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A Role for Adrenal Steroids in Regulation of Epithelial Sodium Channels in Cushing 's syndrome and Hypertension; Possible Target for Treatment by Protease Inhibitors

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ABSTRACT

The epithelial sodium channel (ENaC) is composed of three homologous subunits α, β, and γ. This channel is involved in the regulation of sodium balance, which influences the peri-ciliary liquid level in the lung, and blood pressure via the kidney. ENaC represents the rate-limiting step in sodium reabsorption and thus plays an important role in the maintenance of sodium balance and extracellular fluid volume. Proteolytic cleavage has an important role in regulating the activity of these channels by increasing their open probability. Specific proteases have been shown to activate epithelial Na+ channels by cleaving channel subunits at defined sites within their extracellular domains. In this article we discuss the role of ENaC in hypersteroid conditions like the Cushing's syndrome and steroid induced hypertension.

Keywords: ENaC, blood vessels, Cushing's syndrome, Protease nexin-1, glucocorticoid

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INTRODUCTION

The epithelial sodium channel (ENaC) is a constitute of three homologous subunits α, β, and γ [1].The intensity of hypertension in Cushing syndrome is an critical predictor of morbidity and mortality, but the underlying cause still remain uncertain.Renal sodium retention may contribute, with sodium balance being restoredat the expense of elevated blood pressure. Impairment of natriuretic capacity and hypokalemic alkalosis in Cushing syndrome may reflect an excess of mineralocorticoid levels, and aldosterone levelscan indeed be high. On the other hand all three ENaC subunits and mineralcorticoid receptor (MR)are expressed in the vasculature of the brain in the endothelia as well as smooth muscle cells. In contrast, Drummond *et al* detected mRNA of all three ENaC subunits by PCR from isolated cerebral vessels but could not detect α-ENaC expression by immunoassay or RT-PCR of freshly isolated smooth muscle cells or vessels[2]. It was postulated that ENaC as stretch receptors in blood vessels may be involved in autoregulation of cerebral blood flow. Increased incidence of ischemic cerebrovascular events appears to occur in individuals with ENaC polymorphisms [3], possibly a consequence of dysregulation of this autoregulation. ENaC in endothelia and blood brain barrier, on the other hand, may affect transport of Na^+ from the plasma into the interstitium. In this study the role of ENaC in the hypersteroid states like Cushing's syndrome is evaluated.

Adrenal steroids and ENaC

The steroids, dexamethasone and aldosterone increase ENaC protein levels. These effects are similar in magnitude, additive,and presumably involve different hormone receptors. Two mechanisms have been suggested to account for induction ofENaC gene expression by adreno-corticosteroids.Aldosterone enhances sodium reabsorption in the kidney by increasing the expression of ENaC and the Na-Clcotransporter in the distal nephron[4,5]. The synthetic steroid dexamethasone has also been shown to alter the activity of ENaC. In the aldosterone-sensitive distal nephron (ASDN), levels of natural glucocorticoids are reduced as they are hydrolyzed by the steroid dehydrogenase enzyme 11betaHSD. However, in cases where there are high circulating glucocorticoid levels (Cushing's syndrome) or loss of 11beta-hydroxysteroid dehydrogenase (11betaHSD) activity by mutation or inhibition, hypertension is manifested by the overactivation of ENaC-mediated Na transport [6].Additional evidence for mineralocorticoid regulation of sodium balance inthe cerebral palsy is provided by the expression of mineralocorticoid-responsive epithelial sodium channels (ENaC) at the basolateralmembrane of CSF-producing epithelial cell. However, in cases where there are high circulating glucocorticoid levels (Cushing's syndrome) or loss of 11beta-hydroxysteroid dehydrogenase (11betaHSD) activity by mutation or inhibition, hypertension is manifested by the overactivation of ENaC-mediated Na transport. It is unclear whether GR, MR or both are involved in these processes. The present study shows that alpha-ENaC protein levels are increased by both dexamethasone and aldosterone independently in mice. Dexamethasone and aldosterone together increased the abundance of the cleaved form of alpha-ENaC without altering channel activity[7].Chronic treatment with aldosterone increases the ENaC subunit primarily and the β and γsubunits to a

lesser extent at mRNA and protein levels.α-ENaC mRNA and protein are constitutively expressed, but that corticosteroids, acting at a glucocorticoid receptor, increase βand γ -ENaC transcription substantially, and α-ENaC to a lesser extent, to account for the increase amiloride-sensitive current

Modulation ofENaCby glucocorticoids in various tissues

Glucocorticoid response elements (GRE) exist upstream of genes for all ENaC subunits in the humans. A GRE is present upstream of bovine α -ENaC, although the presence of a GRE for β or γ -ENaC has not yet been shown clearly. Corticosteroid regulation of ENaC at the molecular level in bovine mammary epithelium takes place in βandγ-subunits and only mildly in the α-subunit. ENaC expression is regulated by glucocorticoids and/or mineralocorticoids in a tissue-specific manner [8,9]. Aldosterone's effects are observed primarily in the kidney with effects also observed in the colon. Glucocorticoids, are supposed to play a central role in regulating ENaC expression in the airway or alveolus and in epithelia lining the male reproductive duct [10]. Exposure to mifepristone precluded both the corticosteroidinduced elevation in amiloride-sensitive *I*sc and the induced changes in α , β , and γ -ENaC mRNA transcription, suggesting that the change in Na+ movement across epithelia in the bovine mammary gland is modulated by corticosteroids via a glucocorticoid receptor-mediated mechanism that is linked to ENaC transcription [11] as shown in Figure 1.

Protease inhibitors and ENaC

Serpins form a covalent bond with the serine residue of the catalytic triad of serine proteases (1). Pharmacologic inhibitors of serine proteases reduce Na transport, but importantly an endogenous inhibitor of prostasin, protease nexin 1 (PN-1) has been identified. PN-1 is a member of the serpin family (serine protease inhibitor) and is an endogenous inhibitor for α-thrombin, plasmin, and plasminogen activators. PN-1 decreases sodium current by ENaC*in vitro* [12,13]. And knockdown of PN-1 gene expression increases baseline sodium current in M-1 cells. Furthermore prostasin and PN-1 appear to be reciprocally regulated by aldosterone (increased prostasin and decreased PN-1) and TGF-β (decreased prostasin and increased PN-1). The expression of prostasin as well as protease nexin-1, an inhibitor of prostasin and other serine proteases, may be regulated by aldosterone [14]. ENaC residence time at the plasma membrane affects the extent of αand γ subunit cleavage. Longer residency times were associated with a greater degree of subunit proteolysis. Several factors, including aldosteronedependent signaling processes, increase ENaC residency time at the plasma membrane. Furthermore, rates of Na+entry or changes in intracellular [Na+] influence proteolytic processing of the αand γsubunits. A reduced rate of Na+ entry led to enhanced cleavage of channel subunits. Mechanisms by which rates of Na+ entry alter channel cleavage remain to be defined. Protease nexin-1 and endogenous prostasin inhibitor, inhibits ENaC activity through suppression of prostasin activity and may hold the key to controlling Cushing's syndrome and hypertension. Distribution of epithelial sodium channels and mineralocorticoid receptors in cardiovascular regulatory centers in rat brain has been reported [15].

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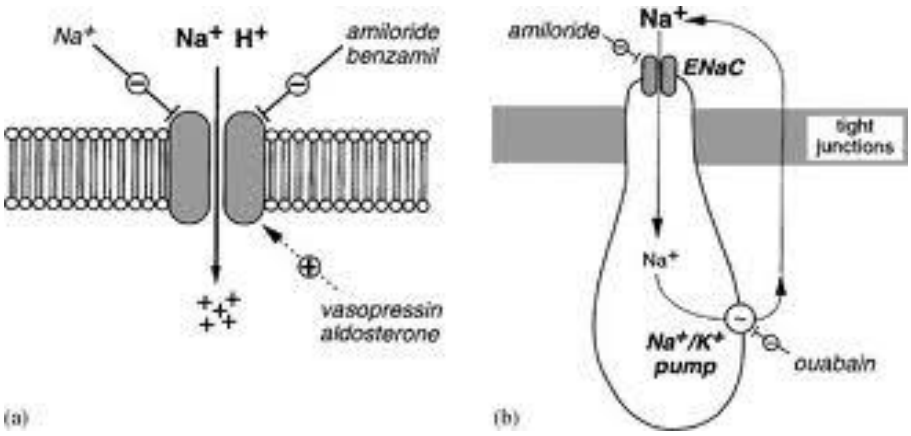
Camostatmesilate (CM), a synthetic serine protease inhibitor, reduced prostasin activity and subsequently decreased ENaC current. Oral administration of CM to Dahl salt-sensitive rats resulted in a significant decrease in blood pressure with an elevation of the urinary sodium/potassium ratio[16]. Thus it seems from several studies that protease inhibitors could modulate the activity of ENaC and could be useful for clinical conditions.

Figure 1: Structure of epithelial sodium channel



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