (Madison, Wisconsin, SAD) na sljedeći način: 5 µL RNA (1 µg), Oligo(dt)primeri (1 µL) i H_2O su inkubirani na 70°C 5 minuta i potom ohlađeni na +4°C. Nakon toga su dodani GoScript reakcijski pufer (4 µL), $MgCl_2$ (2 µL), smjesa nukleotida (1 µL), inhibitori ribonukleaze (0,5 µL), GoScript reverzna transkriptaza (1 µL) i H_2O (6,5 µL). Reakcijska smjesa je zatim inkubirana na 25°C (5 min), 42°C (60 min) i 70°C (15 min).

TaqMan Real-Time PCR test (ThermoFisher Scientific) korišten je za kvantitativno određivanje izražaja ionskih transportera i kanala za vodu. Probe specifične za akvaporine 1 (AQP1; Mm00431834_m1), 3 (AQP3; Mm01208559_m1) i 5 (AQP5; Mm00437578_m1), izmjenjivače natrij/vodik 1 (NHE1; Mm00444270_m1) i 3 (NHE3; Mm01352473_m1), elektroneutralni natrij/hidrogenkarbonatni kotransporter 1 (NBCn1; Mm01310972_m1), članove 3 (Slc26a3; Mm00445313_m1) i 6 (Slc26a6; Mm00506742_m1) obitelj 26 Slc transportera te transmembranskog regulatora provodljivosti cistične fibroze (CFTR; Mm00445197_m1) korišteni su za kvantificiranje izražaja gena. Kao kontrolni gen koristili smo beta-aktin (ActB; Mm00607939_s1). Reakcijska smjesa koja je sadržavala TaqMan Master Mix (10 µL), označene početnice (1 µL), cDNA (1 µL) i H_2O (8 µL) dodana je u jažice pločice s 96 jažica, kratko centrifugirana (5 min, 250 g) te je pločica postavljena u 7500 Real-Time PCR System (ThermoFisher Scientific). Dobiveni rezultati normalizirani su prema tkivnoj ekspresiji ActB-a.

Statistička analiza

Za testiranje rezultata koji imaju normalnu distribuciju korišten je Kolmogorov-Smirnov test. Studentovi ne-pareni *t* testovi korišteni su za svaki učinak u usporedbi s vlastitom kontrolom. Podaci su prikazani kao srednja vrijednost ± SEM. $p < 0,05$ smatra se statistički značajnim. Korelacija je izračunata pomoću Pearsonovog testa korelacije. Za statističke analize korišten je statistički softver GraphPad Instat (GraphPad Software, Boston, MA, SAD).

Results

Uroguanylin decreases the pilocarpine stimulated saliva flow rate and changes saliva ion composition

The ion content of the saliva of seven months old WT mice, upon pilocarpine stimulation is: pH = 7.94 ± 0.03; [Na^+] = 48 ± 5 mmol/L; [K^+] = 38 ± 4 mmol/L; [Cl^-] = 86 ± 3 mmol/L (n = 6 - 8).

UGN was applied i.p. and the pilocarpine stimulated saliva production and saliva composition was determined 1 h after application. UGN inhibits salivary production in WT mice (WT Control: 8.9 ± 0.4; WT UGN: 7.5 ± 0.3 mL/g body weight/15 min, n = 8, p = 0.022). In animals which do not have GC-C, this effect was abolished (GC-C WT UGN: 7.6 ± 0.5; GC-C KO UGN: 9.2 ± 0.3 mL/g body weight/15 min, n = 6, p = 0.023) (Figure 1A). UGN also decreased a pH (WT Control: 7.94 ± 0.03; WT UGN: 7.68 ± 0.06, n = 6 and 7 respectfully, p = 0.004, which is an increase in H^+ concentration from 11.5 to 20.1 nM/L), but this effect is still present in GC-C KO mice (GC-C WT: 7.67 ± 0.07; GC-C KO: 7.75 ± 0.06 n = 7 and 6 respectfully, p = 0.419), suggesting involvement of GC-C independent signalling pathway.

UGN also changed saliva ion composition by increasing Na^+ and Cl^- concentration (Na^+: WT Control: 48 ± 5; WT UGN: 60 ± 3 mmol/L, n = 8, p = 0.046; Cl^-: WT Control: 86 ± 3 WT UGN: 95 ± 2 mmol/L, n = 7 and 8 respectively, p = 0.030). Those effects are still present in GC-C KO mice (Na^+: GC-C WT: 64 ± 6; GC-C KO: 60 ± 3 n = 7 and 6 respectfully, p = 0.562; Cl^-: GC-C WT: 99 ± 5; GC-C KO: 94 ± 3 n = 7 and 6 respectfully, p = 0.483). UGN had no effect on the saliva concentration of the K^+ (Figure 2).

Positive correlation of the salivary flow vs pH and K^+ concentrations in UGN stimulated mice

Passing through the ducts of salivary glands Na^+ and Cl^- are absorbed and K^+ and HCO_3^- are secreted. In the human parotid gland, the concentration of Na^+ and Cl^- is in positive and K^+ is in negative correlation to saliva flow rate (33). In this study, after pilocarpine stimulation of salivary production in 7 months old WT control mice, there is no statistically significant correlation of ion concentration to salivary flow (Table 1).

Upon UGN stimulation, pH and K^+ concentration in pilocarpine stimulated saliva were in positive correlation to salivary flow (Table 1, $p < 0.05$ statistically significant correlation). The changes in pH could occur due to changes in the function of H^+ or HCO_3^- transporters.

Rezultati

Urogvanilin smanjuje brzinu protoka sline stimuliranu pilokarpinom i mijenja njenzin ionski sastav

Sadržaj iona u slini sedmomjesečnih WT miševa, nakon stimulacije pilokarpinom, iznosi: pH = 7,94 ± 0,03; [Na^+] = 48 ± 5 mmol/L; [K^+] = 38 ± 4 mmol/L; [Cl^-] = 86 ± 3 mmol/L (n = 6 - 8).

UGN je primijenjen i.p. a pilokarpinom stimulirana proizvodnja sline i sastav sline određeni su 1 sat nakon primjene. UGN inhibira proizvodnju sline u WT miševa (WT Kontrola: 8,9 ± 0,4; WT UGN: 7,5 ± 0,3 mL/g tjelesne mase/15 min, n = 8, p = 0,022). Kod životinja koje nemaju GC-C ovaj učinak nije primijećen (GC-C WT UGN: 7,6 ± 0,5; GC-C KO UGN: 9,2 ± 0,3 mL/g tjelesne mase/15 min, n = 6, p = 0,023) (Slika 1A). UGN je također snizio pH (WT Kontrola: 7,94 ± 0,03; WT UGN: 7,68 ± 0,06, n = 6 odnosno 7, p = 0,004, što je povećanje koncentracije H^+ s 11,5 na 20,1 nM/L), ali je ovaj učinak UGN-a još uvijek prisutan u GC-C KO miševa (GC-C WT: 7,67 ± 0,07; GC-C KO: 7,75 ± 0,06 n = 7 odnosno 6, p = 0,419), što upućuje na aktivaciju GC-C neovisnog signalnog puta.

UGN je također promijenio ionski sastav sline povećanjem koncentracije Na^+ i Cl^- (Na^+: WT Kontrola: 48 ± 5; WT UGN: 60 ± 3 mmol/L, n = 8, p = 0,046; Cl^-: WT Kontrola: 86 ± 3 WT UGN: 95 ± 2 mmol/L, n = 7 odnosno 8, p = 0,030). Ti su učinci još uvijek prisutni u GC-C KO miševa (Na^+: GC-C WT: 64 ± 6; GC-C KO: 60 ± 3 n = 7 odnosno 6, p = 0,562; Cl^-: GC-C WT: 99 ± 5; GC-C KO: 94 ± 3 n = 7 odnosno 6, p = 0,483). UGN nije imao utjecaja na koncentraciju K^+ u slini (Slika 2).

Pozitivna korelacija protoka sline u odnosu na pH i koncentraciju K^+ u miševa stimuliranih UGN-om

Prolaskom kroz izvodne kanaliće žlijezda slinovnica reapsorbiraju se Na^+ i Cl^-, a izlučuju K^+ i HCO_3^-. U ljudskoj parotidnoj žlijezdi koncentracija Na^+ i Cl^- je u pozitivnoj, a K^+ u negativnoj korelaciji s protokom sline (33). U ovom istraživanju, nakon stimulacije proizvodnje sline pilokarpinom u 7 mjeseci starih WT kontrolnih miševa, ne postoji statistički značajna korelacija koncentracije iona u odnosu na protok sline (Tablica 1).

Nakon primjene UGN-a, pH i koncentracija K^+ u slini stimuliranoj pilokarpinom bili su u pozitivnoj korelaciji s protokom sline (Tablica 1, $p < 0,05$ statistički značajna korelacija). Promjene u pH mogu nastati zbog promjena funkcije transportera za H^+ ili HCO_3^-.

Table 1. Positive correlation of the salivary flow and pH and K^+ concentrations in UGN stimulated mice.
Tablica 1. Pozitivna korelacija protoka sline u odnosu na pH i koncentraciju K^+ u miševa stimuliranih UGN-om.

Ion vs flow • Ioni vs protok		pH	Na^+	K^+	Cl^-
WT	r =	0.7921	-0.0522	0.2047	0.1296
Control • Kontrola	p =	0.0603	0.9021	0.6268	0.7817
WT	r =	**0.5320**	0.4845	**0.5582**	0.4931
UGN	p =	**0.0412**	0.0672	**0.0306**	0.0618
GC-C KO	r =	0.4584	-0.2265	0.0824	-0.4250
UGN	p =	0.3605	0.6659	0.8766	0.4008

GC-C – guanylate cyclase C • gvanilat ciklaza C, GC-C KO – mice missing GC-C • miševi kojima nedostaje GC-C, UGN - uroguanylin • urogvanilin, WT – wild type of mice • divlji tip miševa

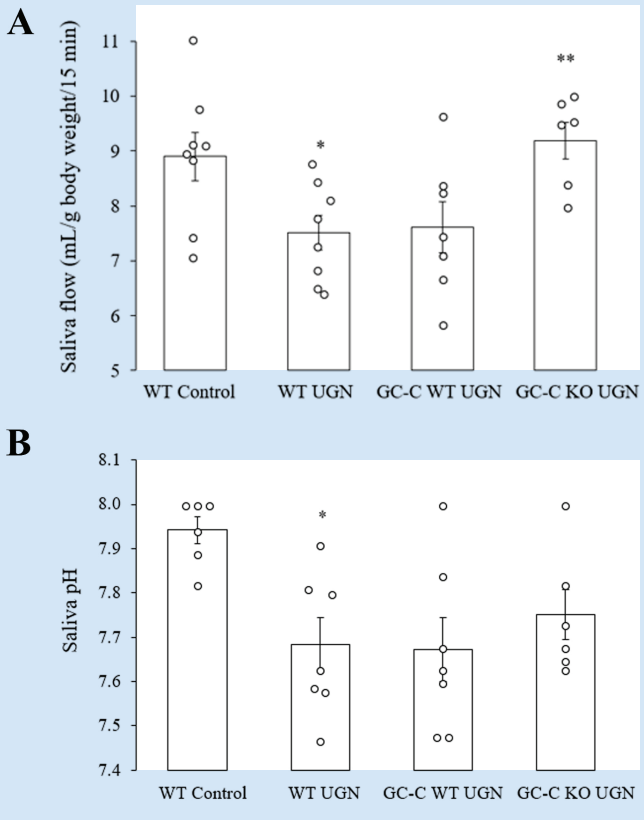

Figure 1. Uroguanylin decreased pilocarpine stimulated saliva flow rate and pH via different signalling pathways. **A:** Effects of uroguanylin (UGN) on saliva production was not present in guanylate cyclase C (GC-C) knockout mice missing GC-C receptor (GC-C KO). **B:** UGN decreased pH, but this effect is still present in GC-C KO mice. The results are presented as mean ± SEM, n = 6-8. *p < 0.05 statistically significant when compared to WT control; **p < 0.05 statistically significant when compared to GC-C WT which are siblings (littermates) of GC-C KO mice but still have GC-C receptor. WT – wild type mice

Slika 1. Urogvanilin je smanjio protok sline stimulirane pilokarpinom i pH sline aktivacijom različitih signalnih putova. **A:** Učinci urogvanilina (UGN) na proizvodnju sline nisu bili prisutni kod životinja kojima nedostaje gvanilat ciklaze C (GC-C KO). **B:** UGN je smanjio pH, ali je taj učinak još uvijek prisutan kod GC-C KO miševa. Rezultati su prikazani kao srednja vrijednost ± SEM, n = 6-8. *p < 0,05 statistički značajno u usporedbi s WT kontrolom; **p < 0,05 statistički značajno u usporedbi s GC-C WT koji su braća (iz legla) GC-C KO miševa, ali još uvijek imaju GC-C receptor. WT – miševi divljeg tipa

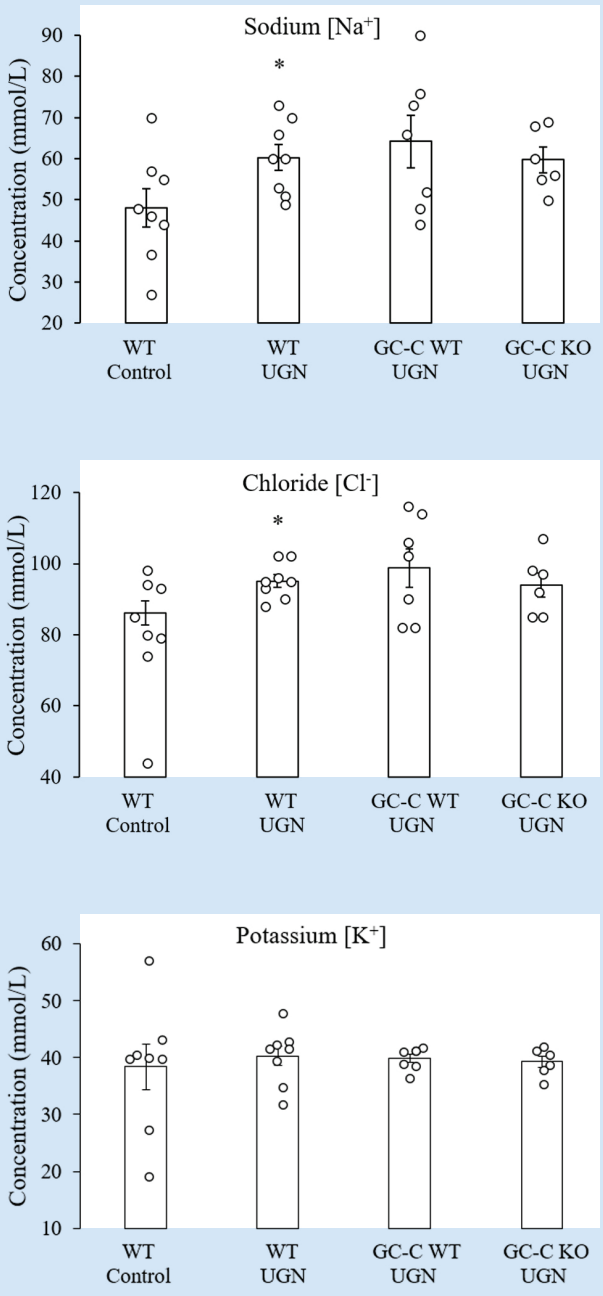

Figure 2. Uroguanylin increased Na^+ and Cl^- concentration via GC-C independent signalling pathway. Uroguanylin (UGN) increased concentration of Na^+ and Cl^- and that effect is still present in GC-C KO mice. UGN did not change K^+ concentration. The results are presented as mean ± SEM, n = 6-8. *p < 0.05 statistically significant when compared to WT control; WT – wild type mice

Slika 2. Urogvanilin je povećao koncentraciju Na^+ i Cl^- aktivacijom GC-C neovisnog signalnog puta. Urogvanilin (UGN) je povećao koncentraciju Na^+ i Cl^- i taj učinak je još uvijek prisutan kod GC-C KO miševa. UGN nije promijenio koncentraciju K^+. Rezultati su prikazani kao srednja vrijednost ± SEM, n = 6-8. *p < 0,05 statistički značajno u usporedbi s WT kontrolom; WT – miševi divljeg tipa

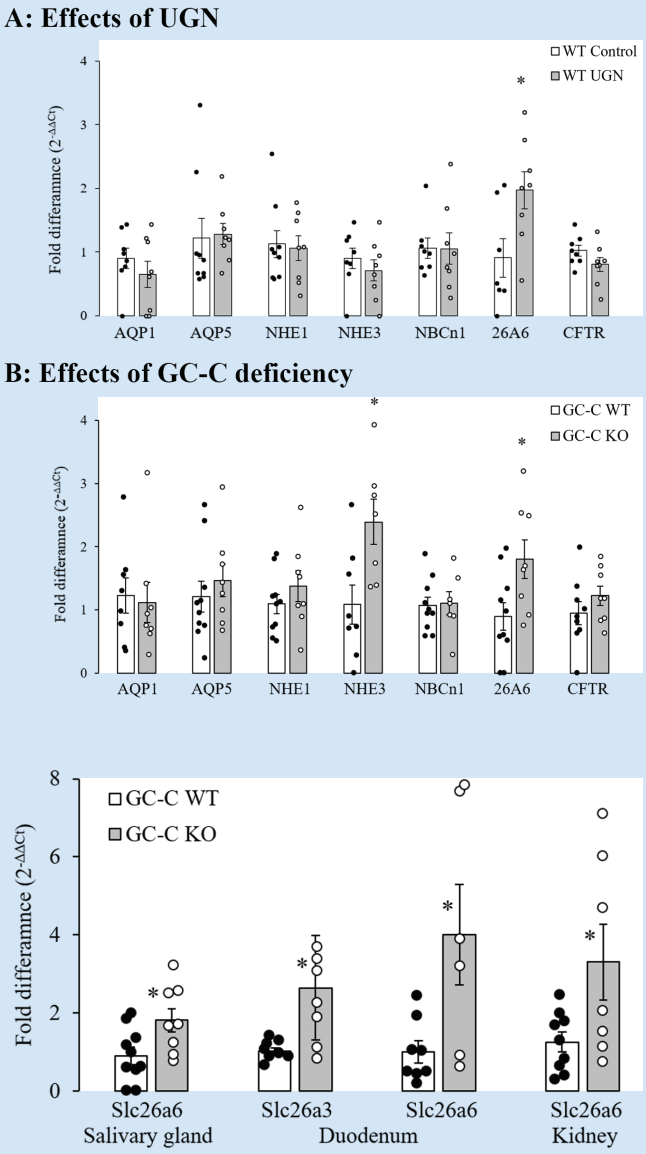

Figure 3. Uroguanylin increased mRNA expression of Slc26a6 in submandibular salivary glands via GC-C independent signalling pathway. **A:** Effects of uroguanylin (UGN) on expression of channels for water (AQPs), sodium-hydrogen exchanger isoform 1 (NHE1) and 3 (NHE3), sodium-bicarbonate cotransporter (NBCn1), solute carrier family 26 member 6 (Slc26a6), and cystic fibrosis transmembrane conductance regulator (CFTR) are shown. *p < 0.05 statistically significant when compared to WT Control animals, n = 7-9. **B:** Missing GC-C in submandibular glands of GC-C KO mice leads to an increase in NHE3 and Slc26a6 expression (*p < 0.05 statistically significant when compared to GC-C WT mice, n = 7-10). The results are presented as mean ± SEM. GC-C KO - guanylate cyclase C (GC-C) knockout mice missing GC-C receptor, 26A6 – Slc26a6, WT – wild type mice

Slika 3. Urogvanilin je povećao izražaj mRNA (glasničke RNK) za Slc26a6 u submandibularnim žlijezdama slinovnicama aktivacijom GC-C neovisnog signalnog puta. **A:** Prikazani su učinci urogvanilina (UGN) na izražaj kanala za vodu (AQP-i), natrij-vodik izmjenjivač izoforme 1 (NHE1) i 3 (NHE3), natrij-hidrogrnkarbonat kotransporter (NBCn1), članove 3 i 6 obitelj 26 Slc transportera te transmembranskog regulatora provodljivosti cistične fibroze (CFTR). *p < 0,05 statistički značajno u usporedbi s kontrolnim životinjama divljeg tipa, n = 7-9. **B:** Nedostatak GC-C u submandibularnim žlijezdama GC-C KO miševa dovodi do povećanja izražaja NHE3 i Slc26a6 (*p < 0,05 statistički značajno u odnosu na GC-C WT miševe, n = 7-10). Rezultati su prikazani kao srednja vrijednost ± SEM. GC-C KO – životinje kojima nedostaje gvanilat ciklaza C (GC-C) receptor, 26A6 – Slc26a6, WT – divlji tip miševa

Figure 4. Expression of members of Slc26 family increased in submandibular salivary gland, duodenum and kidney cortex of animals missing GC-C. *p < 0.05 statistically significant when compared to GC-C WT mice (n = 6-10). The results are presented as mean ± SEM. GC-C KO - guanylate cyclase C (GC-C) knockout mice missing GC-C receptor

Slika 4. Povećan izražaj članova obitelji Slc26 u submandibularnoj žlijezdi slinovnici, duodenumu i korteksu bubrega životinja kojima nedostaje GC-C. *p < 0,05 statistički značajno u odnosu na GC-C WT miševe (n = 6-10). Rezultati su prikazani kao srednja vrijednost ± SEM. GC-C KO – životinje kojima nedostaje gvanilat ciklaza C (GC-C) receptor

Uroguanylin increased expression of Slc26a6 in submandibular salivary glands via GC-C independent signalling pathway

In our study, the effects of the peptide hormone UGN on expression of mRNA for channels for water (AQP 1, 3 and 5), sodium-hydrogen exchanger isoform 1 (NHE1) and 3 (NHE3), sodium-bicarbonate cotransporter (NBCn1), members of solute carrier family 26 (a3 and a6) and cystic fibrosis transmembrane conductance regulator (CFTR) were determined. The expression of AQP3 and Slc26a3 was not found in mouse submandibular salivary glands.

Of all tested AQPs and ion transporters, one hour after i.p. application of UGN expression of Slc26a6 increased two times (p = 0.027). Similarly, the Slc26a6 expression was higher in GC-C KO mice compared to their GC-C WT littermates (p = 0.024) (Figure 2).

It is not surprising that, although the UGN application did not change NHE3 expression in submandibular salivary glands (p = 0.416), GC-C KO mice had two-time higher expression of NHE3 than GC-C WT animals (p = 0.016).

Urogvanilin povećava izražaj Slc26a6 u submandibularnim žlijezdama slinovnicama djelovanjem na GC-C neovisni signalni put

U našem istraživanju određeni su učinci peptidnog hormona UGN-a na izražaj mRNA za kanale za vodu (AQP 1, 3 i 5), natrij-vodik izmjenjivač i to izoforme 1 (NHE1) i 3 (NHE3), natrij-hidrogenkarbonat kotransporter (NBCn1), članove a3 i a6 obitelji 26 Slc transportera te transmembranski regulator provodljivosti cistične fibroze (CFTR). Izražaj AQP3 i Slc26a3 nije pronađen u mišjim submandibularnim žlijezdama slinovnicama.

Od svih testiranih AQP-ova i ionskih transportera, jedan sat nakon i.p. primjene UGN-a izražaj Slc26a6 se povećao dva puta (p = 0,027). Slično, izražaj Slc26a6 bio je viši u GC-C KO miševa u usporedbi s GC-C WT miševima iz istog legla (p = 0,024) (Slika 2).

Iako primjena UGN-a nije promijenila izražaj NHE3 u submandibularnim žlijezdama slinovnicama (p = 0,416), ne iznenađuje da su GC-C KO miševi imali dva puta viši izražaj NHE3 od GC-C WT životinja (p = 0,016).

GC-C regulates expression of Slc26a6 in salivary glands, kidney cortex and duodenum

To determine if effects of UGN are unique for salivary glands, we investigated differences in expression of Slc26a6 in duodenum and kidney cortex of GC-C KO mice, where, in physiological conditions, the GC-C dependent signalling pathway for UGN exists. Similar to submandibular salivary gland, expression of Slc26a6 was higher in duodenum and kidney cortex of GC-C KO mice than in GC-C WT mice ($p = 0.022$ and $p = 0.038$, respectively, Figure 4). In duodenum exist another Cl^-/HCO_3^- exchanger, Slc26a3, and its expression is also higher in animals missing GC-C (Figure 4).

Discussion

The effects of the natriuretic peptides on salivary glands, which includes guanylin peptides and other naturistic peptides such as the atrial natriuretic peptide (ANP), as an agonist of guanylate cyclase A (GC-A), has been known for a while (34). After parasympathetic stimulation, in submandibular glands, ANP increases salivary secretion, decreases Na^+ and increases K^+ but in parotid glands ANP increases salivary flow rate, and Na^+ and K^+ concentration (35). Therefore, the aim of this study was to determine the effects of GC-C agonist, UGN, on saliva flow rate and ion composition.

In this study saliva flow rate upon pilocarpine stimulation and ion composition corresponded to previously published results (36, 37). As we know, decrease in saliva flow rate leads to a decrease in Na^+ and Cl^- concentration, pH and an increase in K^+ concentration. UGN decreased saliva flow rate and pH, but also increased saliva concentration of Na^+ and Cl^- which could not be explained by changes in saliva flow rate.

The effects of the UGN on flow rate is GC-C dependent since the UGN effect is gone in GC-C KO mice compared to their GC-C WT littermates which is not the case for the changes of pH, Na^+ and Cl^- concentration (Figure 1 and 2). Furthermore, upon UGN stimulation both pH and K^+ concentration was in positive correlation to saliva flow rate. Correlation was not found in GC-C KO mice suggesting the involvement of GC-C signalling pathway (Table 1).

To determine the possible mechanism of UGN action in salivary glands, the expression of ion transporters and AQPs were determined in submandibular glands of WT and GC-C KO mice. In our study mRNA for AQP3 was not found although it has been previously shown (38). Expression of mRNA for all tested isoforms of AQPs did not differ between WT Control and WT UGN animals as well as between GC-C WT and GC-C KO mice (Figure 3). The possible mechanism of decrease in salivary flow rate due to UGN action remains unclear. This mechanism possibly involves translocation of AQPs in apical membrane of the cells but not changes in mRNA expression tested in this study.

Even though, the one member of Slc26 family, also Cl^-/HCO_3^- exchanger, pendrin (Slc26a4), is down regulated by UGN (39), previous research has not shown any involvement of UGN in regulation of Slc26a3 nor Slc26a6. In our study, one hour after i.p. application, UGN increased expres-

GC-C regulira izražaj Slc26a6 u žlijezdama slinovnicama, kori bubrega i duodenumu

Kako bismo utvrdili jesu li učinci UGN-a jedinstveni za žlijezde slinovnice, istražili smo razlike u izražaju Slc26a6 u duodenumu i korteksu bubrega GC-C KO miševa, gdje za UGN, u fiziološkim uvjetima, postoji GC-C ovisan signalni put. Slično kao u submandibularnoj žlijezdi slinovnici, izražaj Slc26a6 je bio viši u duodenumu i korteksu bubrega GC-C KO miševa u odnosu na izražaj u GC-C WT miševa ($p = 0,022$ odnosno $p = 0,038$, slika 4). U duodenumu postoji još jedan Cl^-/HCO_3^- izmjenjivač, Slc26a3, čiji je izražaj također bio viši u životinja kojima nedostaje GC-C (Slika 4).

Rasprava

Poznati su već neko vrijeme učinci natrijuretskih peptida (što uključuje gvanilinske peptide i druge naturijuretske peptide kao što je atrijski natrijuretski peptid (ANP), agonist gvanilat ciklaze A (GC-A)) na žlijezde slinovnice (34). ANP povećava izlučivanje sline submandibularnim žlijezdama nakon parasimpatičke stimulacije i smanjuje koncentraciju Na^+, ali povećava koncentraciju K^+. U parotidnim žlijezdama, ANP povećava volumen stvorene sline, te povećava koncentraciju Na^+ i K^+ (35). Stoga je cilj ovog istraživanja bio utvrditi učinke GC-C agonista, UGN-a, na brzinu protoka sline i njen ionski sastav.

U ovoj studiji brzina protoka sline i njezin ionski sastav nakon stimulacije pilokarpinom odgovarali su prethodno objavljenim rezultatima (36, 37). Kao što znamo, smanjenje protoka sline dovodi do smanjenja koncentracije Na^+ i Cl^-, pH i povećanja koncentracije K^+. UGN je smanjio protok sline i pH, ali i povećao koncentraciju Na^+ i Cl^- u slini što se ne može objasniti pukim promjenama u protoku sline.

Učinci UGN-a na brzinu protoka ovise o aktivaciji GC-C-a jer učinak UGN-a ne postoji kod GC-C KO miševa u usporedbi s GC-C WT miševima iz istog legla. To nije slučaj za promjene koncentracije pH, Na^+ i Cl^- (Slika 1 i 2). Nadalje, nakon stimulacije UGN-om i pH i koncentracija K^+ bile su u pozitivnoj korelaciji s brzinom protoka sline. Korelacija nije pronađena u GC-C KO miševa što upućuje na uključenost GC-C signalnog puta (Tablica 1).

Kako bi se odredio mogući mehanizam djelovanja UGN-a na žlijezde slinovnice, određen je izražaj ionski transportera i AQP-a u submandibularnim žlijezdama WT i GC-C KO miševa. U našem istraživanju mRNA za AQP3 nije pronađena iako je prethodno prikazana (38). Izražaj mRNA za sve testirane izoforme AQP-a nije se razlikovao između WT Kontrolne i WT UGN skupine životinja kao ni između GC-C WT i GC-C KO miševa (Slika 3). Mogući mehanizam smanjenja brzine protoka sline zbog djelovanja UGN-a ostaje nejasan. Ovaj mehanizam vjerojatno uključuje premještanje AQP-a u apikalnu membranu stanica, ali ne i promjene u izražaju mRNA testirane u ovoj studiji.

Iako je jedan član obitelji Slc26, također Cl^-/HCO_3^- izmjenjivač, pendrin (Slc26a4), smanjeno UGN-om (39), dosadašnja istraživanja nisu pokazala ulogu UGN-a u regulaciji Slc26a3 niti Slc26a6. U našoj studiji, jedan sat nakon i.p. primjene, UGN je povećao izražaj mRNA za Slc26a6, među-

sion of mRNA for Slc26a6, however, GC-C KO mice also showed an increase in mRNA expression when compared to their WT littermates suggesting possible effects of several signalling pathways for UGN on Slc26a6 expression. There are few possible explanations for this hypothesis. Protein kinase C (activated by Ca^{2+}) inhibits Slc26a6 function in the intestine cell line (40) but the downstream part of Ca^{2+} signalling pathway increase expression of Slc26a6 on the apical membrane of pancreatic or salivary ducts cells (41) accordingly to effects of UGN presented in this study. Furthermore, Slc26a6 is located in acinar cells as well as in ductal cells of submandibular salivary glands (42) which suggests possible effects of i.p. UGN on acinar cells via GC-C independent signalling pathway.

GC-C is located at apical membrane of the ductal cell of salivary glands (24). Since the expression of Slc26a6 is increased in GC-C KO mice, its activation might lead to decrease in its expression. The involvement of GC-C in regulation of Slc26a6 was confirm in the duodenum and kidney cortex (Figure 4). In addition to effects on Slc26a6, in duodenum of GC-C KO mice expression of Slc26a3 was higher than in their WT littermates.

It is known that UGN, when activating GC-C, inhibits NHE3 in apical membrane of kidney and intestine cells (10, 43). It seems that the regulation is similar in salivary glands ducts as well because NHE3 expression is higher in submandibular glands of GC-C KO mice (Figure 3). Furthermore, it is not surprising, when UGN was applied i.p., mRNA expression for NHE3 did not change since the GC-C is located in apical membranes of salivary duct cells (24) which i.p. UGN cannot reach.

Conclusion

The most important conclusions of this research are: UGN, when applied i.p., change the salivary flow rate, pH and ion concentrations suggesting its possible involvement in regulation of postprandial salivary glands function. The changes of ion composition of saliva by GC-A agonists do not match changes by GC-C agonist (UGN). The effects of the UGN on saliva flow rate is GC-C dependent which is not the case for the changes of H^+, Na^+ and Cl^- concentration. In mice missing GC-C, expression of Slc26a3 and/or a6 was higher in salivary glands, duodenum and kidney cortex suggesting wildly spread regulation of this ion transporter by GC-C. I.p. applied UGN did not decrease NHE3 expression which is higher in GC-C KO mice

Conflict of interests:

The authors report no conflict of interests.

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tim, GC-C KO miševi također su pokazali povećanje izražaja mRNA u usporedbi s WT miševa iz istog legla, što upućuje na moguće djelovanje nekoliko različitih signalnih putova za UGN na izražaj Slc26a6. Nekoliko je mogućih objašnjenja za ovu hipotezu. Protein kinaza C (aktivirana Ca^{2+}) inhibira funkciju Slc26a6 u staničnoj liniji crijeva (40), ali nizvodni dio Ca^{2+} signalnog puta povećava izražaj Slc26a6 na apikalnoj membrani stanica gušterače ili kanala slinovnice (41) u skladu s prikazanim učincima UGN u ovoj studiji. Nadalje, Slc26a6 je lokaliziran u acinusnim stanicama i u stanicama izvodnih kanalića submandibularnih žlijezda slinovnica (42) što upućuje na moguće učinke i.p. UGN-a na acinusne stanice aktivacijom GC-C neovisnog signalnog puta.

GC-C se nalazi na apikalnoj membrani stanica izvodnih kanalića žlijezda slinovnica (24). Budući da je izražaj Slc26a6 povećan u GC-C KO miševa, aktivacija GC-C-a može dovesti do smanjenja izražaja Slc26a6. Uključenost GC-C u regulaciju Slc26a6 potvrđena je u duodenumu i korteksu bubrega (Slika 4). Osim učinaka na Slc26a6, izražaj Slc26a3 u duodenumu GC-C KO miševa bio je veći nego u WT životinja iz istog legla.

Poznato je da UGN, kada aktivira GC-C, inhibira NHE3 u apikalnoj membrani stanica bubrega i crijeva (10, 43). Čini se da je regulacija slična i u kanalićima žlijezda slinovnica jer je izražaj NHE3 veći u submandibularnim žlijezdama GC-C KO miševa (Slika 3). Nadalje, nije iznenađujuće, kada je UGN primijenjen i.p., izražaj mRNA za NHE3 nije se promijenio jer je GC-C smješten u apikalnim membranama stanicama izvodnih kanalića slinovnica (24), a do njega i.p. primijenjeni UGN ne može doći.

Zaključak

Najvažniji zaključci ovog istraživanja su: UGN, kada se primjenjuje i.p., mijenja brzinu protoka sline, pH i koncentraciju iona u slini što upućuje na njegovu moguću uključenost u regulaciju postprandijalne funkcije žlijezda slinovnica. Agonisti GC-A ne mijenjaju ionski sastav sline jednako kao i prikazani agonist GC-C-a (UGN). Učinci UGN-a na brzinu protoka sline ovise o aktivaciji GC-C-a, što nije slučaj za promjene koncentracije H^+, Na^+ i Cl^-. U miševa kojima nedostaje GC-C, izražaj Slc26a3 i/ili a6 bio je veći u žlijezdama slinovnicama, duodenumu i korteksu bubrega što upućuje na široko rasprostranjenu regulaciju ovog ionskog transportera djelovanjem GC-C-a. I.p. primijenjeni UGN nije smanjio izražaj NHE3 koji je veći u GC-C KO miševa.

Sukob interesa:

Autori navode da nema sukoba interesa.

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Doprinos autora: **D. J.** – osmislio i izveo pokuse (sijalometrija, prikupljanje tkiva, ionski sastav sline, qPCR), analizirao podatke, napisao izvorni nacrt rada; **M. R.** - izvela pokuse (sijalometrija, prikupljanje tkiva, qPCR); **I. M. B.** - izvela pokuse (sijalometrija, prikupljanje tkiva); **I. P.** – izvela pokuse (ionski sastav sline); **A. D.** – administracija projekta, dizajnirala sve pokuse, analizirala podatke, pregledala i uredila ovaj rukopis

Sažetak

Cilj rada: Gvanilinski peptidi smatraju se isključivo unutarnjim regulatorima lučenja žlijezda slinovnica. Stoga je cilj ovog istraživanja utvrditi učinke sistemskog urogvanilina (UGN) na protok sline i njen ionski sastav te uključuju li ti učinci aktivaciju gvanilat ciklaze C (GC-C). **Materijali i metode:** Ovo istraživanje je provedeno na 7 mjeseci starim C57Bl6NCrl (divlji tip, WT) miševima te miševima kojima nedostaje GC-C (GC-C KO). Količina stvorene sline i njen ionski sastav određeni su nakon stimulacije pilokarpinom uz primjenu UGN-a (30 µg/životinji) ili fiziološke otopine i.p.. Izražaj mRNA (glasničke RNK) za AQP-e, NHE, NBCn1, Slc26a3/a6 i CFTR određena je qPCR-om u submandibularnim žlijezdama slinovnicama. **Rezultati:** Kada se na UGN primijeni i.p., dolazi do smanjenja brzine protoka sline stimulirane pilokarpinom i povećava koncentracija Na^+, H^+ i Cl^-. U GC-C KO miševa, UGN nema učinak na brzinu protoka sline, dok su koncentracije Na^+, H^+ i Cl^- iste u slini GC-C KO miševa u usporedbi sa WT miševima iz istog legla. UGN je povećao izražaj Slc26a6 dok je i kod GC-C KO miševa Slc26a6 imao veći izražaj u odnosu na WT miševe, što pretpostavlja uključenost GC-C neovisnog signalnog puta za UGN. Razlika u Slc26a6 kod GC-C KO miševa nije jedinstvena za žlijezde slinovnice jer je također pronađena u duodenumu i u bubrežnoj kori. **Zaključci:** Učinci UGN-a preko bazolateralne membrane stanica žlijezda slinovnica do danas nisu razmatrani. U našoj studiji, UGN, kada se primijeni i.p., smanjuje brzinu protoka sline, pH i mijenja ionski sastav sline. Stoga bi UGN u plazmi sat vremena nakon obroka mogao imati fiziološku i patološku važnost (razvoj karijesa, upale ili demineralizacije), a inhibicija sistemskih učinaka UGN-a mogla bi se smatrati novim pristupom u liječenju tih stanja.

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MeSH pojmovi: podčeljusna žlijezda slinovnica; enterotoksin receptori; slinjenje; pilokarpin
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