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Research Report

Blockade of ENaCs by amiloride induces c-Fos activation of the area postrema

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ABSTRACT

Epithelial sodium channels (ENaCs) are strongly expressed in the circumventricular organs (CVOs), and these structures may play an important role in sensing plasma sodium levels. Here, the potent ENaC blocker amiloride was injected intraperitoneally in rats and 2 h later, the c-Fos activation pattern in the CVOs was studied. Amiloride elicited dose-related activation in the area postrema (AP) but only ~10% of the rats showed c-Fos activity in the organum vasculosum of the lamina terminalis (OVLT) and subfornical organ (SFO). Tyrosine hydroxylase-immunoreactive (catecholamine) AP neurons were activated, but tryptophan hydroxylase-immunoreactive (serotonin) neurons were unaffected. The AP projects to FoxP2-expressing neurons in the dorsolateral pons which include the pre-locus coeruleus nucleus and external lateral part of the parabrachial nucleus; both cell groups were c-Fos activated following systemic injections of amiloride. In contrast, another AP projection target – the aldosterone-sensitive neurons of the nucleus tractus solitarius which express the enzyme 11-β-hydroxysteriod dehydrogenase type 2 (HSD2) were not activated. As shown here, plasma concentrations of amiloride used in these experiments were near or below the IC_{50} level for ENaCs. Amiloride did not induce changes in blood pressure, heart rate, or regional vascular resistance, so sensory feedback from the cardiovascular system was probably not a causal factor for the c-Fos activity seen in the CVOs. In summary, amiloride may have a dual effect on sodium homeostasis causing a loss of sodium via the kidney and inhibiting sodium appetite by activating the central satiety pathway arising from the AP.

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1. Introduction

Epithelial sodium channels (ENaCs) facilitate movement of sodium across luminal epithelia such as found in the kidney and airways (Althaus, 2013; Alvarez de la Rosa et al., 2013; Kusche-Vihrog et al., 2013; Soundararajan et al., 2010; Warnock et al., 2014). Less is known about the ENaCs that are present in the brain (Giraldez et al., 2013). These nonvoltage-gated ion channels have been localized in five different types of brain cells: astrocytes, choroid plexus cells, ependymal cells, endothelial cells, and neurons (Amin et al., 2005; Miller and Loewy, 2013). ENaCs present in the choroid plexus regulate the [Na^+] of the cerebrospinal fluid (Amin et al., 2009; Nakano et al., 2013; Wang et al., 2010). ENaCs expressed in brain endothelial cells function much like those found in peripheral endothelial cells (Kim et al., 2012; Kusche-Vihrog et al., 2013), serving to stiffen mechanically these cells and trigger the release of the nitric oxide which acts on its underlying smooth muscle to induce local vasodilation (Kusche-Vihrog et al., 2013). However, the role of ENaCs that are expressed in astrocytes (Miller and Loewy, 2013) and neurons (Amin et al., 2005; Miller et al., 2013; Teruyama et al., 2012) remains elusive.

ENaCs are densely concentrated in the neurons of the sensory circumventricular organs (CVOs) (Amin et al., 2005; Miller et al., 2013) which include the organum vasculosum of the lamina terminalis (OVLT), subfornical organ (SFO) and area postrema (AP). Like all CVOs, these structures lack a blood–brain barrier and thus, their neurons and glial cells are continuously exposed to the same chemical environment as found in the plasma. On the basis of this anatomical property, we hypothesized that ENaC-expressing CVO neurons may function as plasma sodium sensors since they become c-Fos activated following peripheral manipulations of plasma sodium levels (Miller and Loewy, 2014; Miller et al., 2013).

Earlier we reported that hypertonic saline intraperitoneal injections induced c-Fos activation of selective regions of the OVLT, SFO, and AP, while sodium deprivation elicited activation of the complimentary OVLT and SFO regions, but had no effect in the AP (Miller et al., 2013). Later studies established that serotoninergic AP neurons were c-Fos activated by sodium repletion or hypertonic saline injections (Miller and Loewy, 2014). Some, but not all, of these neurons expressed ENaCs (Miller and Loewy, 2014). In addition, we identified a unique group of ENaC-γ expressing astrocytes that define the lateral border of the CVOs (Miller and Loewy, 2013), and presumably have the same vascular environment as the main part of the CVOs, functioning as an important glial–neuronal interface perhaps for monitoring the ionic content of the cerebrospinal fluid.

Here we tested whether ENaCs per se potentially play a functional role in the CVOs by injecting the highly selective ENaC-blocking drug – amiloride into the peritoneal cavity, and after two hours, examining whether this drug would have any effect on the CVOs. This experiment was begun as a pilot project, and since amiloride *blocks* ENaCs, we initially had assumed it would have no or a minimal effect on the CVOs. If this was established, our plan was to use this drug in subsequent experiments to determine whether it would block c-Fos activation of the CVOs which occurs following hypertonic saline intraperitoneal injections (Miller et al., 2013). We reasoned that if amiloride blocked the c-Fos activation that occurs after hypertonic saline, these data would support the hypothesis that ENaCs localized in the CVOs may play a role in the brain sodium sensing system.

Instead, we were surprised to find that amiloride itself activated the AP in a dose-related manner. Moreover, it also caused activation of a group of synaptically-related neurons that lie in the dorsolateral pons; these neurons express the transcription factor Forkhead protein (FoxP2) and receive a direct input from the AP (Stein and Loewy, 2010). The activation pattern of both groups of neurons (AP and FoxP2 neurons) was correlated with changes in circulating and central levels of amiloride. To determine the possible mechanism of actions, we measured the effect of amiloride on blood pressure and vascular resistance in conscious rats, and found that this drug caused no changes in these cardiovascular parameters. While other possibilities could explain our findings, including the release of hormones from peripheral organs such as the kidney, we propose that the CNS effects observed here were due to the direct action of amiloride on AP neurons and offer a cellular mechanism to explain this phenomenon.

2. Results

Our first goal was to determine whether systemic injections of amiloride would induce c-Fos activation of the CVOs, and as the study progressed, we found that the AP was the principal site where c-Fos activity was most consistently elicited. To refine these studies, we next measured the plasma and cerebrospinal fluid (CSF) levels of amiloride after intraperitoneal injections of graded doses of this drug. Once we established a "dose–response" relationship for amiloride and c-Fos activity in the AP, we analyzed the second-order AP projection targets and determined whether any of them were activated. Briefly, the FoxP2 neurons of the dorsolateral pons were activated by amiloride while the aldosterone-sensitive (HSD2) neurons of the NTS were not.

Fig. 1 shows the total density of cells versus the density of ENaC positive cells in the AP. In a group of 3 rats, we found that ENaC positive cells represent 93% of the total AP cell population. There was no difference between the number of ENaC positive cells and the total number of AP cells.

Fig. 2 illustrates the amiloride concentrations found in the plasma and CSF following intraperitoneal injections of this drug.

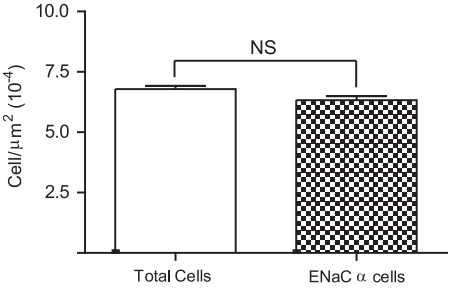

Fig. 1 – Bar graphs demonstrating that the majority (93%) of AP cells express ENaCs. NS, not statistically significant.