Stanniocalcin 1 effects on the renal gluconeogenesis pathway in rat and fish

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Vanessa Schein [a, b, c, *], Luiz C. Kucharski [a], Pedro M.G. Guerreiro [c], Tiago Leal Martins [a], Isabel Morgado [c], Deborah M. Power [c], Adelino V.M. Canario [c], Roselis S.M. da Silva [a]

[a] Pos-Graduate Program in Biological Sciences, Department of Physiology, Institute of Health Sciences, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500, 90050-170, Porto Alegre, RS, Brazil
[b] Pos-Graduate Program in Health Sciences, Department of Obstetrics and Gynecology, School of Medicine, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2400, 90035-003, Porto Alegre, RS, Brazil
[c] CCMAR — Centre for Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal

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ABSTRACT

The mammalian kidney contributes significantly to glucose homeostasis through gluconeogenesis. Considering that stanniocalcin 1 (STC1) regulates ATP production, is synthesized and acts in different cell types of the nephron, the present study hypothesized that STC1 may be implicated in the regulation of gluconeogenesis in the vertebrate kidney. Human STC1 strongly reduced gluconeogenesis from ^{14}C-glutamine in rat renal medulla (MD) slices but not in renal cortex (CX), nor from ^{14}C-lactic acid. Total PEPCK activity was markedly reduced by hSTC1 in MD but not in CX. $Pck2$ (mitochondrial PEPCK isoform) was down-regulated by hSTC1 in MD but not in CX. In fish ($Dicentrarchus\ labrax$) kidney slices, both STC1-A and -B isoforms decreased gluconeogenesis from ^{14}C-acid lactic, while STC1-A increased gluconeogenesis from ^{14}C-glutamine. Overall, our results demonstrate a role for STC1 in the control of glucose synthesis via renal gluconeogenesis in mammals and suggest that it may have a similar role in teleost fishes.

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1. Introduction

In mammals, the kidney contributes to glucose homeostasis through the gluconeogenesis processes, glucose filtration, reabsorption, and consumption. The kidney is responsible for up to 20% of all glucose production, contributing to about 40% of gluconeogenesis (Gerich et al., 2001; Mather and Pollock, 2011). While the poorly vascularized and relatively hypoxic renal medulla is a site of glycolysis, the renal cortex is a site of gluconeogenesis (Mather and Pollock, 2011). The proximal tubule is the only nephron segment that contains the key gluconeogenic enzymes. High rates of glucose synthesis from pyruvate has been measured in the straight portion of the proximal tubule of micro-dissected rabbit nephron segments (Guder and Ross, 1984; Maleque et al., 1980; Marsenic, 2009; Monteil et al., 1995; Schoolwerth et al., 1988). As a result, the net equilibrium of glucose is a balance between renal glucose release by the cortex and renal glucose uptake by the renal medulla (Gerich et al., 2001; Mather and Pollock, 2011).

The hormonal control of renal gluconeogenesis in mammals is largely accomplished by insulin and catecholamines, whereas glucagon has little or no effect (Kurokawa and Massry, 1973; Mather and Pollock, 2011). In addition, parathyroid hormone (PTH) also stimulates gluconeogenesis from a range of substrates in isolated segments of the rabbit renal cortex provided levels of external calcium are high (Nagata and Rasmussen, 1970; Rasmussen et al., 1986; Rasmussen and Nagata, 1970).

The kidney is also important organ for glucose homeostasis in the teleost fishes (Mommsen et al., 1985). The enzymes required for gluconeogenesis have been reported (Kirchner et al., 2008) and phosphoenolpyruvate carboxykinase (PEPCK) activity is highly specific to gluconeogenic tissues, such as liver and kidney (Knox

et al., 1980; Suarez and Mommsen, 1987; Walton and Cowey, 1979, 1982).

Stanniocalcin 1 (STC1) secreted by the Corpuscles of Stannius (CS) is an established hypocalcemic factor in bony fish from where it was first isolated (Wagner et al., 1986, 1991). Recently two fish STC1 isoforms encoded by paralogue genes have been identified: *STC1-A* strongly expressed in the CS and *STC1-B* only faintly expressed in various tissues (Schein et al., 2012). STC1-A stimulates phosphate reabsorption by renal proximal tubules, resulting in an increase in serum phosphate that combines with excess calcium to deposit into bone and scales (Lu et al., 1994). Mammalian STC1 shares a high degree of amino acid sequence similarity (approximately 65–70%) with the fish STC1-A and STC1-B (Schein et al., 2012).

Mammalian STC1 exhibits paracrine/autocrine signaling (Huang et al., 2009; Stasko and Wagner, 2001a,b). Although several studies have been demonstrated a pleotropic role for stanniocalcins (Bouras et al., 2002; Filvaroff et al., 2002; Huang et al., 2009; Law and Wong, 2010; Tamura et al., 2011; Tohmiya et al., 2004; Varghese et al., 2002; Westberg et al., 2007), little is known about a putative role of these proteins on carbohydrate metabolism. The collecting duct cells of mammalian kidney express the highest level of STC1 (De Niu et al., 1998; Haddad et al., 1996; Turner et al., 2010; Wong et al., 1998). STC1 targets nephron mitochondria to regulate ATP production by promoting respiratory uncoupling and calcium uniport activity (Basini et al., 2009; McCudden et al., 2002). *In situ* hybridization and immunocytochemistry revealed STC1 expression to be higher in the renal inner medulla than in the renal cortex (Deol et al., 2001; Turner et al., 2010; Wong et al., 1998).

Here we hypothesized that STC1 may be implicated on gluconeogenesis regulation in the vertebrate kidney. We investigated the effect of recombinant human STC1 (hSTC1) (i) on gluconeogenesis in the rat renal cortex and in the outer and inner renal medulla; and (ii) on the activity and gene expression of key gluconeogenic enzymes. We have carried out a parallel study on the teleost fish European seabass (*Dicentrachus labrax*) kidney using fish STC1 recombinant isoforms to test the hypothesis of STC1 function conservation across vertebrates.

2. Material and methods

2.1. Animals

Male Wistar rats were housed in plastic cages (three animals each), and received water and pelleted food *ad libitum*. They were maintained under standard laboratory conditions (controlled temperature of 21 °C, 12 h light/12 h dark). Special care was taken to minimize the number of animals used and their suffering. All animal procedures used in this study were in accordance with the Principles of Laboratory Animal Care (COBEA – Brazilian College of Animal Experimentation), and the experimental protocol was approved by the UFRGS Animal Care Committee.

European sea bass (*Dicentrarchus labrax*) were purchased from a local fish farm (TIMAR Cultura de Águas, Olhão, Portugal) and maintained at the Ramalhete experimental station of the University of Algarve (Faro, Portugal) under the natural light/dark cycle in a 1000 L tank containing sea water (35‰) at 21 ± 2 °C. Fish were fed once a day with commercial fish food (Provimi, Portugal). All animal care and experimental procedures were done in accordance with the national legislation for the use of laboratory animals ("Group-1" license issued by the Ministry of Agriculture, Rural Development and Fisheries of Portugal).

2.2. Experimental procedure

The rats (250–300 g) were euthanased by decapitation, the kidneys were immediately excised, weighed and placed in Petri dishes with cold Krebs ringer bicarbonate (KRB) buffer, pH 7.4 (in mM: 118 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 24.8 $NaHCO_3$ and 0.1 phenylmethylsulphonyl fluoride – PMSF). The renal cortex and medulla were separated and sliced fresh (800–1000 μm) with a slicer apparatus (Jahn et al., 2011) according to the methodology initially developed by Krebs et al. (1963). In the renal medulla, the outer and inner medulla regions were maintained intact – gluconeogenesis is highest in segment 3 (S3) of the proximal straight tubule (Maleque et al., 1980), which descends toward the renal medulla, in a perpendicular to the cortical surface of the kidney.

The fishes (both sexes) (weight 123.40 ± 4.7 g, length 22.2 ± 0.3 cm) were euthanased by decapitation. The kidneys were excised and weighed, placed in Petri dishes with cold oxygenated physiological fish buffer (FB) (in mM: NaCl 150.6; NaH_2PO_4 ($2H_2O$) 0.4; $NaHCO_3$ 4.2; KCl 4.0; $MgSO_4$ 1.0; Hepes 25.0; $CaCl_2$ 1.98; 1% protease inhibitor cocktail P2714 (Sigma) pH 7.7; 340 mOsm/Kg H_2O) (Munoz et al., 1999) and sliced fresh (800–1000 μm) with a slicer apparatus.

2.3. Hormones

Recombinant human STC1 (hSTC1) was purchased from Biovender Inc. (Prague, Czech Republic). Human and rat STC1 share 99% identity and the recombinant hSTC1 has been previously used in studies in rat (Law et al., 2012; Moreau et al., 2012; Ookata et al., 2001). Recombinant fish STC1-A and STC1-B isoforms (from puffer fish, *Tetraodon nigroviridis*) were produced and kindly provided by Dr. Mirjam Czjzek (Groisillier et al., 2010). *T. nigroviridis* and *D. Labrax* STC-1A and STC-1B share 87%, and 94% identity, respectively, based on comparisons of translated nucleotide sequences (Supplementary Fig. 1 and Table 1).

2.4. Gluconeogenesis assay

Lactate, glutamine and glycerol are the main renal gluconeogenesis precursors (Mitrakou, 2011). The rate of glucose synthesis was determined by measuring the conversion of ^{14}C-lactic acid or ^{14}C-glutamine into medium ^{14}C-glucose. Rat renal cortex (CX) (n = 4–8/group) and outer and inner medulla (MD) (n = 4–8 group) slices (90 ± 10 mg) and fish kidney slices (50 ± 10 mg) (n = 4–13 group) were placed in sterile 24-well culture plates. They were pre-incubated for 15 min without hormones in 1 ml KRB (rat) or FB (fish), respectively, equilibrated with O_2:CO_2 (95:5, v/v) at 37 °C (rat) or 23 °C (fish), under constant shaking in a Dubnoff incubator. The rat MD or CX slices were then transferred to new plates and incubated with 0.01, 0.1 or 10 ng/ml hSTC1 – within the range of physiological levels (De Niu et al., 2000; Sazonova et al., 2008) – in KRB pH 7.4 for 1 h (the time was determined in preliminary experiments) at 37 °C. The incubation was performed in the presence of: (1) 0.15 μCi [U-^{14}C]-L-lactic acid (172 mCi/mmol, DuPont, NEN Products, Boston, MA, USA), plus 10 mM of lactic acid; or (2) 0.15 μCi[U-^{14}C]-glutamine (142 mCi/mmol, GE Healthcare Life Science, Piscataway, NJ, USA) plus 10 mM of glutamine and equilibrated with O_2:CO_2 (95:5, v/v). After addition of unlabeled substrate (glutamine or lactic acid 10 mM), the incubation medium pH was measured. Previous studies showed that under experimental conditions 5% CO_2 did not affect gluconeogenic capacity (Oliveira et al., 2004). The fish slices were similarly incubated in FB for 30 min at 23 °C using fish STC1-A (fSTC1-A) and STC1-B (fSTC1-B). Rat and fish control tissue slices were incubated in parallel without

hormones (control). Following incubations, the medium was deproteinized and the ^{14}C-glucose was separated by thin-layer chromatography using: n-butanol/95%; ethyl alcohol/5.4%; acetic acid in water (75:47.4:27.6, v/v/v). The spot corresponding to glucose, localized by spraying with anisaldehyde reagent (95% ethyl alcohol/concentrated sulfuric acid/p-methoxybenzaldehyde 18:1:1, v/v/v), was marked, scraped off, and dissolved in toluene: triton (2:1)-PPO-POPOP. Radioactivity was measured in a LKB Wallac scintillation counter with a color quench correction curve. The values of gluconeogenic activity are given as lactic acid or glutamine converted to glucose per g tissue per min (μmol g^{-1} min^{-1}) (Oliveira and Da Silva, 1997).

2.5. PEPCK enzyme assay

PEPCK activity was determined in rat kidney CX and MD slices (90 ± 10 mg; n = 3–6 rats/group) incubated with and without hormone as previously. At the end of incubation, tissues were rinsed twice in cold KRB buffer, blotted on filter paper, and homogenized in 0.25 M sucrose plus 0.1 mM PMSF 0.5:3:0.003 (w/v/v), with a teflon pestle homogenizer. The PEPCK (E.C. 4.1.1.32) activity assay was based on the exchange reaction between H^{14}CO$_3^-$ and oxaloacetate and carried out according to Migliorini et al. (1973) modified by Oliveira and Da Silva (1997). The PEPCK activity was expressed as nmol H^{14}CO$_3^-$ incorporated into oxalacetate mg^{-1} protein min^{-1}. Total protein was determined by the Bradford method (Bradford, 1976) using bovine albumin as the standard.

2.6. Quantitative-PCR of PEPCK genes

Rat kidney CX or MD slices samples (250 ± 20 mg; n = 3–6 rats/group) were incubated with and without hSTC as described above. At the end of incubation, slices were rinsed twice in cold KRB buffer, blotted on filter paper and total RNA extracted following the Trizol protocol (Invitrogen, Carlsbad, CA, USA). RNA integrity, purity and concentration were checked by electrophoresis in 1% agarose gel and spectrophotometry. cDNAwas synthesized using the SuperScript III RT Kit (Invitrogen, Carlsbad, CA, USA) using 0.5 μg of total RNA treated with DNAse I (Amplification Grade kit, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Specific primers were designed for the target genes pck1 (NM_198780.3; forward: 5′ tctgatccagaccttccaaaag 3′; reverse: 5′ caaacttcctcacttcctgg 3′; amplicon length 165 bp), pck2 (NM_001108377.2; forward: 5′ tgctatgtacctccccgg 3′; reverse: 5'agcctcagtcccatcacaaata 3′; amplicon length: 199 bp) and for the reference gene 18S (forward: 5'gtctgccctatcaactttc 3′; reverse: 5′ttccttggatgtggtagc 3′, product length: 119 bp) using the IDT Design Software (Integrated DNA Technologies Inc., USA). The primers for pck1 and pck2 were designed between exons 1 and 2. Quantitative reverse-transcription polymerase chain reaction (qPCR) reactions were performed in triplicate and contained 0.1 μM of each specific primer, 10× diluted cDNA and Platinum$^{®}$ SYBR$^{®}$ Green qPCR Super Mix-UDG with ROX (Invitrogen Corp., USA). The qPCR thermal cycling conditions were: 2 min at 50 °C, 2 min at 95 °C, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s. Under these conditions, qPCR produced a single product at a specific melting temperature for each gene analyzed. Control reactions were performed to verify that no amplification occurred without cDNA. The mean Ct values from triplicate measurements were used to calculate expression of the target gene, with normalization to internal control (18S) using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

2.7. Statistical analysis

Results are expressed as means ± standard error of the mean (SEM). Statistical differences in gluconeogenesis, PEPCK activity and mRNA expression experimental groups compared to control were evaluated using one-way ANOVA followed by Dunett's test. The differences between gluconeogenic activity in rat CX and MD kidneys control groups and between gluconeogenesis activity from ^{14}C-acid lactic and ^{14}C-glutamine in fish kidney control groups were analyzed by Student's t-test. P < 0.05 was taken as the criterion of significance. All tests were carried out using the statistics module in SigmaPlot 11.0.

3. Results

3.1. Gluconeogenesis in rat kidney

There was no statistically significant difference between the gluconeogenesis rate in CX and MD control groups when incubated with ^{14}C-glutamine or ^{14}C-lactic acid (P > 0.05) (Fig. 1).

The gluconeogenesis rate from ^{14}C-glutamine in CX was not affected by the hSTC1 at any dose tested (Fig. 1A). In the MD, the rate of glucose synthesis from ^{14}C-glutamine was not modified by 0.01 ng/ml hSTC1 but it was 40% lower at 0.1 ng/ml (P < 0.05) when compared to the control group (Fig. 1A).

The gluconeogenesis rate from ^{14}C-lactic acid in CX or MD slices incubated with 0.01 or 0.1 ng/ml of hSTC1 was similar to the control group (Fig. 1B).

3.2. PEPCK total activity and mRNA expression in rat kidney

PEPCK activity was two-fold higher (P < 0.05) in CX than in MD rat kidneys. In the presence of 0.01 and 0.1 ng/ml hSTC1 PEPCK activity was markedly reduced in MD (respectively, by 70% and 83%) but not in the CX (Fig. 2A).

hSTC1 at 0.1 and 10 ng/ml did not modify transcription of pck1 (which encodes the cytosolic isoform of PEPCK enzyme) in the CX (Fig. 3A). In contrast, 10 ng/ml hSTC1 up-regulated pck1 gene expression 2.7-fold (P < 0.05) (Fig. 3A) in MD. The pck2 (which encodes the mitochondrial isoform of PEPCK enzyme) transcription in the CX increased 1.7-fold (P < 0.05) in presence of 10 ng/ml of hSTC1 (Fig. 3B). On the other hand, in the MD either 0.1 or 10 ng/ml hSTC1 reduced pck2 transcription 3-fold (P < 0.05) (Fig. 3B).

3.3. Gluconeogenesis in fish kidney

In fish kidney, the gluconeogenesis rate from ^{14}C-acid lactic in control groups (7.25 ± 0.343 μmol^{-1} g^{-1} min^{-1}) was significantly higher (P < 0.01) than that from ^{14}C-glutamine (3.64 ± 0.471 μmol^{-1} g^{-1} min^{-1}; Fig. 4).

The gluconeogenesis rate from ^{14}C-glutamine in fish kidney increased by 50% compared to control (P < 0.05) when 0.01 ng/ml of fish STC1-A was present in the incubation medium, but not at higher concentrations (Fig. 4A). STC1-B at the concentrations tested did not affect significantly the gluconeogenesis rate although at 10 ng/ml it was close to statistical significance (P = 0.059) (Fig. 4A). In contrast, with ^{14}C-lactic acid as precursor, both STC1-A and B decreased the rate of glucose synthesis in fish kidney (P < 0.05) (Fig. 4B). STC1-A was only effective at 0.01 ng/ml, while STC1-B was effective at all concentrations tested - 33% reduction at 0.01 ng/ml and 50% at 10 ng/ml (Fig. 4B).

4. Discussion
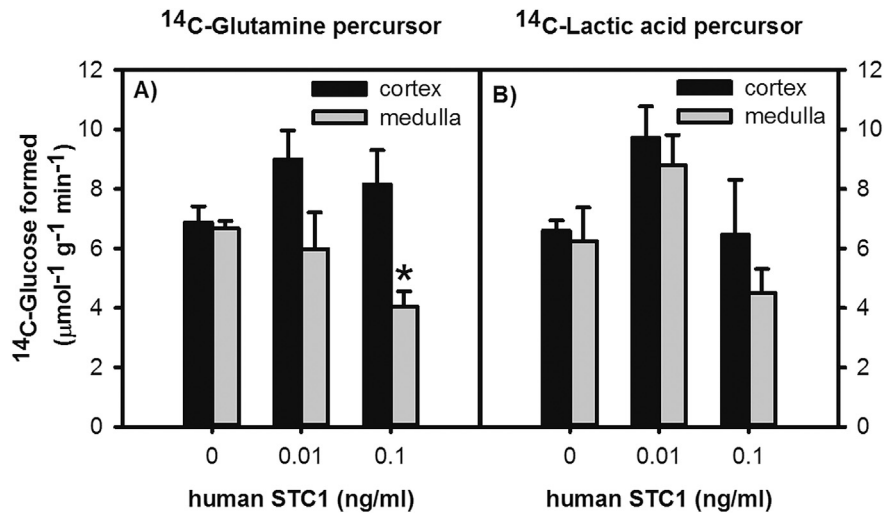
Several studies have shown a pleotropic role for STC in

Fig. 1. Effect of human STC1 on rat kidney gluconeogenesis. Bars indicate gluconeogenesis using ^{14}C-Glutamine (A) or ^{14}C-L-Lactate (B) as precursor to form ^{14}C-glucose in renal cortex and outer and inner renal medulla after tissue incubations with different doses of hSTC1 (n = 4–8). Data is presented as the mean ± SE. * means $P < 0.05$.
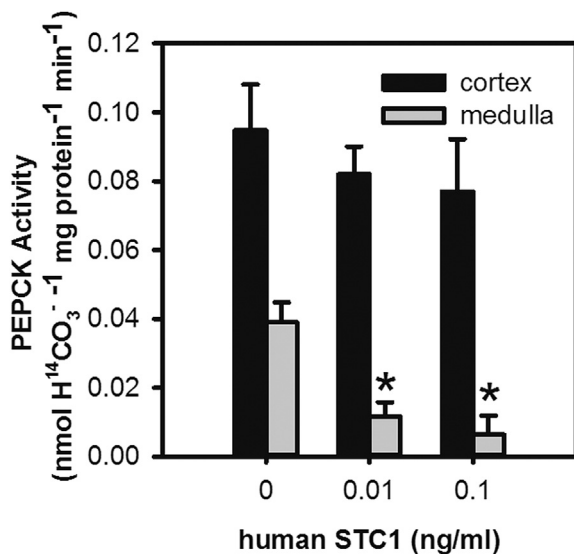

Fig. 2. PEPCK total activity analysis in renal cortex (A) and outer and inner renal medulla (B) of rats. PEPCK activity is more prominent in renal cortex than in renal medulla. There was a significant decrease in PEPCK activity in the renal medulla but not in the renal cortex when tissues are treated with recombinant human STC1 (n = 3–8). Data is presented as the mean ± SE. * means $P < 0.05$.

mammals and fish, but the present study was the first to demonstrate an effect of STC1 on kidney gluconeogenesis.

The gluconeogenesis rate from lactic acid was similar to glutamine in control rat renal medulla (without hormone), indicating that both substrates were converted to glucose in the S3 segment of the proximal tube. In human, it has been shown that the three segments (S1, S2 and S3) of the proximal tubule can synthesize glucose from lactate and glutamine at physiological concentrations, whereas the S2 and S3 segments synthesize more glucose from the same lactate concentration than S1 (Conjard et al., 2001; Mather and Pollock, 2011).

hSTC1 decreased the incorporation of ^{14}C-glutamine into ^{14}C-glucose in rat renal medulla, but in cortex gluconeogenesis activity was not affected by the hormone. These results are consistent with the presence of STC1 receptors and high levels of immunoreactive

STC1 in the S3 segment of the renal proximal tubule (Haddad et al., 1996; James et al., 2005; McCudden et al., 2002; Wong et al., 1998). Moreover, in normal rats and mice STC1 mRNA levels were higher in inner medullary kidney than in cortex (Turner et al., 2010). These data suggest that STC1 is produced and secreted by cells in the inner renal medulla and it is sequestered and acts in the outer renal medulla to control glutamine metabolism, consistent with paracrine effect of STC family proteins (Huang et al., 2009; Stasko and Wagner, 2001a, b).

Gluconeogenesis and ammoniagenesis have been considered a serial reaction of glutamine metabolism, especially in the S3 segment of the proximal tubule (Abu Hossain et al., 2011; Goorno et al., 1967; Goto et al., 1984; Taylor and Curthoys, 2004). Glutamine is the major circulating amino acid and it is metabolized in the proximal tubule to produce NH_4^+, HCO_3^- and glucose (Abu Hossain et al., 2011). The renal catabolism of glutamine is sustained by increased expression of the genes that encode mitochondrial glutaminase and glutamate dehydrogenase, and the cytosolic PEPCK (Taylor and Curthoys, 2004). When the renal medulla was incubated in presence of hSTC1, the rate of ^{14}C-glutamine incorporation into glucose, total PEPCK activity, and *pck2* mRNA levels decreased. In contrast, *pck1* gene expression increased only at 10 ng/ml hSTC1. It is well established that changes in the rate of *pck1* transcription is critical to establish the overall activity of the enzyme in rat kidney (Taylor and Curthoys, 2004; Yang et al., 2009). However, transcription was not affected by the hSTC1 concentrations used to test gluconeogenesis. Therefore, the reduction of total PEPCK activity induced by different concentrations of hSTC1 in renal medulla is likely to result from a post-translational effect together with a decrease in *pck2* transcription. While several studies have established that the control of cytoplasmic PEPCK appears to be regulated at a transcriptional and translational level (Stark et al., 2009; Yang et al., 2009), evidence for regulation of mitochondrial PEPCK is scarce, and related to fasting (Monteil et al., 1995). More recently, it was demonstrated that hepatocyte mitochondrial PEPCK silencing lowers plasma glucose and raises plasma lactate in normal health adult rats (Stark et al., 2014). The present study is the first to establish specific regulation of mitochondrial PEPCK by the STC1 protein.

Anaplerotic and cataplerotic reactions in the kidney metabolism are essentially balanced. Renal ammoniagenesis and gluconeogenesis are tightly interlocked and dependent upon balanced
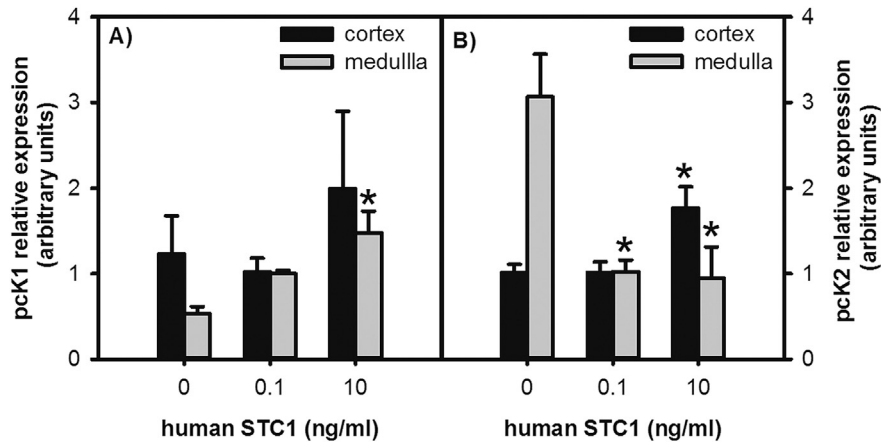
Fig. 3. Semi-quantitative analysis of *pck1* (PEPCK cytosolic isoform) and *pck2* (PEPCK mitochondrial isoform) gene expression by real-time RT-PCR in renal cortex (A and B) and outer and inner renal medulla (C and D) of rats, respectively. Values are the ratios of the normalized Ct values of measuring samples from the recombinant human STC1 treatment tissues to those of control samples (n = 3–4). Tissue expression levels were analyzed using rRNA 18S as the reference gene. Data is presented as the mean ± SE. * means $P < 0.05$.
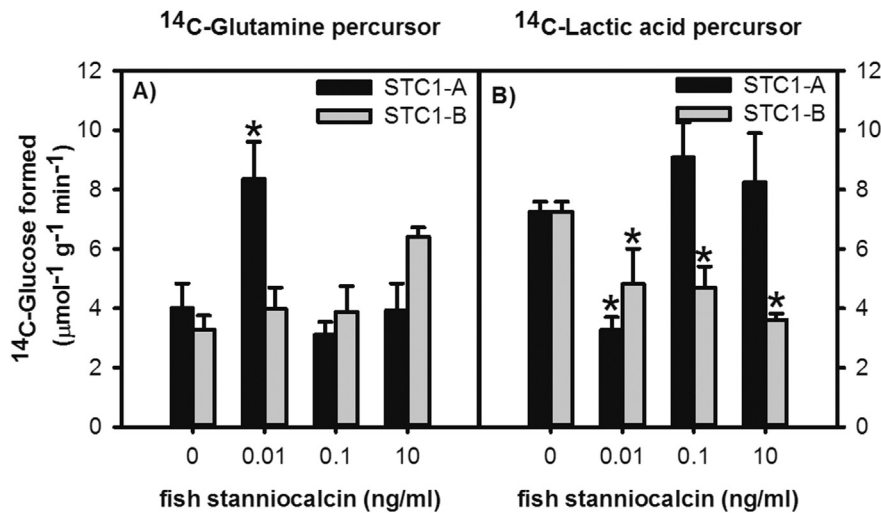

Fig. 4. Effect of recombinant fish STC1 homologues (fSTC1-a or fSTC1-b) into fish kidney gluconeogenesis. Bars indicate gluconeogenesis using ^{14}C-Glutamine (A and B) or ^{14}C-L-Lactate (C and D) as precursor to form ^{14}C-glucose after tissue incubation with different doses of fSTC1 (n = 4–13). Data is presented as the mean ± SE. * means $P < 0.05$.

anaplerotic reactions to replenish the α-ketoglutarate in the tricarboxylic acid (TCA) cycle and cataplerotic reactions to drain remnant 4-carbon metabolic intermediates from the cycle to synthesize glucose (Fig. 5). Lactate oxidation in the cytosol generates NADH which requires phosphoenolpyruvate produced in the mitochondria to balance the cytosolic redox state and then NADH is subsequently transported to the cytosol. Thus, we propose that the STC1-induced reduction of transcriptional and translational mitochondrial PEPCK activity increases the transport of malate to the cytosol, where it is oxidized to oxaloacetate to generate NADH. Since one NADH molecule is consumed per triose phosphate converted to glucose, two molecules of NADH produced with the reduction in mitochondrial PEPCK activity increase the NADH level in cytosol, creating a further imbalance in the cytosolic redox state. Our data show that the incorporation of ^{14}C-lactic acid into glucose does not decrease in the presence of different concentrations of hSTC1. A likely explanation is that cytosolic NADH can be transferred into mitochondria through the malate-aspartate shuttle; thereby transferring the electrons to the respiratory chain to balance the cytosolic redox state and the levels of reactive oxygen species (ROS). This model (Fig. 5) concurs with a role of STC1 in

cellular stress as found in isolated macrophages (Ellard et al., 2007; Wang et al., 2009). However, elucidation of this hypothesis requires further studies to determine the effects of STC1 on oxidative balance in renal medulla. Furthermore, mitochondrial PEPCK may play a role in the integration of multiple pathways in kidney glucose metabolism as suggested for mammalian liver (She et al., 2000; Stark et al., 2014).

Metabolic acidosis, PTH and epinephrine increase renal glutamine gluconeogenesis inducing the levels of PEPCK-C (cytosolic PEPCK), mitochondrial glutamate dehydrogenase and glutaminase, in contrast to insulin which suppresses renal glucose production in humans and animals (Mather and Pollock, 2011; Mitrakou, 2011; Nissim et al., 1995; Taylor and Curthoys, 2004). The increased activity of mitochondrial glutamate dehydrogenase and glutaminase is related to a pH-induced stabilization of their mRNA, while the lower pH induces PEPCK-C gene transcription in the kidney cortex (Curthoys and Gstraunthaler, 2001). This significant catabolism of glutamine greatly increases the quantity of α-ketoglutarate that enters the TCA cycle (anaplerosis). Since the cycle does not completely oxidize four and five carbon compounds to carbon dioxide, it is critical that the anions that enter the cycle are removed
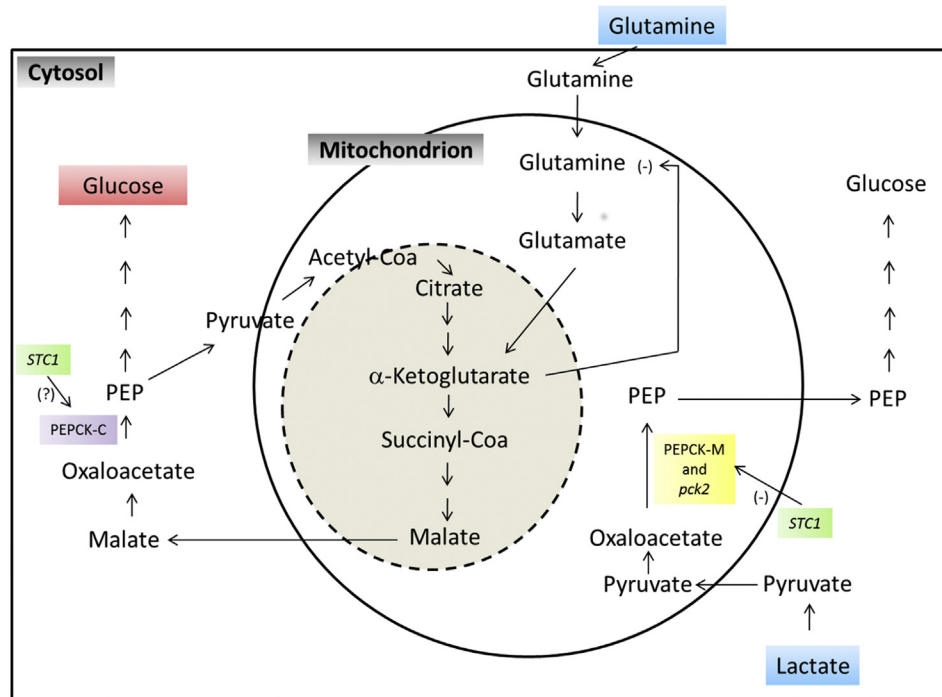
Fig. 5. Schematic representation of stanniocalcin-mediated gluconeogenesis molecular responsive-mechanisms in rat renal medulla identified in the present study. Substrates are shown in blue, down-regulated metabolites in red, stannicalcin-1 (STC1) in green, phosphoenolpyruvate carboxykinase-cytosolic isoform (PEPCK-C; *pck1*) in purple and phosphoenolpyruvate carboxykinase-mitochondrial isoform (PEPCK-M; *pck2*) in yellow. (−) indicates inhibition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(cataplerosis). The carbon chain of glutamine (α-ketoglutarate) leaves the mitochondria as malate, which is ultimately converted to glucose via gluconeogenesis (Chakravarty et al., 2005).

At what stage is glutamine metabolism in the outer renal medulla controlled by hSTC1? Our data show that, in presence of the hSTC1, glucose synthesis from ^{14}C-glutamine decreases by 40%, in renal medulla from fed rats without metabolic acidosis. Moreover, some studies have demonstrated that STC1 enhanced mitochondrial calcium uptake (Ellard et al., 2007; Yeung et al., 2012). Rise in the calcium mitochondrial concentration increases oxoglutarate dehydrogenase activity, hence the glutamine metabolism. One explanation for the decrease in glucose synthesis from glutamine, in presence of the hSTC1, is an increase in the conversion of the carbon chain of glutamine to phosphoenolpyruvate and further to pyruvate, via pyruvate kinase, for subsequent oxidation in the TCA cycle as acetyl-CoA, to generate energy molecules. Nevertheless, the bicarbonate generation is not affected, because both pathways generate two HCO_3^- ions per mole of α-ketoglutarate (Taylor and Curthoys, 2004). Therefore, the effect of the hSTC1 on the glutamine metabolism provides a high degree of metabolic flexibility necessary for the maintenance of homeostasis.

Although experiments that determine the activities of mitochondrial enzymes glutaminase and glutamate dehydrogenase are required, our results show that STC1 participates in the regulation of glutamine metabolism in outer renal medulla and therefore in acid-base balance and glucose homeostasis in mammals.

It has been shown that on a gram per gram tissue basis the gluconeogenic capacity of the kidney exceeds that of the liver (Krebs, 1963) and that in the postprandial state, renal gluconeogenesis increases by approximately 2-fold (Mitrakou, 2011). Furthermore, the kidney is responsible for up to 20% of all glucose production, contributing to about 40% of gluconeogenesis (Mather and Pollock, 2011). The decrease in glucose synthesis by STC1 may

be important to reduce renal glucose contribution, especially in type-2 diabetes mellitus patients.

Our data also revealed that the renal cortex and medulla respond differently to hSTC1, possibly due to the higher level of STC1 gene expression in inner renal medulla than in renal cortex (Turner et al., 2010). Also, in rats submitted to water deprivation the cortical and medullary renal STC1 genes respond differently (Turner et al., 2010).

While hSTC1 decreased gluconeogenesis from glutamine in rat kidney medulla, STC1-A had the opposite effect in fish kidney. In addition, the effect of STC1-A on renal gluconeogenesis was related to the precursor: STC-A increased the incorporation of ^{14}C-glutamine into glucose and, in contrast, decreased that of ^{14}C-lactic acid. The two STC1 isoforms caused a decrease in gluconeogenesis from ^{14}C-lactate in fish kidney (Fig. 4B). As fishes have lipids as primary energy source, this could be related to the increased conversion of lactate to pyruvate and, via pyruvate dehydrogenase, to acetil-CoA to be oxidized in the TCA cycle or converted to fatty acid (Tocher, 2003). However, the mechanisms and significance of these processes will need further investigation. In teleost fishes, the kidney contains high activity of glutamate dehydrogenase and glutaminase, and the glutamine metabolism is closely connected to the elimination of ammonia (Ip and Chew, 2010; Van Waarde, 1983). Thus, the significant increase in glucose from glutamine upon STC1-A (Fig. 4A) stimulation could be related to ammonia release (ammoniagenesis) and consequently increase the glucose production via gluconeogenesis.

In fish, the primary function of STC1 is related to calcium homeostasis, whereby STC1 (isoform A) is secreted by the CS in response to increased calcium in the blood protecting the animal against toxic hypercalcaemia (Schein et al., 2012; Wagner et al., 1986). Interestingly, *T. nigroviridis* kidney expresses both *STC1-A* and *STC1-B* (Schein et al., 2012) suggesting a potential paracrine

action related to renal gluconeogenesis. Furthermore, the CS are also usually located in close proximity on the ventral surface of the kidney. Altogether, these results suggest a degree of conservation of STC1 function on glucose metabolism from fish to mammals. This indicates that the most important features for this process may be structurally linked to the more conserved N-terminal region, since the C-terminal region is variable (Supplementary Fig. 1). However, the role of STC1-A and -B on the gluconeogenesis pathway in fish kidney needs more investigation.

5. Conclusion

Our results demonstrate a role for STC1 in the glucose synthesis control via renal gluconeogenesis in mammals and probable in teleost fishes, indicating conservation of function. STC1 inhibits gluconeogenesis in the rat renal medulla through a mechanism balancing the cytosolic redox state and pH in which mitochondrial PEPCK has a key role and uses glutamine as a source of carbon. In fish, STC1 seems to regulate preferentially lactic acid rather than glutamine as carbon source for gluconeogenesis. This may be linked with specificities of diet. In conclusion, STC1 appears to have an important role in the carbohydrate metabolism regulation, in particular gluconeogenesis from glutamine in the kidney, across the vertebrates.

Author contributions

Conceived and designed the experiments: VS LCK RSMS AVMC. Performed the experiments: VS LCK TLM PMGG. Analyzed the data: VS LCK RSMS AVCM. Contributed reagents/materials/analysis tools: VS IM DMP RSMS AVCM. Wrote the paper: VS RSMS AVM.

Competing interests

The authors have declared that no competing interests exist.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.mce.2015.07.010.

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