Phycocyanobilin and Dietary Nitrate May Exert Natriuretic Effects That Suppress Production of Marinobufagenin

Mark F. McCarty, Catalytic Longevity, markfmccarty@gmail.com

Abstract

Dietary nitrate and the phycocyanobilin (PhyCB) chromophore of spirulina have anti-hypertensive potential; the former boosts endogenous production of the vasodilator nitric oxide (NO), while the latter can aid the synthesis and stability of NO by suppressing NADPH oxidase-mediated production of superoxide. While these agents likely have some utility for lessening peripheral resistance, they can also be expected to exert a natriuretic effect within the renal medulla. In the medullary thick ascending loop of Henle (mTAL), NO/cGMP inhibit sodium transport by decreasing the activity of the Na-K-2Cl cotransporter. Conversely, superoxide boosts the activity of this cotransporter. NO/cGMP also promote natriuresis by enhancing the sensitivity of Na-K-ATPase to inhibition by the natriuretic hormone marinobufagenin (MBG). In the inner medullary collecting duct, NO synthase 1 activity plays a physiological role in promoting natriuresis. Dietary nitrate could be expected to give rise to NO somewhat selectively in the renal medulla, as this portion of the kidney is relatively hypoxic. Medullary oxidative stress, primarily stemming from NADPH oxidase activation, is prominent in salt-sensitive rats, and is suspected to be a key mediator of salt sensitive hypertension; PhyCB should quell this oxidative stress via inhibition of NADPH oxidase. If dietary nitrate and PhyCB do indeed promote natriuresis, the consequent favorable impact on sodium/fluid retention would likely diminish adrenal secretion of MBG, and hence mitigate the hypertensive and vascular hypertrophic effects of this hormone, believed to be a key mediator of salt-sensitive hypertension and its complications. These agents may also act directly on the target organs of MBG to lessen its pathogenic impact. Supplemental citrulline and high-dose folate may have the potential to promote natriuresis by supporting renal NO synthase activity. A potassium-rich diet and lifestyle choices which moderate diurnal insulin secretion may also counter salt sensitivity by promoting natriuresis.

Anti-Hypertensive Potential of Dietary Nitrate and Phycocyanobilin

It is now appreciated that dietary nitrate, after reduction to nitrite by oral bacteria and re-absorption, can be further reduced by spontaneous interaction with several proteins – including xanthine oxidoreductase, NO synthase, and hemoglobin or myoglobin – to yield NO within the body’s tissues.1-3 Moreover, this reaction proceeds more avidly in tissues that are hypoxic or acidotic – a protective mechanism, inasmuch as such tissues would stand to benefit most from the vasodilatory impact of NO.4-6 Not surprisingly, an increase in dietary nitrate of sufficient magnitude has been shown to decrease blood pressure; this effect may be somewhat larger in hypertensives, likely owing to the fact that, for unknown reasons, their erythrocytes tend to express greater xanthine dehydrogenase activity.7,9 In hypertensive patients, an oral dose of 3.5 mmol nitrate was followed 3-4 hours later by reductions of 12 and 10 mm Hg in systolic and diastolic pressure, respectively.9
Although the impact of chronic nitrate supplementation on blood pressure control has not yet been reported, such supplementation clearly achieves sustained reductions in blood pressure in rodent models of hypertension.\(^{10-12}\) Moreover, in a rat model of salt-induced hypertension (uninephrectomy with high-salt diet), dietary administration of nitrate not only moderated blood pressure, but also markedly suppressed renal fibrosis, albuminuria, and pathological changes in the glomeruli and tubules typically seen with this model, and at the highest dose also prevented cardiac hypertrophy and fibrosis.\(^{10}\) A systemic antioxidant impact of dietary nitrate was also observed. The renal protection observed in this model may have been partially attributable to NO produced with the kidney itself, as nitrate supplementation elevated levels of nitrosylation products in both the cortex and medulla.

Another nutraceutical with potential for blood pressure control is spirulina. Spirulina is the richest natural source of phycocyanobilin (PhyCB), which has been found to be orally effective as an inhibitor of certain NADPH oxidase complexes, mimicking the physiological activity of its homolog bilirubin in this regard.\(^{13-17}\) As is well known, the superoxide produced by NADPH oxidase within the vasculature can suppress vascular NO activity, both by direct quenching, and by uncoupling of NO synthase.\(^{18}\) Hence, it is logical to suspect that PhyCB administration in a sufficient dose would boost the effective bioactivity of vascular NO, at least in subjects with vascular oxidative stress.\(^{19}\) Although spirulina has received little if any study in rodent models of hypertension to date, an open clinical study in which 4.5 g spirulina was administered to 36 subjects for 6 weeks, observed average reductions of 10/8 mm Hg and 11/6 mm Hg, in males and females, respectively.\(^{20,21}\) Of related interest is a recent report that orally administered PhyCB can prevent glomerulosclerosis in diabetic mice, an effect associated with strong antioxidant activity.\(^{14}\)

In light of the fact that that PhyCB can mimic the antioxidant activity of bilirubin, it is pertinent that Gunn rats, in which unconjugated bilirubin is chronically elevated owing to a failure of hepatic conjugation, are relatively resistant to hypertension provoked by either DOCA-salt or chronic infusion of angiotensin II.\(^{22,23}\) The ability of systemic induction of heme oxygenase-1 to moderate blood pressure rises in rodent models of hypertension may be attributable, in part, to the biliverdin/bilirubin generated by such induction.\(^{24-31}\) Surprisingly, blood pressure has been little studied in human subjects with Gilbert’s syndrome, in whom plasma levels of free bilirubin are constitutively elevated. However, a recent prospective epidemiological study in Korea found that normotensive subjects with serum bilirubin in the top quartile were about 30% less likely than subjects with lower bilirubin to develop hypertension during follow up.\(^{32}\) In patients with pre-existing hypertension, moderately elevated serum bilirubin is associated with reduced risk for albuminuria and carotid atherosclerosis; it is also associated with a lesser incidence of end-stage renal disease in patients with IgA nephropathy.\(^{33-35}\)

**Natriuretic Impact on Renal Medulla**

It is logical to suspect that the anti-hypertensive effects of dietary nitrate and of bilirubin (and likely PhyCB) are attributable in part to a direct vasodilatory impact of improved NO bioactivity. However, there is also reason to suspect that renal effects of these agents could contribute to blood pressure control. In particular, the NO generated from dietary nitrate in the kidneys, and the antioxidant impact of PhyCB, have the potential to promote natriuresis in the renal medulla – more specifically, in the thick ascending loop of Henle (mTAL), responsible for about 25% of sodium absorption, and in the inner medullary collecting ducts (IMCD). It is notable that the renal medulla tends to be relatively hypoxic – a
circumstance that would be expected to boost NO generation specifically within the medulla when dietary nitrate is administered.\textsuperscript{4-6}

NO and the superoxide (produced primarily by activated NADPH oxidase) appear to have a yin/yang oppositional relationship with respect to regulation of natriuresis in the renal medulla – NO bioactivity promotes natriuresis, whereas superoxide and its derivative hydrogen peroxide promote sodium reabsorption.\textsuperscript{37-39} The effects of NO in this regard in the mTAL are mediated by cGMP; by activating phosphodiesterase II, cGMP decrease cAMP levels and thereby suppresses the activity of the apical Na-K-2Cl co-transporter, crucial to efficient sodium reabsorption in the TAL.\textsuperscript{40, 41} Sodium reabsorption by the mTAL also depends on basolateral Na-K-ATPase activity (“the sodium pump”); this activity is partially inhibited by physiological concentrations of the natriuretic hormone marinobufagenin (MBG), whose secretion from the adrenal gland is evoked when the brain detects sodium/fluid overload.\textsuperscript{42-44} cGMP generated by the activity of atrial natriuretic peptide in the TAL has been shown to sensitize the Na-K-ATPase to inhibition by MBG; this effect is mediated PKG.\textsuperscript{45, 46} It is logical to suspect that cGMP stemming from local NO synthase activity or from spontaneous reduction of circulating nitrite within the medulla could likewise enhance the impact of MBG on the sodium pump. Hence, NO within the mTAL may promote natriuresis both by reduction of Na-K-2Cl cotransporter activity, and by sensitizing sodium pumps to inhibition by MBG. When blood pressure rises, synthesis of NO within the renal medulla rises concurrently, likely owing to increased shear stress on endothelium of the descending vasa recta, and on mTAL epithelium; this phenomenon is likely to be a key mediator of the pressure-natriuresis response.\textsuperscript{47-49}

The renal medulla is particularly susceptible to oxidative stress, as it has high capacity for superoxide generation, but diminished expression of antioxidant enzymes. Increased medullary oxidative stress, mediated primarily by NADPH oxidase, is observed in salt-sensitive rats, and can be provoked by elevated blood pressure, high-salt diets, diabetes, and angiotensin II infusion.\textsuperscript{50-56} NO has been reported to oppose NADPH oxidase activation in the mTAL via a cGMP-PKG-dependent mechanism.\textsuperscript{57} Superoxide promotes sodium retention by enhancing Na-K-2Cl cotransporter activity in the mTAL – an effect diametrically opposed to that of NO.\textsuperscript{58} Superoxide, by directly quenching or inhibiting the synthesis of NO, would also be expected to blunt the natriuretic impact of MBG on the Na-K-ATPase. Part of the impact of superoxide on mTAL function appears to be mediated by hydrogen peroxide; infusion of hydrogen peroxide or a catalase inhibitor into the renal medulla induces an acute rise in blood pressure.\textsuperscript{59} Conversely, chronic medullary infusion of the NADPH oxidase inhibitor apocynin leads to a marked reduction of blood pressure in Dahl salt-sensitive rats, without influencing blood pressure in a consomic rat strain that is not salt-sensitive.\textsuperscript{59, 60} Taylor and colleagues note that “because salt-sensitive rats share many of the abnormalities seen in human hypertension, administration of a specific NADPH oxidase inhibitor or a H2O2 scavenger may also be effective in treating salt-sensitive hypertension in human patients.”\textsuperscript{51}

Nitric oxide, produced by the neuronal form of NO synthase (NOS1), has been shown to suppress sodium reabsorption in the inner medullary conducting duct.\textsuperscript{61-64} An increase in blood pressure provokes an increase in the NOS1 activity of the IMCD; this may be driven by shear stress, and requires endothelin activity, and is an additional mediator of the pressure-natriuresis response. Currently, little appears to be known about how NO promotes natriuresis in the IMCD. Although the IMCD expresses NADPH
oxidase, the impact of oxidative stress on function of the IMCD has been little studied. It is logical to
suspect, however, that superoxide would act to oppose NO’s natriuretic effect on these collecting ducts.

**Implications for Salt-Sensitive Hypertension and its Complications**

In aggregate, these considerations suggest that co-administration of adequate doses of dietary nitrate and
of spirulina/PhyCB could be expected to promote medullary natriuresis by boosting NO and diminishing
oxidants within the mTAL and IMCD. This, in turn, would be expected to lessen the impact of a salty
diet or of fluid overload on adrenal production of MBG. Although MBG exerts an adaptive natriuretic
effect, it also functions as an inhibitor of sodium pumps in vascular smooth muscle – an effect which may
be largely responsible for increased vascular resistance in salt-sensitive hypertension. Moreover,
MBG also can exert a hormone-like activity via binding to sodium pumps; this activity promotes tissue
oxidative stress, fibrosis, and hypertrophy, and may explain why salt-sensitive individuals are more prone
to cardiac hypertrophy, independent of blood pressure. Arguably, MBG may also be a mediator of
the pro-oxidative endotheliopathy associated with hypertension. In addition, suppression of oxidative
stress and an enhancement of NO bioactivity in target tissues would seem likely to oppose the vascular
hormonal effects of MBG that promote remodeling. Hence, to the extent that dietary nitrate and
spirulina could lessen MBG secretion and oppose its pathogenic hormonal activity, they would be
expected to protect the vascular system in ways that are partially independent of blood pressure
modulation.

**Ancillary Potential of Citrulline and High-Dose Folate**

Endogenous NO synthase activity in the renal medulla clearly plays a physiological role in the regulation
of natriuresis. It seems likely that, when the medulla is under oxidative stress, this activity could be
compromised either by uncoupling of the enzyme (reflecting peroxynitrite-mediated oxidation of its
cofactor tetrahydrobiopterin), or by an increase in the functional antagonist assymetric dimethylarginine
(ADMA). Within vascular endothelium, high-dose folic acid promotes recoupling of NO synthase,
presumably owing to the ability of its reduced metabolite tetrahydrofolate to scavenge peroxynitrite-
derived oxidants; it therefore would be of interest to determine whether high-dose folate could
promote natriuresis in the oxidatively-stressed medulla (in Dahl salt-sensitive rats, for example). Clinically, a single 30 mg oral dose of folate was found to induce a modest reduction of blood pressure in
patients with coronary disease - albeit this effect likely is partly attributable to improved NO synthase
function in vascular endothelium. When elevated ADMA contributes to suboptimal NO synthase
activity in the renal medulla – there appears to be little research addressing this point – supplemental
citrulline would be expected to exert a corrective effect by raising tissue levels of arginine.

**Modulation of Natriuresis by Insulin and Dietary Potassium**

Other dietary factors can influence natriuresis. A potassium-depleted diet promotes sodium retention,
whereas a high potassium intake tends to induce natriuresis in the context of a salty diet. (When
sodium intake is low, potassium may modestly increase sodium retention via stimulation of aldosterone
production.) These finding help to explain why an increase in dietary potassium tends to lower blood
pressure when concurrent sodium intake is high, but has little influence on blood pressure when sodium
intake is low. How potassium intake influences natriuresis remains unclear; some research suggests
that renal NO production may be higher, and Na-K-2Cl cotransporter activity lower, on potassium-rich
Intriguingly, a high-potassium diet is associated with a reduction in stroke risk independent of blood pressure; this may reflect a favorable impact of slightly elevated plasma potassium on the vascular endothelium. There is virtually universal agreement that an ample dietary potassium intake can mitigate the pathogenic effects of salty diets. Increased intakes of both potassium and nitrate may be key to the anti-hypertensive utility of the DASH diet.

Conversely, insulin acts directly on the kidneys to promote sodium retention. The salt-sensitivity and increased proneness to hypertension associated with metabolic syndrome is attributable, in part, to the fact that the kidney retains its sensitivity to insulin in metabolic syndrome, such that the compensatory hyperinsulinemia in this syndrome is associated with a net increase in renal insulin activity and consequently in sodium retention. Hence dietary and lifestyle measures which lessen diurnal insulin secretion – by promoting good insulin sensitivity of muscle and liver, or by lessening the meal-driven stimulus to insulin secretion – tend to oppose salt sensitivity. Such measures include prevention/reversal of abdominal obesity, aerobic exercise training, vegan or Mediterranean diets low in saturated fat, and reliance on lower-glycemic-index starchy foods.

References


(47) Ortiz PA, Hong NJ, Garvin JL. Luminal flow induces eNOS activation and translocation in the rat thick ascending limb. II. Role of PI3-kinase and Hsp90. Am J Physiol Renal Physiol 2004 August;287(2):F281-F288.


(56) Yang J, Lane PH, Pollock JS, Carmines PK. Protein kinase C-dependent NAD(P)H oxidase activation induced by type 1 diabetes in renal medullary thick ascending limb. *Hypertension* 2010 February;55(2):468-73.


(64) Hyndman KA, Boesen EI, Elmarakby AA et al. Renal Collecting Duct NOS1 Maintains Fluid-Electrolyte Homeostasis and Blood Pressure. *Hypertension* 2013 April 22.


