Mini-review: regulation of the renal NaCl cotransporter by hormones

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1Molecular Physiology Unit, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico; and 2Department of Nephrology and Mineral Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

Rojas-Vega L, Gamba G. Mini-review: regulation of the renal NaCl cotransporter by hormones. Am J Physiol Renal Physiol 310: F10–F14, 2016. First published October 28, 2015; doi:10.1152/ajprenal.00354.2015.—The renal thiazide-sensitive NaCl cotransporter, NCC, is the major pathway for salt reabsorption in the distal convoluted tubule. The activity of this cotransporter is critical for regulation of several physiological variables such as blood pressure, serum potassium, acid base metabolism, and urinary calcium excretion. Therefore, it is not surprising that numerous hormone-signaling pathways regulate NCC activity to maintain homeostasis. In this review, we will provide an overview of the most recent evidence on NCC modulation by aldosterone, angiotensin II, vasopressin, glucocorticoids, insulin, norepinephrine, estradiol, progesterone, prolactin, and parathyroid hormone.

angiotensin II; NCC; norepinephrine; prolactin; PTH

IN THE DISTAL CONVOLUTED TUBULE (DCT), Na+ and Cl– reabsorption is fine tuned by the electroneutral Na+/Cl– cotransporter (NCC) (13) expressed in the apical membrane and target of the thiazide-type diuretics (11). Thiazides are the first-line pharmacological therapy for the management of arterial hypertension (10, 34). Inactivating mutations of the SLC12A3 gene (NCC) result in Gitelman syndrome, featuring arterial hypotension with hypokalemia, metabolic alkalosis, and hypocalcemia (48), while NCC activation by mutant kinases (56) or ubiquitin ligases (3, 25) produce pseudohypoaldosteronism type II, a mirror image disease featuring hypertension, hyperkalemia, metabolic acidosis, and hypercalcemia. Thus NCC activity is not only critical for salt balance and blood pressure regulation, but also for potassium, calcium, and acid-base handling in the kidney (30).

It is known that expression/activity of NCC is affected by two known posttranslational mechanisms (14). Phosphorylation by the kinase STE-20 proline-alanine-rich (SPAK) in the NCC amino-terminal domain (38) [which in turn is modulated by the “with no lysine kinases” (WNKs)] is associated with the activation of the cotransporter (35, 39), and ubiquitylation by either a direct effect of the HECT-type E3 ubiquitin ligase complex, Nedd4-2, which reduces the amount or activity of NCC in the plasma membrane (2, 41), or an indirect effect of the RING-type ubiquitin ligase complex, KLHL3 and Cul3, that modulates the ubiquitylation of WNKs (3, 25).

Regulation of NCC activity by the master rheostats of the body, the hormones, has been extensively studied over the years. It has been established that NCC is a pleiotropic protein in the sense that many different stimuli can modulate its activity. The goal of this short review is to provide a glimpse of the most recent evidences of NCC control by hormones (Table 1) (Fig. 1).

Aldosterone

Aldosterone was the first hormone found to have an effect on NCC activity. This was demonstrated in rats by direct aldosterone infusion and as a response to a low-sodium diet (LSD) (18). Increased activity of NCC in response to aldosterone is due to increased expression and phosphorylation of the cotransporter (1, 16, 27) by a mineralocorticoid receptor-dependent SPAK-mediated phosphorylation of NCC (9, 22). The mechanism has not been elucidated yet. Some evidence suggests that it could be through modulation of WNK4 activity and/or SGK1 activation (43, 54), and a recent study showed that WNK1 is a target of Ned4-2, an effect of the ligase that is inhibited by SGK1 (42). Inhibition of Ned4-2 ligase by SGK1 phosphorylation could also explain the increased expression of NCC since it has been observed that Ned4-2 knockdown in mice results in overexpression of NCC, suggesting a tonic reduction of the cotransporter by Ned4-2 (41). Additionally, WNK3, an activator of NCC, also inhibits Ned4-2 by a mechanism that appears to be different to SGK1 (24).

ANG II

It was first reported in 2007 that administration of captopril, an ANG II-receptor antagonist, in rats acutely promoted the redistribution of apical NCC to cytoplasmic vesicles and that the coadministration of ANG II reversed this effect (45). In Xenopus laevis oocytes ANG II promoted NCC activation by a WNK4- SPAK-dependent mechanism (44). Later, it was observed that in rats exposed to a LSD, NCC apical expression increased in an aldosterone-independent fashion (12). Supporting this, another work showed that chronic ANG II infusion
increased the thiazide-sensitive distal salt reabsorption that was not prevented by spironolactone (57). The aldosterone-independent action of ANG II was demonstrated by the ANG II-induced NCC phosphorylation in previously adrenalectomized rats (53). The positive effect of ANG II on SPAK and NCC is lost in the total WNK4 knockout mice, confirming a WNK4-dependent mechanism (5). In addition, the WNK4-dependent effect of ANG II has been observed also in mDCT15 and mpkDCT cells (21, 51, 53). Finally, another mechanism by which ANG II promotes NCC activity is by preventing the KLHL3 recognition of WNK4, thus promoting WNK4 accumulation, which in turn can increase the activity of NCC (47).

Vasopressin

It has been known for a long time that vasopressin receptors are expressed in the DCT (32), but the demonstration of a direct effect on NCC was not obtained until 2010 by two independent groups that reported that vasopressin increased NCC activity. NCC was activated in Brattleboro rats infused with an agonist of vasopressin receptors (32, 36). This effect was also observed in isolated cells from the DCT through what appeared to be a WNK-SPAK-related pathway (36) (Fig. 1). Later, it was also observed that switching the expression of SPAK isoforms (28) by vasopressin had differential stimulatory effects along the nephron, being the full-length isoform the responsible of phosphorylating NCC (33, 38, 46).

Glucocorticoids

The evidence of NCC activation by adrenal steroids was initially reported before the cloning of NCC cDNA, when the specific binding of [3H]metolazone to renal cortex proteins was used as an index of NCC expression/activity. It was observed that the binding decreased after rat adrenalectomy and that

Table 1. Summary of the hormones that regulates NCC

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Receptor</th>
<th>Activity</th>
<th>Proposed pathway</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>MR (nuclear receptor)</td>
<td>↑</td>
<td>SGK1/Nedd4-2</td>
<td>18, 9, 2, 1, 22, 29, 16, 43, 41, 27, 54, 24.</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>V2R (metabotropic)</td>
<td>↑</td>
<td>SPAK</td>
<td>32, 36, 28, 33, 38, 46.</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>GR (nuclear receptor)</td>
<td>↑</td>
<td>Unknown</td>
<td>7, 54, 17.</td>
</tr>
<tr>
<td>Insulin</td>
<td>IR (tyrosine kinase receptors)</td>
<td>↑</td>
<td>PI3K/Akt</td>
<td>50, 49, 23, 6.</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>β2-AR (metabotropic)</td>
<td>↑</td>
<td>PKA/histone acetylation/WNK4</td>
<td>31, 52.</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Unknown</td>
<td>↑</td>
<td>Unknown</td>
<td>55, 40.</td>
</tr>
<tr>
<td>Prolactin</td>
<td>PrLR (class 1 cytokine receptors)</td>
<td>↑</td>
<td>Unknown</td>
<td>40.</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Unknown</td>
<td>↑</td>
<td>Unknown</td>
<td>40.</td>
</tr>
<tr>
<td>PTH</td>
<td>PTH1R (metabotropic)</td>
<td>↓</td>
<td>RasGRP1/ERK1/2</td>
<td>19, 26, 20, 51.</td>
</tr>
</tbody>
</table>

Fig. 1. Illustration of a transverse view of a distal convoluted tubule segment (DCT2) with all the hormones known to regulate the Na+/Cl− cotransporter (NCC). See text for additional details. The hormone receptors are located in the basolateral membrane. PRLR, prolactin receptor; PRA, progesterone receptor A; GPER-1, G protein estrogen receptor 1; ER-α, estrogen receptor α; α1-AR, α1-adrenergic receptor; V2R, vasopressin receptor 2; IR, insulin receptor; PTH1R, parathyroid hormone receptor 1; GR, glucocorticoid receptor; AT1, angiotensin receptor 1; MR, mineralocorticoid receptor.
replacement therapy with glucocorticoids restored NCC density back to control levels (7). A few years later, it was reported that both aldosterone and dexamethasone increased NCC expression and activity in adrenalectomized rats (54). However, the latter did not prove an aldosterone-independent effect of glucocorticoids on NCC. A more recent preliminary report showed glucocorticoid-positive regulation of NCC activity is dependent on diurnal rhythm (17).

**Insulin**

It is known that obesity, diabetes, and metabolic syndrome are often associated with hypertension, and it was hypothesized that one potential mechanism could be that insulin stimulates NCC activity (50). Three groups reported this type of modulation. In 2011, Sohara et al. (49) provided evidence that insulin indeed activates NCC and SPAK by a phosphinositol 3-kinase (PI3K)-dependent pathway in mpKDCT cells. Koomers et al. (23) reported that NCC phosphorylation is increased in a metabolic syndrome animal model (Zucker rats) and that these animals have a higher hydrochlorothiazide sensitivity than control rats. More recently, NCC activation and phosphorylation induced by insulin were confirmed in X. laevis oocytes (6). Moreover, supporting this hypothesis there is evidence showing that the effect of insulin is direct and not mediated by another hormonal system in kidney ex vivo perfusion techniques (6).

**Norepinephrine**

In 2011, a study showed that chronic adrenergic activation by β2-adrenergic agonists causes salt-sensitive hypertension associated with activation of NCC, which in turn was due to an isoproterenol-induced epigenetic effect on the glucocorticoid receptor that modulated WNK expression (31). The positive effect of norepinephrine on NCC activity through stimulation of β-adrenergic receptors was confirmed by another group showing that OSR1 kinase, but not SPAK, is required for such an effect (52).

**Female Hormones**

Sexual dimorphic regulation of NCC has been studied intermittingly over the years. In 1994, experiments in rats showed that the sensitivity to thiazides and [3H]metolazone binding to renal proteins were higher in female than in male rats (8). This difference was reversed in gonadectomized female rats. Later on, a positive effect of estradiol on NCC expression was observed by immunogold electron microscopy (55). In 2006, a study in lean and obese Zucker rats showed increased NCC expression in the kidney of female vs. male rats (38). Recent evidences from our group demonstrated higher NCC and SPAK activity/phosphorylation in female than in male rats and mice. This increase is reduced after ovariectomy, and it is due to positive effects of estrogens, progesterone, and also prolactin on NCC (40). The estradiol activation of NCC was related to changes in SPAK isoform expression (40). The prolactin effect was demonstrated using ex vivo kidney perfusion, thus excluding other hormonal systems that could be implicated (40). Interestingly, these findings were confirmed in humans since expression/phosphorylation of NCC in urinary exosomes was higher in women than in men (40).

**Parathyroid Hormone**

Evidence of NCC suppression by diacylglycerol (DAG) through activation of Ras guanyl-releasing protein 1 (Ras-GRP1) suggested a physiological regulation of NCC by DAG (20). In this regard, Ko et al. (19) observed that parathyroid hormone (PTH) suppresses NCC function and surface expression through DAG activation of RasGRP1 and the ERK1/2 MAPK pathway. The expression of PTH receptors in DCT have been documented (26). Thus it is likely that PTH-induced reduction in NCC activity results in diuresis seen with elevated levels of PTH (19). This could potentially explain increased calcium reabsorption induced by PTH, since it is known that the lower the NCC activity, the higher the calcium reabsorption (15).

In summary at first glance the pleiotropy in the activation/phosphorylation of NCC seems redundant, but the context in which every hormone stimulates its activation is unique, even when the cross talk between the networks could lead to integrative and additive responses. The utility of the multihormone NCC activation system exists in the point that distal reabsorption is not subjected to the tubuloglomerular feedback, and despite the low level of salt reabsorption by NCC important changes in urinary sodium excretion can be produced (4). Next, studies must be centered on the development and integration of new models to study the effect of two or more hormones on NCC dynamics in the same period of time.

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