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Regulation of Salt and Volume Transport along the Nephron during Acute Systolic Hypertension

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INTRODUCTION

Hypertension is one of the most important risk factors for cardiovascular diseases including myocardial infarction, stroke, congestive heart failure, end stage renal disease and peripheral vascular disease (Burt et al, 1995; Cowley, 1997; Cowley & Roman, 1996). Furthermore, hypertension is a very common condition, the prevalence in the US adult population is estimated at 24% (Burt et al, 1995). It is well known and accepted that renal function is altered in hypertension. The alterations may be either responsible for the disease or may be homostatic compensations to the elevated pressure. Cowley summarizes six lines of evidence that indicate that alteration of renal function is essential for the development and/or maintenance of hypertension: kidneys play a dominant role in long term regulation of blood pressure, experimental hypertension models all invole some maneuver to reduce renal excretory function, the kidney requires a higher arterial pressure to excrete a sodium and volume load in every known genetic model of hypertension, all antihypertension drugs promote sodium and volume excretion, when renal function is impaired an increase in arterial pressure is essential to restore fluid and electrolyte balance, and blood pressure goes the kidney in renal transplantation (Cowley & Roman, 1996). The phenomenon whereby an increase in arterial pressure provokes an increase in sodium excretion is known as pressure-natriuresis. Since it occurs in the absence of a change in the filtered load, it involves a decrease in net tubular sodium reabsoeption (Fig. 1). In humans, and in all genetic rat models of hypertension, the pressure natriuresis response is blunted and reset toward higher pressures (Cowley & Roman, 1996; Navar & Majid, 1996).

Role of the proximal tubule (PT) in autoregulation

Acute increases in arterial pressure elicit rapid natriuretic and diuretic responses that occur in the absence of changes in renal blood flow (RBF) or glomerular filtration rate (GFR). This autoregulation of RBF and GFR is mediated by an increase in chloride delivery to the macula densa, which provokes an increase in afferent arteriolar resistance (Briggs & Schnermann, 1996). This response is known as tubuloglomerular feedback (TGF) (Fig. 1). The increased volume flow at the macula densa during acute hypertension, in the face of a constant reabsorption in the proximal tubule (Chou & Marsh, 1986, 1987, 1988; Kinoshita & Knox, 1990).

Role of the Thick Ascending Limb of the Loop of Henle (TALH) in autoregulation

During acute hypertension there is normally a significant increase in delivery of salt and volume to the

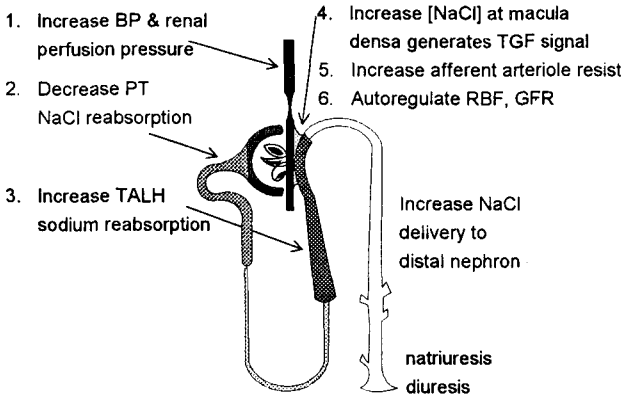

Fig. 1. Downstream shift in sodium reabsorption from PT to TALH during acute hypertension.

TALH resulting from the inhibition of transport in the PT. While one might postulate that there is further inhibition of sodium transport in the TALH to amplify the pressure natriuresis response, the study of Chou and Marsh (Chou & Marsh, 1986) suggests that there is actually an increase in the fraction of sodium reabsorption in this region during hypertension: they report a 40% increase in Cl^- at the macula densa and a 13% increase in volume flow to the distal tubule. We refer to the decrease in PT sodium and volume reabsorption followed by an increase in TALH sodium and volume reabsorption as a "downstream shift" in reabsorption during hypertension. The increase in transport in the TALH serves to control fluid delivery to the distal nephron, and reduces the amount of $NaHCO_3$ that has to be absorbed distally.

Our aim has been to determine the cellular mechanisms responsible for the normal decrease in sodium transport that occurs in PT, as well as the mechansims reponsible for the normal increase in TALH sodium transport that occur in response to acute hypertension in normotensives and use this information to understand the changes that occur in chronic hypertension. This brief review summarizes our recent findings (Zhang et al, 1998a, 1998b, 1996; Magyae et al, 1997).

Cellular mechanisms to acutely regulate sodium transport in PT and TALH

Active sodium reabsorption across renal epithelia is mediated by apical entry via sodium-coupled transporters such as Na/H exchangers (NHE-3) and Na-phosphate cotransporter (NaPi) in the PT and NHE-3 and the Na-K-2Cl co-transporter (NKCC2) in the TALH followed by active uphill extrusion via basolateral sodium pumps (Na,K-ATPase). The rapid changes in sodium transporter activity in response to acute hypertension (decrease in PT, increase in TALH) may be due to: 1) change in the activity of transporters in the apical and/or basolateral plasma membranes mediated by covalent regulation of the transporters themselves or association with regulator proteins, 2) trafficking of transporters between plasma membranes and endosomal stores, or 3) rapid degradation of transporters. There is evidence for all three types of transport regulation along the nephron: 1) phosphorylation of sodium pumps has been reported to change ATPase and transport activity (Aperia et al, 1996, 1994; Middleton, 1996), and phosphorylation of NHE-RF (NHE regulatory factor) regulates transport activity of NHE-3 (Weinman et al, 1993), 2) there is evidence for trafficking of apical membrane proteins between the brush border and a large pool of subapical endosomes (Nielson, 1993), as well as reversible wholesale internal retraction of microvilli with ATP depletion and repletion (Golenhofen et al, 1995), and 3) Na-Pi are internalized and degraded following acute high Pi diet (Lotscher et al, 1997).

RESULTS

Proximal tubule

Acute systolic hypertension, as short as 5 min, inhibits proximal tubule (PT) sodium reabsorption, which increases chloride delivery to the macula densa, the error signal for tubuloglomerular feedback (TGF) autoregulation of renal blood flow (RBF) and glomerular filtration rate (GFR), and contributes to pressure natriuresis. In contrast to the response in the PT, the thick ascending limb of the loop of Henle (TALH) impacts TGF during arterial hypertension by reabsorbing more salt to limit salt delivery to the distal nephron, while delivering increased $[Cl^-]$ to the macula densa.

We have focussed on determining the molecular mechanisms responsible for the "downstream shift" in sodium reabsorption. Sprague Dawley rats were subjected to an acute increase in arterial pressure (from 100 mmHg baseline to 150 mmHg) by constricting arteries for 5 minutes, and in some cases blood pressure was then restored for up to 40 minutes (Zhang et al, 1998b). This acute hypertension provokes a 5 fold increase in urine output (V) and a 3 fold increase

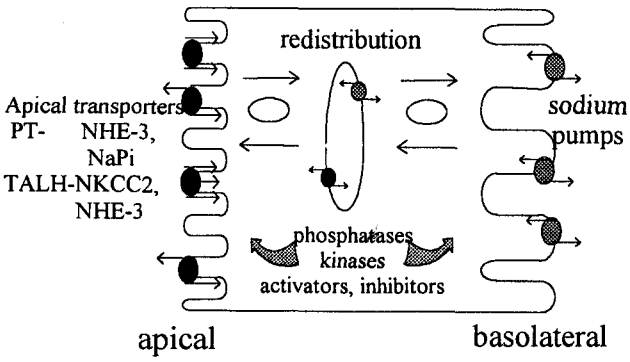

Fig. 2. Regulation of renal sodium transport in PT and TALH.

in the clearance of endogenous lithium, an inverse measure of PT sodium reabsorption. We analyzed the cellular responses after subcellular fractionation of the renal cortex as summarized in Fig. 3. After analyzing a large panel of membrance markers (Zhang et al, 1998a, 1998b, 1996), we concluded that the decrease in PT salt and fluid reabsorption can be attributed to parallel and reversible changes in sodium transporters

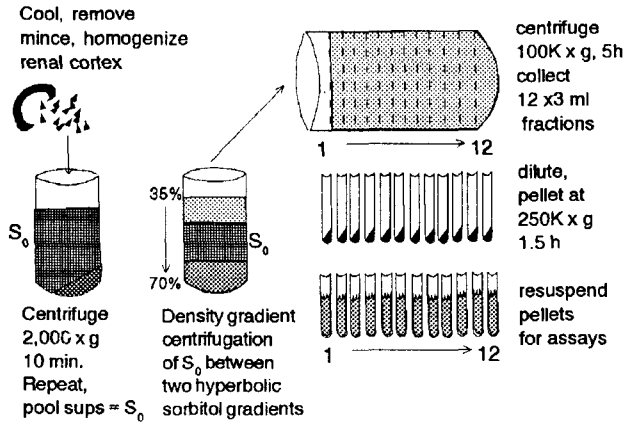

Fig. 3. Subcellular fractionation.

in both apical and basolateral membranes. Apical Na^+/H^+ exchanger isoform 3 and Na^+ Pi cotransporters rapidly translocate from brush border microvilli to intramocrovillar or sub-apical membranes, and basolateral Na,K-ATPase activity is significantly depressed. The results for NHE-3 redistribution and Na,K-ATPase inhibition are summarized in Fig. 4. These responses were reversed about 40 minutes after blood pressure was restored by releasing the arterial clips (Zhang et al, 1998b). The delay in the restoration response implies that it is not pressure per se (which restores immediately) but more likely a chemical signal that decays with time after the stimulus is removed.

Hypertension model

The response appears to be chronically activated in the adult Spontaneously Hypertensive Rat (SHR) where apical sodium transporters are located in membrances with the density of sub apical stores, and Na,K-ATPase activity is supressed compared to normotensive adult SD (Fig. 4).

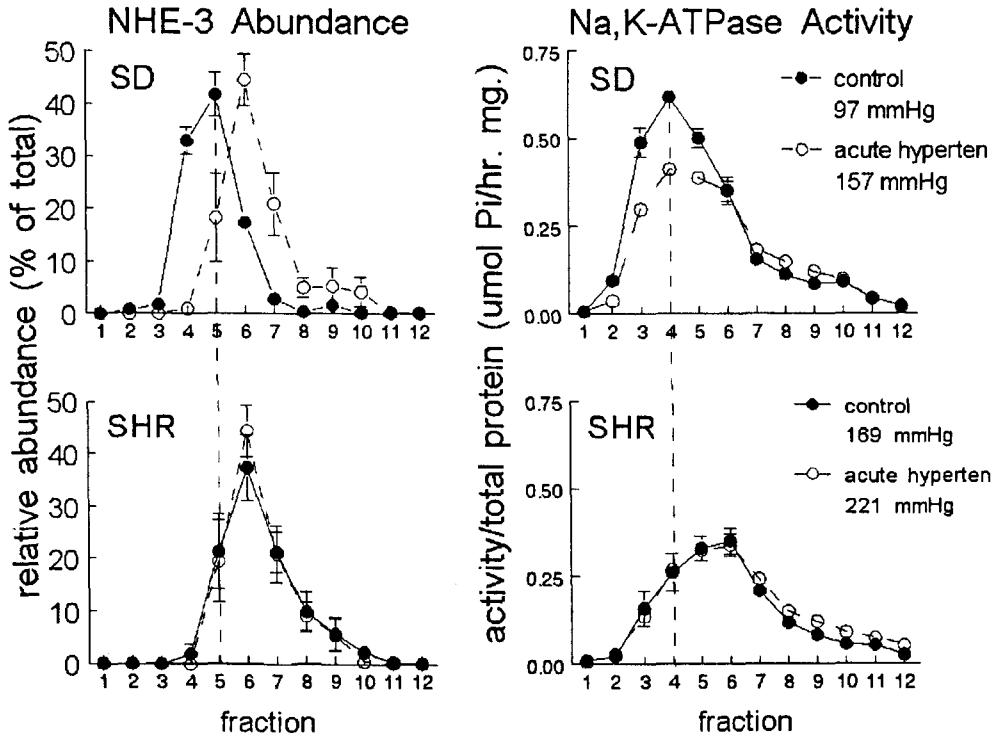

Fig. 4. Effect of acute hypertension on density distribution of apical NHE-3 and activity of basolateral Na,K-ATPase in Sprague Dawley (SD) and Spontaneously Hypertensive Rats (SHR).

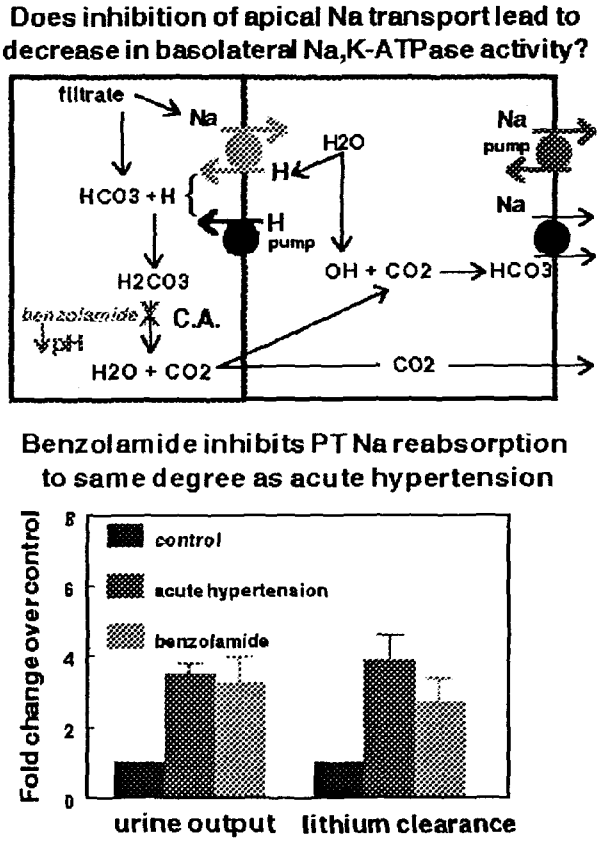

Fig. 5. Does inhibition of apical Na transport lead to decrease in basolateral Na,K-ATPase activity?

Are the apical and basolateral responses linked?

To determine whether the inhibition of the basolateral Na,K-ATPase was linked to the apical response, apical Na^+ transport in the PT was inhibited (without changing blood pressure) a with the carbonic anhydrase inhibitor benzolamide. We chose a dose that elevated both urine output and lithium clearance to the same degree as the acute hypertension protocol (Fig. 5). This treatment did not provoke a change in Na,K-ATPase activity, leading us to conclude that the inhibition of sodium pump activity during acute hypertension was not secondary to suppressed Na^+ uptake.

Inhibition of cytochrome P450

Since cytochrome P450 dependent arachidonate metabolites participate in the regulation of renal sodium transport and blood pressure (Rahman et al, 1997), we tested the hypothesis that these renal responses to acute hypertension would be prevented

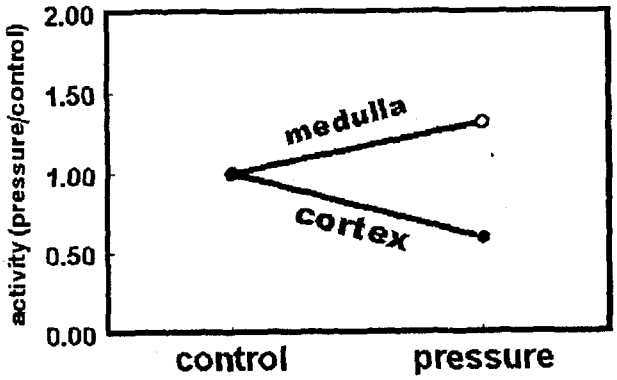

Fig. 6. Reciprocal effect of 5 min hypertension on renal cortex vs medulla Na,K-ATPase.

if cytochrome P450 metabolism were inhibited by cobalt chloride ($CoCl_2$) (Zhang et al, 1998a). Four groups of rats (n=4-5) were studied: 1) sham operated, 2) 50 mg $CoCl_2$/kg s.c. for 2 days, 3) acute hypertension by constricting arteries for 5 min, 4) acute hypertension after $CoCl_2$ treatment as in 3). Renal cortex was analyzed after sorbitol density gradient fractionation. $CoCl_2$ treatment alone did not significantly affect V, CLi, maximal activity of Na,K-ATPase or subcellular distribution of NHE-3 containing membranes. In non-$CoCl_2$ treated animals, acute hypertension provoked 3-4 fold increase in V and CLi, 33% inhibition of renal cortex Na,K-ATPase activity and redistribution of NHE-3 out of the apical membrane peak. In $CoCl_2$ treated animals, V and CLi increased only 2 fold during acute hypertension, there was no inhibition of Na,k-ATPase activity, and no redistribution of NHE-3 immunoreactivity to higher density membranes. These findings demonstrate $CoCl_2$ treatment both attenuates the natriuresis/diuresis and abolishes Na,K-ATPase inhibition and NHE-3 redistribution during acute hypertension, evidence that these responses may be mediated by cytochrome P450 arachidonate metabolites such as 20-HETE.

Loop of henle

In contrast to the PT response, there is an increase in salt and volume transport in the TALH that is associated with a significant increase in Na,K-ATPase activity in the outer medulla (Fig. 6). Thus, acute hypertension drives a parallel increase in Na,K-ATPase in outer medulla and decrease in Na,K-ATPase activity in cortex. The effect of acute hypertension on apical transporters of the TALH (NKCC2, NHE-3)

remains to be established. The reciprocal modulation of Na,K-ATPase activity in PT vs TALH during acute hypertension contributes the driving force for activating TGF while minimizing changes in delivery of Na^+ and volume to the hormone sensitive distal nephron.

DISCUSSION

Acute regulation of sodium pump activity

There is a burgeoning literature on mechanisms responsible for short term regulation of Na,K-ATPase activity (Aperia et al, 1996, 1994; Beron et al, 1997; Bertorello & Katz, 1993). Pathways linked to both generation of protein kinase C (PKC) and/or cyclic-AMP dependent protein kinase A are postulated to regulate Na,K-ATPase activity by changing the α catalytic subunit phosphorylation status. However, phosphorylation has been associated with both decreased activity (Aperia et al, 1994; Chibalin et al, 1995; Middleton et al, 1993; Satoh et al, 1993a, 1993b), and increased activity (Carranza et al, 1996a, 1996b), and no change in activity (Beron et al, 1997). There is also evidence that PKC causes a withdrawal of sodium pumps from the basolateral membranes even if there is mutation of the phosphorylation site (Beron et al, 1997). Proximal tubule Na,K-ATPase activity is also inhibited (whether directly or indirectly is not known) by activation of phospholipase A2 which stimulates production of arachidonate metabolites of cytochrome P-450 such as 20-HETE (Aperia et al, 1996; Nowicki et al, 1997; Ominato et al, 1996). Although the precise signaling mechanisms remain to be elucidated for the responses to altered blood pressure, our results indicate that the inhibition of the sodium pump activity in PT is due to structural modification of the pump itself or an associated regulator, rather than solely mediated by trafficking of active pumps to a new location, because the data demonstrate significant changes in total ATPase activity that persist through membrane fractionation and phase partitioning, and our results implicate a role of cyt P450 aa metabolism to 20-HETE in the response (Zhang et al, 1998).

Altered natriuretic responses in hypertension

As discussed in the introduction, an altered natriuretic response to an elevation in blood pressure is the hallmark of hypertension. The Spontaneously Hypertensive Rat (SHR) has numerous renal defects that could account for the development of hypertension. In regards to this project, the PT of SHR fail to respond normally to the natriuretic hormone dopamine (Kinoshita et al, 1989), and they have enhanced tubuloglomerular feedback (TGF) response (reviewed in Cowley & Roman, 1997). Our results indicate a distribution of apical sodium transportes in SHR is the same as in acutely hypertensive SD (Magyar et al, 1997).

In summary, our findings to date suggest that the dynamic regulation of proximal tubule and loop of Henle sodium transport by fluctuations in blood pressure may be mediated by changes in sodium transporter characteristics at both the apical and basolateral membranes: 1) by reversible inhibition of basolateral Na,K-ATPase activity in the PT and activation in the TALH, and 2) relocation of a set of apical proteins, including NHE-3 and NaPi, consistent with redistribution to intermicrovillar cleft region and/or internalization to endosomal pools in the PT. The reciprocal modulation of Na,K-ATPase activity in PT and TALH contributes the driving force for activating TGF, while minimizing changes in delivery of salt and water to the hormone sensitive distal nephron.

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