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The renal stanniocalcin-1 gene is differentially regulated by hypertonicity and hypovolemia in the rat

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ABSTRACT

Stanniocalcin-1 (STC-1) is made by kidney collecting duct cells for autocrine and paracrine targeting of nephron cell mitochondria. Here, the ligand stimulates respiratory uncoupling and calcium uniport activity. However, the underlying purpose of these actions and how the renal gene is regulated are poorly understood. In a previous study, we described the time-dependent, stimulatory effects of water deprivation on renal STC-1 mRNA levels in both rats and mice. In cortical kidney, STC-1 mRNA levels were increased 8-fold by 72 h of water deprivation, whereas the gene response in outer and inner medulla was less pronounced (2–4 fold). Gene induction occurred equally in males and females and was accompanied by increased mitochondrial STC-1 protein levels. As water deprivation increases extracellular fluid (ECF) tonicity and at the same time reduces ECF volume, the present study examined the individual effects of hypertonicity and hypovolemia on renal gene activity in rats. Hypertonicity, whether induced by mannitol, glucose or NaCl, uniquely stimulated the cortical gene, to the extent that transcript levels were positively correlated with serum osmolality. This was in contrast to high dietary sodium, which had no bearing on cortical or medullary transcript levels. The situation was reversed in the case of hypovolemia. Inner medullary gene expression was uniquely induced by hypovolemia (low sodium diet or polyethylene glycol) such that transcript levels were positively correlated with hematocrit, while cortical gene activity was unaffected or reduced. Hence, the cortical and medullary genes proved to be differentially regulated by changing ECF tonicity and volume, respectively. The findings are therefore indicative of cortical and medullary STC-1 having separate roles in the renal control of ECF balance.

1. Introduction

The in vivo actions and regulation of stanniocalcin-1 (STC-1) in mammalian kidney have not received a lot of attention to date. A role in kidney function is of particular interest from a phylogenetic standpoint. STC-1 was first identified in fishes, in endocrine glands that are embryologically derived from the pronephros. STC-1 in fish has stimulatory effects on renal phosphate transport, as has also proven to be the case for the mammalian hormone (Olsen et al., 1996; Wagner et al., 1997). However, the phosphate-conserving effects of STC-1 in mammalian kidney are not as striking as they are in fish, and are not accompanied by concomitant changes in serum levels of calcium or phosphate (Lu et al., 1996; Wagner et al., 1997; Gerritsen and Wagner, 2005). Similarly, in contrast to fishes, changes in serum levels of calcium and phosphate in mammals have only modest effects on renal STC-1 gene expression (Deol et al., 2001; Honda et al., 1999; Ookata et al., 2001; Yahata et al., 2003). For these reasons, there is growing speculation on our part that the principal role of STC-1 in mammalian kidney may lie outside the realm of mineral metabolism.

In a recent study, we monitored renal levels of STC-1 mRNA in a number of physiological models unrelated to mineral metabolism that included unilateral nephrectomy, over-hydration and water deprivation. Unilateral nephrectomy was examined because of the growing evidence of a role for STC-1 in cellular stress, in terms of its ability to promote respiratory uncoupling (Ellard et al., 2007; Wang et al., 2009), and lower the levels of reactive oxygen species (ROS) and intracellular calcium (Ellard et al., 2007; Zhang et al., 2000; Wang et al., 2009). In the week following unilateral nephrectomy, there are significant increases in blood flow, GFR, oxygen consumption, and energy usage by the remaining kidney (Dicker and Shirley, 1971). As ROS production is positively correlated with oxidative phosphorylation (Brookes, 2005) we reasoned that unilateral nephrectomy could be scenario under which STC-1 gene expression might be enhanced. This proved not to be the case, however, suggesting that enhanced energy requirements do not necessarily presage an increase in STC-1 synthesis, at least within the kidney. On the other hand, a change in hydration status – specifically water deprivation – had marked, stimulatory effects on renal STC-1 transcript levels in both male and female rats and mice. In

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cortical kidney, transcript levels were significantly elevated after 24 h of water deprivation and rose progressively to an 8-fold maximum by 72 h. In comparison, the inner medullary gene response was less pronounced and delayed in comparison; transcript levels were induced 3-fold after 48 h, but not any further by more prolonged periods of water deprivation.

The effects of water deprivation on renal STC-1 gene expression were remarkable in the sense that they far outstripped those obtained through manipulations of serum calcium, phosphate or vitamin D (Deol et al., 2001; Honda et al., 1999; Ookata et al., 2001; Yahata et al., 2003). More intriguingly, the cortical and medullary gene responses differed markedly in both their timing and amplitude. The cause of this differential induction could be the fact that water deprivation first of all entails a rise in ECF osmolality, followed then by a drop in ECF volume. As such, the cortical and inner medullary genes may have been respectively responding to the rising hypertonicity and progressive hypovolemia. The purpose of the present study was to address the cause of the differential gene regulation seen during water deprivation, using the male rat as a model system. Our findings clearly show that the cortical STC-1 gene is uniquely induced by rising serum hypertonicity and/or osmolality, and is generally unresponsive to changes in ECF volume. In contrast, the inner medullary gene proved to be uniquely induced by hypovolemia, and was unresponsive or suppressed by rising ECF osmolality. These findings presage distinct roles for renal STC-1 in the cortical and medullary responses to perturbations in ECF composition and volume.

2. Materials and methods

The animal studies employed adult male Wistar rats (200–300 g), adult male Wistar–Kyoto rats (20 weeks), spontaneously hypertensive (SHR) rats (20 weeks) or adult female C57Bl/6 mice. All animal studies were conducted in accordance with Canadian Council on Animal Care guidelines as approved by the Animal Use Sub-Committee at the University of Western Ontario. All chemicals and reagents were obtained from Sigma–Aldrich, Canada unless stated otherwise.

2.1. Hypertonicity, high dietary Na^+ and SHR studies

The effects of hypertonicity were assessed in three different paradigms. To assess the effects of acute hypertonicity, rats (250–300 g) were given single injections of either 1.5 M NaCl or 1 M mannitol (25 ml/kg, i.p.) both of which have been previously shown to effectively elevate blood plasma osmolality (Ghodsi et al., 1999; Bitoun et al., 2001). Upon injection, mannitol raises blood plasma osmolality and produces a rapid diuretic and natriuretic response, similar to that observed following hypertonic saline injection (Better et al., 1997). Rats that received 1.5 M NaCl were also given 5% NaCl in the drinking water to sustain the high serum Na^+ levels, whereas controls were injected with 25 ml/kg of saline (0.15 M NaCl) and maintained on regular water. Rats that received mannitol had their water removed for the entire experimental period, whereas mannitol controls were saline injected (25 ml/kg) and maintained without water. A third control group was not injected and maintained on regular water to assess the effects of an i.p. injection on renal STC-1 gene expression. To assess the time-course effects of hypertonicity, rats were given single injections of 1.5 M NaCl (25 ml/kg, i.p.) and sacrificed 3, 6 and 12 h post injection. Timed controls received saline alone (25 ml/kg, i.p.).

To assess the effects of chronic hyperglycemia, groups of C57BL/6 mice received tail vein injections of streptozotocin (STZ) or buffer alone (3 successive daily injections of 80 mg/kg in 0.2 ml of sodium citrate buffer, pH 4.5). The development of hyperglycemia following STZ treatment was assessed via measures of urinary glucose. Mice were then sacrificed three and ten days after the third STZ injection.

To assess the effects of a chronic Na^+ load in the absence of hypertonicity, 2 groups of rats were placed on a high Na^+ diet containing either 4% NaCl (TD 92034) or 8% NaCl (TD 92012) for 2 weeks. Control rats were maintained on normal lab diet containing 0.6% NaCl (TD 99414). The dietary formulations were obtained from Harlan Teklad (Madison, WI). All rats had free access to tap water.

2.2. Hypovolemia studies

Two experimental paradigms were employed to assess the effects of hypovolemia on renal STC-1 mRNA levels in the rat. The first paradigm entailed dietary sodium restriction with or without concomitant furosemide treatment. Two groups of rats (250–300 g) were placed on a Na^+-deficient diet (Harlan, TD 90228; 0.01% sodium) for 7 days and had free access to Na^+-free water (Weiner et al., 1971). To accelerate the Na^+ depletion and consequent hypovolemia, one of the Na^+-deficient groups was given injections of the loop diuretic, furosemide, for the first two days of the study (10 mg/kg body weight in 1.7% ethanolamine, i.p.), while the other group received solvent alone. Control rats were maintained on a Na^+ replete diet (Harlan, TD 99414; 0.6% sodium) and regular tap water. The second paradigm employed intraperitoneal injections of polyethylene glycol 6000 (PEG). Rats were injected with 30% PEG dissolved in 0.9% saline (16.5 ml or 5 g PEG/kg body weight) and sacrificed 8 h later (Stricker and Verbalis, 1986; Frankmann et al., 1987; Kondo et al., 2004).

2.3. Hypertension and renal STC-1 gene expression

To complement our studies on angiotensin II blockade (Turner et al., 2010) which decreases blood pressure during dehydration (Burnier et al., 1983), we assessed the effects of high blood pressure on renal STC-1 gene expression in SHR rats. In this case, the levels of expression were compared in 20 week old male Wistar–Kyoto and SHR rats, at which point there is frank hypertension in SHR rats alone (Kimura et al., 1989; O'Sullivan and Harrap, 1995).

2.4. Collection and preparation of tissue samples

Animals were euthanized with CO_2 gas at the end of each experiment. Upon sacrifice, both kidneys were rapidly removed and placed on ice and blood was collected by cardiac puncture. Samples of cortical and inner medullary kidney were immediately placed in TRIzol (Invitrogen, Carlsbad, CA, USA) and homogenized with a motorized pestle. Total RNA was then isolated according to the manufacturer's recommendations (Invitrogen, Carlsbad, CA, USA). Extracted RNA was resuspended in 50–100 μl of DEPC-treated water, aliquoted and stored at −80 °C until use. Total RNA was assessed for purity using 260/280 absorbance ratios and quantified using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.5. Quantitative PCR

Relative STC-1 mRNA levels of experimental and control groups were determined using TaqMan STC-1 gene expression assay and TaqMan one-step RT-PCR master mix (Applied Biosystems, Foster City, CA, USA). The expression of STC-1 was computed relative to the expression of a reference gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), using the comparative cycle threshold method (delta-delta-C_T). PCR reactions were 15 μl in volume and contained 25 ng of total RNA with a 260/280 absorbance ratio of not less than 1.8. Each reaction was performed in quadruplicate on a clear 384 well plate that was sealed with an adhesive film prior to analysis. Reverse transcription was first carried out for 30 min at 48 °C followed by an enzyme activation phase of 10 min at 95 °C. Next the amplification reaction was run for 40 cycles alternating between 95 °C and 60 °C for 15 s and 1 min, respectively. All steps were preformed on an ABI Prism 7900 HT sequence detector. Sequence Detection Software 2.0 (Applied Biosystems, Foster City, CA, USA) was used for analysis. Efficiencies above 90% were deemed acceptable.

2.6. Osmolality measurements

After collection, blood was allowed to clot over night at 4 °C. Samples were then centrifuged at 3000 rpm and the clear supernatant was obtained for analysis. Osmolality was measured on a VAPRO 5520 vapor pressure osmometer (Wescor Inc.) using a 10 μl sample.

2.7. Statistical analysis

All control and experimental data were expressed as statistical means ± SEM. All data were evaluated by two-tailed, paired *t*-tests or one-way analysis of variance (ANOVA) for comparison of three or more groups. A Dunnett's post hoc test was used to compare two or more means to a common control mean. A Tukey post hoc test was used as multiple comparison test. Groups with a p value <0.05 were considered statistically significant. All statistical analyses were preformed with GraphPad Prism software (GraphPad Software Inc.).

3. Results

3.1. Hypertonicity

Hypertonicity was effectively induced by injecting NaCl or mannitol to produce a significant increase in blood plasma osmolality (Fig. 1A). It also caused reductions in body weight in both groups (Table 1), the effects of which were statistically significant in mannitol-treated rats. Twelve hours after the induction of hypertonicity, cortical STC-1 mRNA levels were increased ~5-fold in both NaCl- and mannitol-treated rats (Fig. 1B). In contrast, neither treatment significantly affected transcript levels in the inner medulla, although an inhibitory trend was evident in both groups (Fig. 1C).

Table 1
Body weight changes in control and hypertonic rats over 12 h.

Treatment group	Initial body weight (mean ± SEM)	Final body weight (mean ± SEM)	Significance (paired *t*-test)
Saline ($n=5$)	366.6 ± 6.1 g	363.2 ± 5.1 g	$p>0.05$
Saline WD ($n=6$)	368.4 ± 7.0 g	365.0 ± 6.5 g	$p>0.05$
NaCl ($n=6$)	388.0 ± 10.2 g	360.4 ± 13.1 g	$p=0.1$
Mannitol ($n=6$)	386.9 ± 9.9 g	378.8 ± 10.4 g	$p<0.05$

The temporal effects of hypertonicity on STC-1 gene induction were also examined. In response to a single injection of 1.5 M NaCl, serum osmolalities became significantly higher at 3 h post-injection and then fell thereafter until they were no longer significantly different than controls by 12 h (Fig. 2A). In response to the rise in serum osmolality, cortical levels of STC-1 mRNA increased in sigmoidal fashion, were significantly higher by 3 h ($p<0.05$) and approached an upper asymptote by 12 h (Fig. 2B; $p<0.01$). In contrast, inner medullary transcript levels did not change in response to the same hypertonic stimulus (Fig. 2C; $p>0.05$). When cortical levels of gene expression and the serum osmolalities of individual rats were subjected to regression analysis there proved to be a significant, positive correlation between the two variables ($r^2=0.27$; $p=0.004$), suggesting that ECF osmolality – directly or indirectly – was responsible in part for stimulating the cortical gene (Fig. 2D).

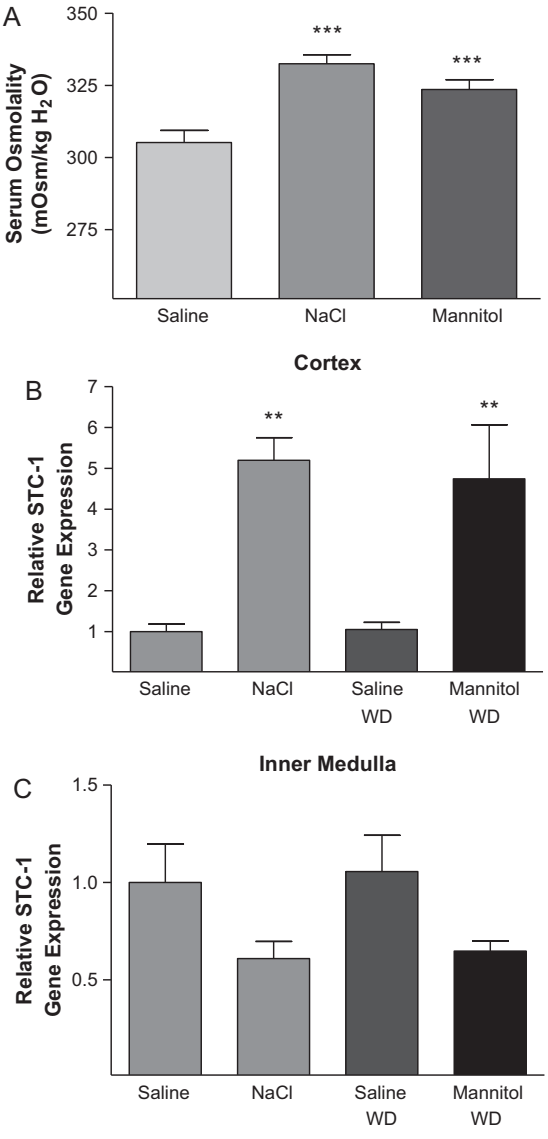

Fig. 1. Cortical and medullary STC-1 mRNA levels are differentially regulated by hypertonicity. (A) Hypertonicity was induced by single injections of NaCl or mannitol as indicated by the significant increases in serum osmolality. (B) In kidney cortex, both NaCl and mannitol increased STC-1 mRNA levels compared with their respective saline controls. (C) In contrast, inner medullary levels of STC-1 mRNA were unchanged in comparison to controls (**$p<0.01$, ***$p<0.001$, ANOVA followed by Dunnett's test; $N=5$–6 rats/group; WD = water deprived).

Fig. 3 illustrates the effects of hypertonicity on renal STC-1 mRNA levels in another context. In normal rats (prior to the induction of NaCl-induced hypertonicity), inner medullary transcript levels were ~6-fold higher than those in the cortex ($p<0.001$). After 12 h of hypertonicity, however, this disparity was abolished by gene induction uniquely in the cortex ($p>0.05$). This was highly reminiscent of what occurs in the kidneys of rats and mice rendered both hypertonic and hypovolemic by 48 h of water deprivation (Turner et al., 2010). The effects of hypertonicity were more pronounced in this case, however, as water deprivation also induces the medullary gene whereas hypertonicity does not. Mannitol and NaCl were not the only impermeant solutes capable of inducing cortical gene expression. The destruction of insulin-producing beta cells and ensuing hyperglycemia had modest, stimulatory effects on cortical STC-1 mRNA levels in mice 10 days after treatment with STZ (Fig. 4; $p<0.05$). However, there were no significant changes in inner medullary gene expression (results not shown).

In contrast to the marked effects of hypertonicity on cortical STC-1 gene expression, two weeks on high sodium diets (4–8% Na^+) had no bearing on gene activity (Fig. 5; $p>0.05$). In this context it is important to point out that serum osmolalities were unchanged in rats on high dietary Na^+ as compared to rats on a normal diet (0.6% Na^+ – 337.3 ± 1.4 mOsm/kg H_2O, 4% Na^+ – 336.8 ± 1.4 mOsm/kg H_2O, 8% Na^+ – 333.8 ± 2.3 mOsm/kg H_2O).

3.2. Hypovolemia

Chronic dietary Na^+-restriction, with and without concomitant furosemide treatment, was the first of two models employed to induce hypovolemia. Serum osmolalities were unchanged among the treatment groups (control – 342.3 ± 2.4 mOsm/kg H_2O; Na^+-restricted – 339.5 ± 6.9 mOsm; Na^+-restricted + furosemide – 351.5 ± 7.4 mOsm). Body weights were significantly reduced, however, in Na^+-restricted, furosemide-treated rats, but not in rats on low Na^+ alone (Table 2). More importantly, Na^+-restriction in combination with furosemide had significant effects on STC-1 mRNA levels. Furthermore, the direction of this effect differed according to zone. Transcript levels were suppressed in the cortex ($p<0.05$; Fig. 6A), but increased 2.5-fold in inner medullary kidney ($p<0.01$; Fig. 6B). The same upward and downward general trends were

Table 2
Body weights of normal and sodium-restricted rats.

Treatment group	Final body weight (mean ± SEM)	Difference from control (%)
Control ($n=7$)	305.6 ± 13.8 g	–
Na^+-restricted ($n=7$)	292.8 ± 11.7 g	−4.0 ± 1.8
Na^+-restricted + furosemide ($n=7$)	267.8 ± 8.0 g**	−11.9 ± 1.7

** $p<0.01$, ANOVA followed by Dunnett's post hoc test.

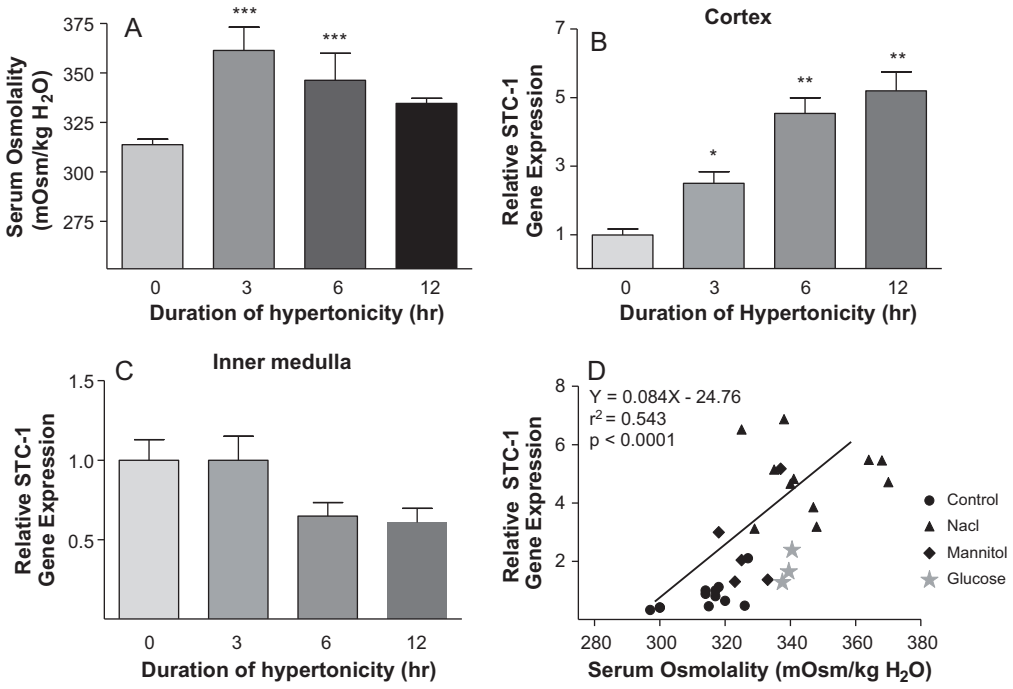

Fig. 2. Time-dependent stimulatory effects of NaCl-induced hypertonicity on renal levels of STC-1 mRNA. (A) A single injection of hypertonic saline significantly increased serum osmolality by 3 h, after which it declined over time. (B) In cortex, STC-1 mRNA levels rose in a time-dependent manner to a 5-fold maximum after 12 h. (C) In inner medulla, hypertonic saline did not significantly affect STC-1 mRNA levels. (D) In NaCl and mannitol treated rats, cortical levels of STC-1 mRNA were positively correlated with serum osmolality ($p < 0.0001$). The data from STZ-treated mice (glucose) is superimposed on the graph for comparison ($^{*}p < 0.05$, $^{**}p < 0.01$, ANOVA followed by Dunnett's test; $N = 6$ rats/group; linear regression analysis was used for panel D).

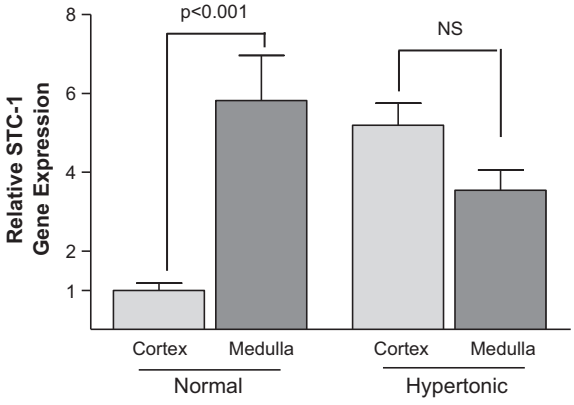

Fig. 3. Comparative STC-1 mRNA levels in cortical and inner medullary kidney of control and hypertonic rats. In control rats, STC-1 mRNA levels were 6-fold higher in inner medulla as compared to cortex. This discrepancy was abolished in hypertonic rats due to selective induction of the cortical gene (ANOVA followed by Tukey's test; $N = 6$ rats/group; NS = non-significant).

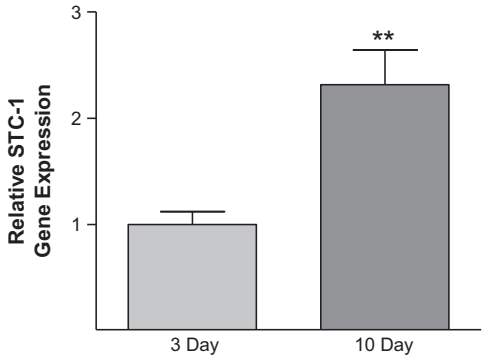

Fig. 4. Comparative STC-1 mRNA levels in normal and hyperglycemic mice. Mice were injected on three successive days with streptozotocin (STZ) and sacrificed 3 and 10 days after the final injection. Ten days after STZ treatment, cortical STC-1 mRNA levels were significantly higher than those in the 3 day group. Table 4 shows that the 10-day group also had significantly higher serum osmolalities and serum glucose levels ($p < 0.01$, paired t-test; $N = 6$ rats/group).

evident in rats on low Na^+ alone, but did not achieve statistical significance ($p > 0.05$; Fig. 6A,B).

The second model of hypovolemia was acute rather than chronic and employed injections of polyethylene glycol 6000 (PEG) to physically absorb extracellular fluid. The efficacy of this was evident by the rise in hematocrit in PEG-treated rats (Table 3). More importantly, PEG treatment had significant effects on STC-1 mRNA levels in a zone-specific manner. In cortical kidney, transcript levels were unaffected by PEG treatment ($p > 0.05$; Fig. 6C). In inner medullary kidney, on the other hand, transcript levels were significantly increased by PEG treatment ($p < 0.05$; Fig. 6D) similar to that seen in Na^+ restricted, furosemide-treated rats (Fig. 6B). When individual hematocrits and inner medullary gene expression levels

Table 3
Hematocrits of saline and PEG treated rats.

Treatment group	Hematocrit (mean ± SEM)
Control ($n = 7$)	42.0 ± 2.6
PEG injected ($n = 7$)	59.6 ± 1.0***

*** $p < 0.001$ unpaired t-test.

Table 4
Serum glucose and osmolalities in STZ-treated mice.

Treatment group	Serum glucose	Serum osmolality
3 day STZ-treated ($n = 3$)	8.7 ± 0.3	320.2 ± 0.67
10 day STZ-treated ($n = 3$)	36.0 ± 1.7**	339.2 ± 0.88***

** $p < 0.01$.
*** $p < 0.001$ unpaired t-test.

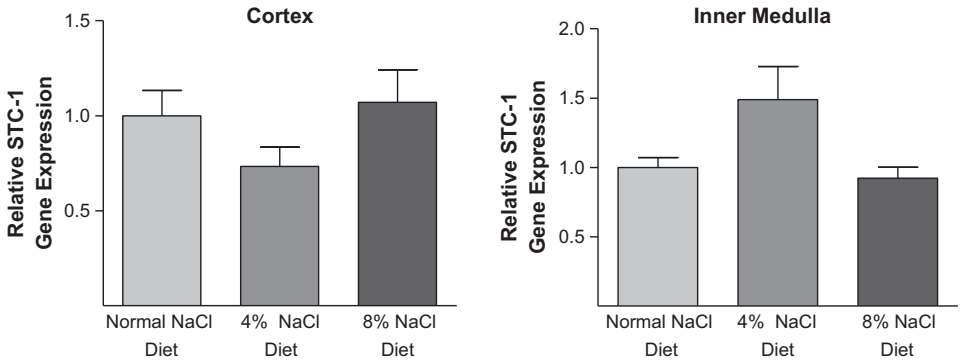

Fig. 5. High sodium diets have no effect on renal STC-1 gene expression. Rats were placed on high Na^+ diets for 2 weeks (4% or 8% NaCl). Renal STC-1 mRNA levels were unchanged in rats on high Na^+ as compared to rats on a normal 0.6% Na^+ diet (ANOVA followed by Dunnett's test; $N = 6$ rats/group).

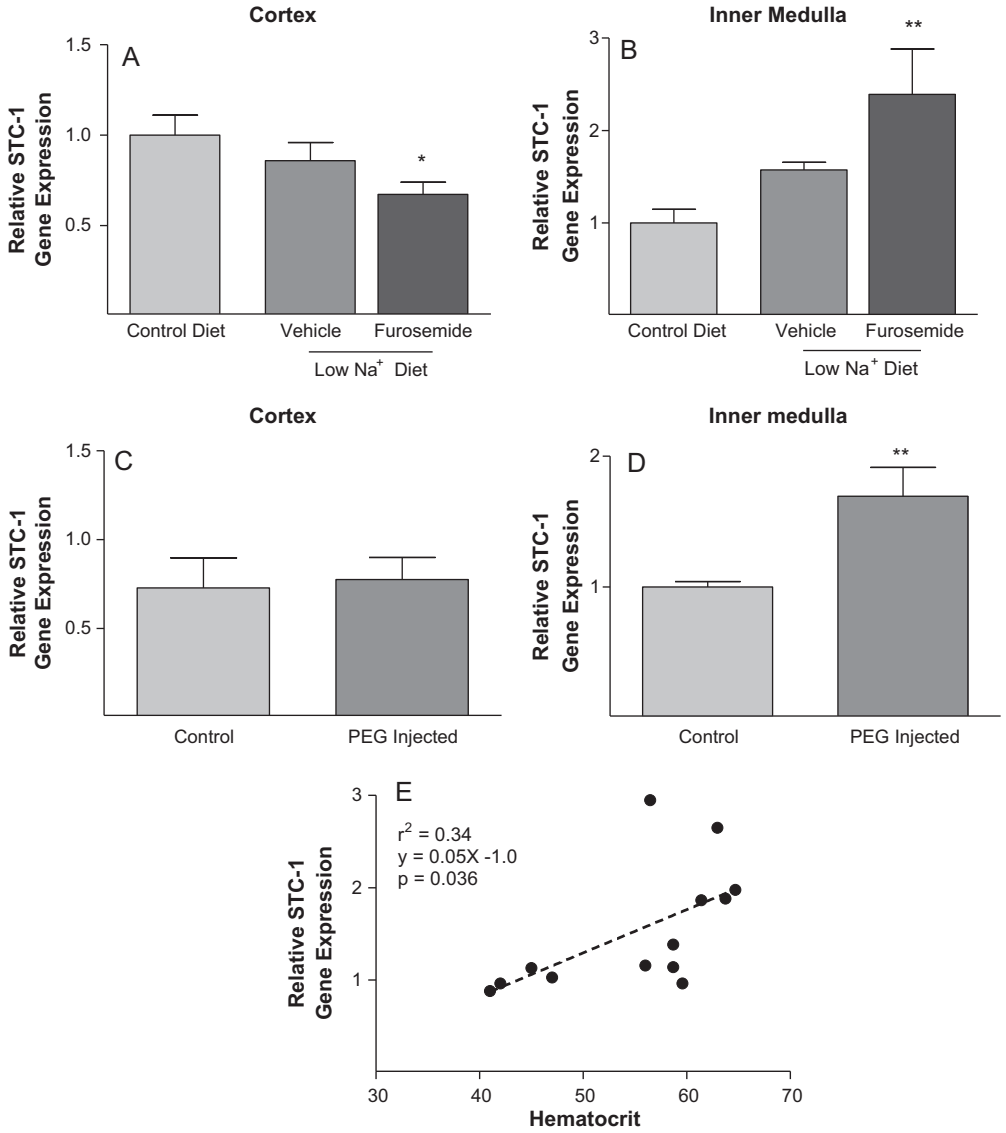

Fig. 6. The cortical and inner medullary STC-1 genes are differentially regulated by hypovolemia. (A) In the cortex, Na^+-restriction alone did not significantly affect STC-1 mRNA levels. However, Na^+-restriction plus furosemide treatment significantly decreased STC-1 mRNA levels. (B) In the inner medulla, STC-1 mRNA levels were increased in Na^+-restricted, furosemide-treated rats, but unchanged in those on low Na^+ alone. (C) In the cortex, injections of polyethylene glycol (PEG) had no effect on STC-1 mRNA levels. (D) In the inner medulla, PEG significantly increased STC-1 mRNA levels. (E) In PEG treated rats, inner medullary STC-1 mRNA levels were positively correlated with hematocrit (**$p < 0.01$, *$p < 0.05$; ANOVA followed by Dunnett's test; $N = 7$ rats/group. C and D – unpaired t-test; $N = 6$ rats/group. Linear regression analysis was used for panel E).

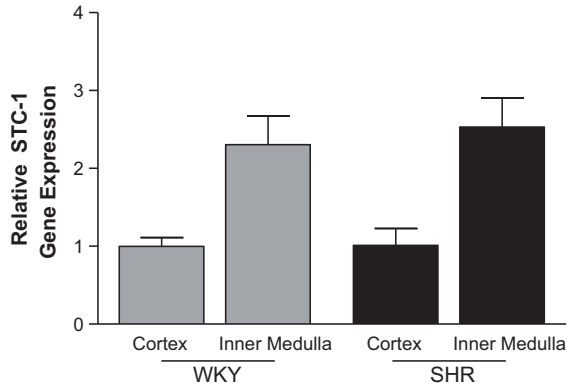

Fig. 7. Renal STC-1 mRNA levels are unaffected by high blood pressure. Renal STC-1 mRNA levels were not significantly different in Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) ($p > 0.05$, paired t-test).

were subjected to regression analysis there proved to be a significant, positive correlation between the two variables ($r^2 = 0.34$; $p < 0.05$), suggesting that ECF volume was responsible in part for stimulating the inner medullary gene (Fig. 6E). And, in contrast to the effects of hypovolemia, high blood pressure had no effect on cortical or inner medulla transcript levels in SHR rats (Fig. 7).

4. Discussion

We have previously shown that water deprivation in rodents has time-dependent, stimulatory effects on STC-1 mRNA levels in both cortical and inner medullary kidney (Turner et al., 2010). However, the effects of water deprivation occurred earlier in the cortex and were much more pronounced in cortex than medulla (8 vs. 3 fold). Hence, it appeared that the two zones might be differentially responsive to the two major consequences of water deprivation on the ECF compartment; hypertonicity and hypovolemia. The present study has now confirmed this to be the case by showing that falling ECF volume (without affecting tonicity) and rising ECF tonicity (without affecting volume) differentially affect the cortical and inner medullary genes, in terms of both direction and magnitude.

4.1. Hypertonicity

In response to progressive water deprivation there is a marked increase in ECF tonicity and a significant decrease in ECF volume (Januszewicz et al., 1986). These represent two separate, physiological stressors that could independently affect renal STC-1 gene expression during dehydration. Of these, hypertonicity is already known to stimulate the gene in kidney cell lines (Sazonova et al., 2008; Sheikh-Hamad et al., 2000). However, it remained to be seen if hypertonicity had similar effects in vivo. To address this, we monitored STC-1 mRNA levels in hypertonic rats, a model in which the hypertonicity of dehydration can be reproduced without the confounding effects of falling ECF volume. The results showed that acute hypertonicity, induced by a single injection of 1.5 M NaCl, increased cortical STC-1 mRNA levels without causing any corresponding upregulation in the inner medulla. This implied that the increase in cortical gene activity following water deprivation (Turner et al., 2010) was specifically in response to the rise in ECF tonicity. The cortical gene response to hypertonicity was also time-dependent (as it was during water deprivation), with the maximum response occurring 6–12 h after induction. The relationship was such that cortical levels of STC-1 mRNA proved to be positively correlated with serum osmolality; as osmolality rose, cortical STC-1 mRNA levels rose in kind.

To rule out the possibility that Na^+ itself had induced the cortical gene, and not hypertonicity per se, mannitol was employed to produce an equivalent degree of hyperosmolality. The results showed that in cortex and medulla, mannitol had the same effects (or lack thereof in medulla) on transcript levels as those obtained with sodium chloride. Reinforcing the importance of hypertonicity, the high serum osmolalities in hyperglycemic, diabetic mice also stimulated cortical gene expression. These findings therefore demonstrate that incremental increases in ECF osmolality, in response to impermeant ions such as Na^+ and glucose (and likely plasma proteins), cause proportional increases in renal cortical STC-1 gene expression, the nature of that proportion being defined by the slope of the regression line.

In contrast to its marked effect on cortical gene activity, there was little evidence of a role for osmolality in medullary gene regulation. This was evident by the absence of any effect of sodium or mannitol loading, in spite of the fact that they have entirely opposite effects on inner medullary interstitial fluid (IF) osmolality. Whereas sodium loading increases IF osmolality, mannitol reduces it (Ullrich et al., 1955; Appelboom et al., 1965; Atherton et al., 1968; Wald et al., 1989) and yet neither solute significantly impacted medullary transcript levels. If anything, STC-1 transcript levels were suppressed in both cases. Thus, it is fair to conclude that changes in ECF osmolality uniquely affect the cortex and have little if any bearing on inner medullary gene activity.

Finally, having determined a relationship between blood plasma osmolality and cortical mRNA expression, we also examined the effects of high dietary Na^+ on renal gene activity by placing rats on high Na^+ diets for up to 2 weeks (4–8% Na^+). However, in spite of these substantial increases in Na^+ intake, there were no changes in ECF osmolality or cortical transcript levels. The findings therefore further reinforced the importance of ECF hypertonicity and/or hyperosmolality in mediating cortical gene activity.

There are at least two mechanisms whereby hypertonicity could stimulate cortical gene activity. The first entails a direct effect of hypertonicity on collecting duct cells. Mannitol, NaCl and glucose all draw cellular water into the ECF compartment, causing cell shrinkage (Alfieri and Petronini, 2007; Stahl, 1965) and which could induce cortical gene expression. Many hypertonicity-responsive genes are induced in this manner, controlled by the TonEBP/NFAT family of transcription factors; notably those involved in renal osmolyte synthesis (Cha et al., 2001; Burg et al., 2007). Atrial natriuretic factor is regulated in this manner. Alternatively, some genes respond to hypertonic stress independently of TonEBP; a case in point being thick ascending limb-derived endothelin-1. The renal STC-1 gene belongs to the latter category, as mice with an impaired TonEBP response (Moeckel et al., 2006) have a normal STC-1 response to water deprivation (Turner et al., 2010). Alternatively, gene upregulation may be in response to arginine vasopressin (AVP). Hypertonicity is a potent stimulus for AVP release and previous studies show that the cortical gene response to water deprivation is attenuated by V2 receptor blockade (Turner et al., 2010). As such, both AVP and hypertonicity are potential regulators of the STC-1 gene, at least within the cortex.

The function of newly synthesized cortical STC-1 could be in the realm of tubular transport. During the hypertonicity of dehydration and hypertonic states like that produced by NaCl loading, it is crucial for the kidneys to excrete Na^+ in order to maintain an isotonic body fluid. The process, known as controlled natriuresis, is regulated by a complex network of systemic and cellular pathways that strive to excrete Na^+ in a minimal amount of water, and it is possible that STC-1 is induced by or aids in their actions. Proximal tubular dopamine, oxytocin, prostaglandins, AVP and systemically derived-angiotensin IV all have proven to be involved in controlled natriuresis (Inoue et al., 1993; Hubbard and Henderson, 1995; Huang et al., 1995, 1996; Czekalski et al., 1996). Oxytocin

has already been implicated in ovarian STC-1 gene activation (Deol et al., 2001) and AVP has a role in inducing the cortical STC-1 gene during water deprivation (Turner et al., 2010). Furthermore, as in the case of AVP, oxytocin secretion is markedly increased in response to both water deprivation and euvolemic hypertonicity (Boone and Deen, 2008; Huang et al., 1995, 1996). Hence, both could be involved in regulating the renal STC-1 gene.

4.2. Hypovolemia

Dietary Na^+ restriction in rats causes a controlled diuresis and a reduction in ECF volume, all the while maintaining a normal ECF tonicity (Brenner and Berliner, 1969; Shirley and Skinner, 1994). As such, this model allowed us to monitor renal STC-1 gene expression in response to hypovolemia (like that produced by water deprivation) in the absence of hypertonicity. The results showed that there were no changes in gene activity of rats Na^+-restricted for 8 days, although downward and upward trends were evident in cortex and medulla, respectively. However, inner medullary gene activity was significantly increased in rats supplemented with furosemide, to the same degree (2.5 fold) as that obtained by water deprivation (Turner et al., 2010). Likewise, the PEG model of hypovolemia increased inner medullary gene expression after just 12 h. Collectively therefore, these findings suggest that falling ECF volume was the main driver of inner medullary gene induction during water deprivation (Turner et al., 2010), while hypertonicity on its own had little or no effect.

If hypovolemia does drive inner medullary gene expression, this would explain why the gene response to water deprivation is delayed here in comparison to that in cortex (48 vs. 12 h), as significant hypovolemia in the rat only occurs after 48 h of water deprivation; the very point at which inner medullary gene expression is increased (Turner et al., 2010). It would also explain why inner medullary gene activity was only increased in Na^+-restricted, furosemide-treated rats; as they were the only group to undergo significant hypovolemia. That being said, the inner medullary gene response to hypovolemia is not always slow, as it would appear from the water deprivation studies and furosemide studies. If the onset of hypovolemia is rapid, as in the case of PEG treatment, the gene is capable of being upregulated in kind. Interestingly as well, the inner medullary gene was only responsive to falling ECF volume, as there were no reciprocal, negative effects of high blood pressure on steady state levels of the transcript.

The present study has also confirmed that there is indeed a major difference between the cortical and inner medullary genes; a difference that first became apparent in water deprivation studies. The cortical gene is capable of being induced to a much greater degree (8 vs. 3 fold) than that in inner medulla. This could be related to the fact that the inner medullary gene has a higher basal level of expression to begin with and hence does not need to be induced to the same extent. More than likely, however, it relates to the gene product having different functions in the two different zones. Finally, in contrast to the changes observed in inner medullary kidney, cortical levels of STC-1 mRNA were either unchanged or marginally reduced by hypovolemia. As such, hypovolemia cannot be viewed as a contributing factor to the gene induction that occurs in cortex following water deprivation (Turner et al., 2010) or hypertonicity as in the present study.

The STC-1 cells that make up fish corpuscles of Stannius arise during development from the pronephros. Moreover, the parallels between the inner medullary gene response to hypovolemia and fish STC-1 are intriguing. Some of the earliest studies on fish corpuscles of Stannius showed that the glands contain a pressor substance (Chester-Jones et al., 1966). This has since been given greater credence by studies showing that hypovolemia is a potent stimulus for STC-1 secretion in fish (Butler et al., 2003), and that the renin–angiotensin response to hypovolemia is markedly attenuated in fish lacking corpuscles of Stannius (Butler and Brown, 2007). Hence, a role in ECF volume control is implied for both the fish and rat inner medullary hormones. Thus far, the cortical gene response to hypertonicity appears to be a uniquely mammalian phenomenon, as fish STC-1 cells are unresponsive to hypertonicity both in vitro and in vivo (Wagner et al., 1989, 1991). And yet, several groups have reported that surgically removing the corpuscles of Stannius results in significant changes in serum levels of both sodium (lower) and potassium (higher), in addition to the more frequently reported effects on serum calcium (Fontaine, 1964; Chester-Jones et al., 1966). Hence it is possible that STC-1 has regulatory effects on ECF composition in both vertebrate classes.

In summary, we have demonstrated that the dehydration-induced upregulation in cortical and inner medullary STC-1 gene expression occurs in response to two separate stimuli – hypertonicity/osmolality and hypovolemia. The cortical gene was responsive to rising ECF tonicity, to the extent that mRNA levels and serum osmolality were positively correlated. Intriguingly however, the correlation was not particularly strong, suggesting that factors other than serum osmolality are driving cortical gene induction. One likely candidate is AVP, which is partly responsible for the cortical gene response to water deprivation (Turner et al., 2010). Moreover, AVP-producing cells are exquisitely sensitive to rising ECF osmolality. The involvement of factors other than ECF volume in inner medullary gene induction is also apparent by the weak correlation between hematocrit and transcript levels in the innermost zone. Histological studies may also reveal marked changes in the distribution of ligand and/or receptor in response to hypertonic and hypovolemic challenges, all of which could lead to a better understanding of renal STC-1 function. Finally, the study has identified both similarities and differences between renal STC-1 in mammals and its homolog in fish corpuscles of Stannius.

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