Salbutamol and salt-sensitive hypertension

Citation for published version:
https://doi.org/10.1016/j.kint.2021.05.023

Digital Object Identifier (DOI):
10.1016/j.kint.2021.05.023

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Kidney International

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Salbutamol and salt-sensitive hypertension

Matthew A Bailey

British Heart Foundation Centre for Cardiovascular Science, The University of Edinburgh.

For Correspondence: Matthew Bailey, PhD, FRSB

Matthew.bailey@ed.ac.uk

British Heart Foundation Centre for Cardiovascular Science
The Queen’s Medical Research Institute
BioQuarter Campus
Edinburgh Medical School
The University of Edinburgh
United Kingdom
EH16 6NT

Word count: 1396

Figures: 1

References: 9
Abstract (75/75)
Salbutamol activates NCC, the NaCl co-transporter of the distal convoluted tubule. Salbutamol, in conjunction with high salt intake, induced hypertension in mice, rescued by thiazide therapy. Phospho-proteomics identified Protein Phosphatase 1/Inhibitor 1 as a distinct regulatory node for NCC activation by salbutamol, which did not activate the transporter in Inhibitor 1 knockout mice. Salbutamol is widely used in respiratory medicine and the acquisition of salt-sensitivity may be relevant to understanding cardiovascular risk in certain patients.

Salt Sensitivity and the Distal Convoluted Tubule
It is certain that most of us habitually eat more salt than the World Health Organization’s recommended upper limit of 5g/day. This dietary salt excess contributes widely to poor health, progressing kidney disease, cardiovascular disease and now recognised as a factor in cognitive decline. Excess salt intake damages cells and tissues through several mechanisms, but hypertension remains a major injurious factor. Blood pressure (BP) is probably increased to some extent by high salt intake and in ~30% of otherwise healthy people, the response is exaggerated. Such individuals are categorised as ‘salt-sensitive’, and salt-sensitivity accrues cardiovascular and mortality risk independent of BP per se. Unlocking the molecular secrets of salt-sensitivity is an important health goal and the focus of intense research effort. Mechanisms of salt-sensitivity remain degree contentious, with vascular, neurogenic and kidney emerging as major themes. The vascular dysfunction hypothesis argues that salt-sensitivity reflects a failure of peripheral vasodilation in the face of salt-induced volume expansion; the neurogenic hypothesis proposes sympathetic hyperactivity is the primary underlying abnormality. The kidney
hypothesis holds that the primary defect is in the regulation of epithelial sodium transport, which is unable to modulate normally with salt intake, causing BP to rise when salt intake is high. Human monogenic hypertension provides archetypes of salt-sensitivity and these disorders indicate that “hyperactive” sodium transport in the aldosterone-sensitive distal nephron is key\(^1\).

The distal convoluted tubule (DCT) reabsorbs 5-10% of the filtered sodium load. The thiazide-sensitive NaCl co-transporter is the major apical route for sodium entry into DCT cells and activity is influenced by multiple hormonal systems and by plasma potassium concentration\(^2\). In addition to regulating the expression of the transporter, regulatory factors engage a cascade of serine/threonine kinases, including with-no-lysine kinase (WNK)1 and WNK4, STE20/SPS1-related proline/alanine-rich kinase (SPAK), and oxidative stress-response (OSR) kinase, to promote phosphorylation at conserved residues in the NH2-terminus of NCC. Such phosphorylation determines trafficking of NCC in the apical membrane of the DCT and is thus widely used as an index of \textit{in vivo} NCC-mediated sodium transport.

In this issue of \textit{Kidney International}, Poulsen \textit{et al.} delineate a molecular framework explaining how salbutamol, a $\beta_2$-adrenoreceptor agonist widely used to relieve symptoms of asthma and COPD, promotes NCC activation, inducing salt-sensitive hypertension\(^3\). Exploiting the parvalbumin-EGFP transgenic mouse, the authors used Fluorescence Activated Cell Sorting to isolate a DCT-enriched cell population from kidney, showing $\beta_2$-adrenoreceptor expression at mRNA and protein levels. Acute salbutamol administration to mice induced rapid phosphorylation of the activating threonine\(^58\) residue in the N-terminus of NCC. Chronic salbutamol infusion was then used to “lock” the transporter in an activated state, \textit{in vivo}. The effect on BP was initially modest- laboratory rodent diets contain modest amounts of sodium and the kidney
was able to accommodate the effect of salbutamol on NCC activity. However, when these mice were switched to a high salt diet, reflective of the upper range of usual human intake, salbutamol-treated mice displayed an exaggerated increase in systolic BP compared with vehicle-treated animals. The BP differential was abolished by thiazide therapy. An earlier study had found that isoproterenol caused thiazide-remediable, salt-sensitive hypertension in wild-type but not β2-adrenoreceptor knockout mice⁴. Together these studies indicate that adrenergic stimulation of DCT sodium transport is an important determinant of BP.

**What is the mechanism?**

β2-adrenoreceptor activation promotes redistribution of K⁺ into cells, causing hypokalaemia. Even modest reductions in plasma K⁺ stimulate NCC phosphorylation/activity² but this was discounted as a causal mechanism here because salbutamol induced NCC phosphorylation in isolated kidney tubules, indicative of a direct effect on β2-adrenoreceptors in the DCT. The signalling network regulating NCC is complex, and the authors first interrogated the Kir4.1/WNK-SPAK/OSR pathway, which drives N-terminal phosphorylation of the transporter². In isolated tubules, the stimulatory effect of salbutamol on NCC phosphorylation was diminished by ~50% following pharmacological blockade of Kir4.1 or WNK kinases. The inference was that β2-adrenoreceptor activation also engaged WNK-SPAK/OSR independent pathways and the investigators turned to large-scale phosphoproteomics to interrogate the phosphorylation response to salbutamol in an immortalised DCT cell line. This response was extensive. Probing this dataset with network/pathway analysis identified several putative regulators of NCC and the authors honed-in on protein phosphatase 1 (PP1), a highly conserved phosphatase active at phosphoserine and phospho-threonine residues, which was strongly
downregulated by salbutamol. PP1 was already known to inhibit NCC by causing dephosphorylation of the transporter’s activating residues\(^2\) and the authors thus proposed a hypothesis in which salbutamol “inhibited the inhibitor”, thereby increasing the net phosphorylation of the transporter. Two lines of evidence support this hypothesis: pharmacological inhibition of PP1 in isolated kidney tubules substantially reduced the stimulatory effect of salbutamol on NCC phosphorylation. *Caveat lector*, inhibition of PPI almost “maxed-out” the phosphorylation of NCC, potentially limiting the pool for any further activation by salbutamol. Second, and perhaps more compelling, the stimulatory effect of salbutamol was absent in kidney slices taken from mice with global genetic deletion of the endogenous PP1 inhibitor, I1.

To summarise this study, Poulsen *et al* have drilled down to the detailed molecular pathways through which \(\beta_2\)-adrenoreceptor activation directly stimulates NCC. Impressively, they have also extended up, showing significant impact on higher-order, *in vivo* cardiovascular physiology. The overall picture (Figure 1) is that \(\beta_2\)-adrenoreceptor activation, most probably via cyclic AMP and protein kinase A, engages I1 to inhibit PP1, preventing tonic dephosphorylation of NCC. Thus, salbutamol turns NCC “on” and, by extension, disconnects DCT salt reabsorption from physiological control. Downregulation of NCC is part of the physiological adaptation to high salt intake. Salbutamol prevents this normal modulation of NCC activity and, in this preclinical study, leads to acquired salt-sensitivity. This is mechanistically analogous to the salt-sensitivity induced by calcineurin inhibitors such as tacrolimus. These immunosuppressant drugs are used to prevent transplant rejection but also activate NCC by preventing N-terminal dephosphorylation\(^2\).

Some gaps remain to be filled. The study relies on threonine\(^6\) phosphorylation to report NCC activity and that chronic salbutamol treatment increases thiazide-sensitive
sodium transport in vivo is inferred, rather than directly demonstrated. Indeed, whether salbutamol reduces sodium excretion and alters sodium/fluid balance remains an important question, particularly since thiazides can reduce BP independently of sodium excretion by effects on peripheral vascular resistance. The Inhibitor-1 knockout mice will provide a valuable tool for these future studies: salbutamol cannot activate NCC in these animals and the hypothesis would be that they are thus protected from acquired salt-sensitivity.

**Translational relevance.**

The first and specific implication for this study reflects the widespread use of β-adrenoreceptor agonists in asthma and COPD. People with asthma are at increased risk of developing cardiovascular disease. The cause is likely complex and multifactorial but use of inhaled β-adrenoreceptor agonists has been associated with increased cardiovascular risk in some studies independent of conventional risk factors. Some caution needs to be applied here as it is difficult to directly compare the systemic salbutamol exposure following episodic, nebulised delivery into the human lung with the continuous, systemic infusion of salbutamol used in this study. Nevertheless, salt-sensitivity is an important cardiovascular risk factor and whether NCC is activated in asthma or COPD warrants more detailed investigation, particularly in the context of long lasting β2-agonists, such as salmeterol.

Second, previous studies indicate that activation of the glucocorticoid receptor has an important, permissive role in β-adrenoreceptor induced salt-sensitivity. This was not the focus of the current study. Nevertheless, we find that glucocorticoid receptor activation determines the diurnal rhythmicity of NCC phosphorylation and exogenous glucocorticoids can introduce non-dipping BP, treatable by thiazides. Inhaled glucocorticoids are also commonly used in asthma and COPD and the synergy
between these two therapeutics may have important ramifications for BP control in some patients. Finally, although the DCT contributes only 7% of the kidney's epithelial cell count, this study and others provides incontrovertible evidence that DCT adrenoreceptor activation profoundly alters the relationship between salt homeostasis and BP. The mechanisms elucidated here have a broader significance, connecting neurogenic and renal concepts to provide a deeper understanding of salt-sensitive hypertension.

REFERENCES


**Disclosure:** the author has no competing interest to declare

**FIGURE LEGEND**

Activation of the thiazide sensitive NaCl cotransporter by controlling phosphorylation of key residues in the N-terminal tail. β2-agonists such as salbutamol, activate adenylate cyclase (AC) to increase cyclic AMP and engage protein kinase A (PKa). Two pka-dependent pathways regulate net phosphorylation of the transporter: WNK-SPAK directly phosphorylate activating residues; phosphorylation of Inhibitor-I (I1), blocks tonic dephosphorylation of NCC by Protein Phosphatase 1 (PP1). Chronic treatment of mice salbutamol, locks NCC “on” and changes the blood pressure category to “salt-sensitive”. With high salt intake, blood pressure rises. This pathway is also relevant to activation of the receptor by endogenous adrenoceptor agonists: sympathetic hyperactivity also activates NCC to cause salt-sensitivity9.