Guanylyl cyclase can't stand the HETE

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Nitric oxide (NO)-sensitive isoforms of heterodimeric guanylyl cyclase (α1β1 and α2β1 soluble guanylyl cyclases, sGC) extend an umbrella of protection throughout the cardiovascular system, with wide-ranging effects on local blood flow, platelet & leukocyte reactivity, vessel morphology, and cardiac structure and function(5; 10). Consequently, dysfunctional sGC signalling precipitates cardiovascular disease in an analogous manner to deficits in NO bioactivity, perhaps illustrated most tangibly in the context of human pathophysiology by genome wide association studies (GWAS) identifying mutations in the \textit{GUCY1A3} and \textit{GUCY1B3} genes, that encode the two principal subunits of the enzyme (sGCα1 and sGCβ1, respectively), to associate with a hypertensive phenotype(6). Pre-clinical models have also borne out a clear relationship between sGC activity, local vascular tone and systemic blood pressure. This is best illustrated by the hypertensive phenotype in mice with genetic deletions of either sGCα1 or sGCβ1(3; 8; 9; 17; 19). Interestingly, the sGCα1 and sGCβ1 null mice exhibit a strain- and sex- specific rise in blood pressure(3; 17), and this phenomenon has shed light on mechanisms critical to the vasoprotective functions of NO-sGC signalling, both in terms of endothelium-dependent dilatation and more global effects on neurohormonal axes. For example, the sex difference in response to sGC deletion dovetails well with studies revealing a marked increase in blood pressure in male endothelial NO synthase (eNOS)/cyclooxygenase-1 double knockout mice, which are unable to synthesise two principal endothelium-derived vasodilators, NO and prostacyclin(14). In these animals, endothelium-dependent relaxation is restricted to that provided by hyperpolarisation pathways (i.e. endothelium-dependent hyperpolarisation, EDH) which are up-regulated by female sex steroids(20); in contrast, the predominant endothelium-derived dilator in males is NO, thereby explaining why deletion of eNOS or sGC results in raised blood pressure. Consequently, sGC KO mice are good models with which to ascertain the role of male sex steroids, primarily testosterone, in the development of hypertension. For example, previous studies capitalising on the sGCα1 KO mice have revealed an androgen-driven increase in renin activity that contributes to the hypertensive phenotype in these animals (2; 3).

In the current issue of this journal, Dordea et al. provide a timely and thought-provoking report identifying a further pathway up-regulated by sGCα1 deletion that underlies the development of hypertension in a sex-specific manner. Via linkage
analysis, the authors identified a quantitative trait locus that associates with elevated blood pressure in male 129S6 mice that is not shared by counterparts on a C57BL6 background nor female animals of either strain. In depth analysis revealed that this region encodes a cluster of the cytochrome P450 (Cyp) 4a isoforms, including Cyp4a10, Cyp4a12 and Cyp4a14, which are thought to represent the primary sources of the lipid vasoconstrictor 20-hydroxy-5,8,11,14-eicostraenoic acid (20-HETE). Further scrutiny revealed that the renal expression of Cyp4a12a and 20-HETE levels paralleled the sex- and strain- specific increases in blood pressure and this hypertensive phenotype could be normalised by administration of the 20-HETE antagonist 20-hydroxyeicosa-6(Z),15(Z)-dienoic acid (20-HEDE); this salutary effect was also functionally apparent in terms of endothelium-dependent dilatation in renal resistance arteries. Furthermore, a key role for testosterone in eliciting these vascular abnormalities, via Cyp4a12a, was established by orchiectomy or treatment with 5α-dihydrotosterone. These findings suggest that in the setting of impaired NO-sGC signalling, 20-HETE plays a causative role in the development of elevated blood pressure.

There is considerable interest in the pathological role of 20-HETE in cardiovascular disease, and in hypertension in particular. Certainly, sex- and strain- differences in the expression and activity of Cyp4a isoforms mirror those observed in sGCα1 KO. That is, renal microvascular arachidonic acid hydroxylation to 20-HETE is higher in male 129S6 mice compared to C57BL6, and this in turn is considerably higher than that observed in females from the same strains(11). The study by Dordea et al. therefore adds a further piece to this puzzle illustrating that loss of NO-sGC signalling uncovers this hypertensive pathway. These findings also marry with a recent report that excessive sGC signalling triggered by iNOS-derived NO suppresses 20-HETE formation (albeit via downregulation of Cyp4A1) in the context of sepsis; in this setting, promoting the vasoconstrictor activity of 20-HETE actually reverses the associated hypotension and might have beneficial activity(18). The mechanisms underlying these reciprocal interactions between NO, sGC and 20-HETE may well exist at more than one level. The present study suggests an effect on Cyp4a12a expression that is sGC-dependent, but such an interface may also occur directly; NO is a good inhibitor of Cyp isoforms through haem nitrosylation, and this phenomenon has been demonstrated specifically for 20-HETE-synthesising Cyp isozymes(16; 21). The differential utilization of EDHF between sexes may also be due to interactions between NO-sGC signalling and 20-HETE, since the latter functionally antagonises EDHF bioactivity(12). Interestingly, sGCα1 KO mice exhibit a less severe phenotype compared to animals with sGCβ1 deletion; this is thought to be due, in part, to compensation by the sGCα2 subunit and that in the absence of the β1 protein, sGCα subunits are rapidly degraded(8; 9). Thus, the effects of sGCα1 knockout, as in the present study, may be underestimating the consequences of exposing the hypertensive effects of 20-HETE since residual NO-sGC signalling remains. Moreover, in cardiovascular disease characterised by decreased NO
bioavailability, the direct effects of NO on 20-HETE-synthesizing Cyp4 isoforms will also be disinhibited, creating a multiple hit on the same adverse pathway.

In the study by Dordea et al., vascular reactivity was investigated in renal arteries in which the expression and activity of Cyp4a12a was shown to be upregulated, and plays a central role in 20-HETE production; it would be interesting to know whether this vascular dysfunction is restricted to the renal vasculature or whether this is a more generalised phenomenon. Indeed, the key sites of action of 20-HETE remain unclear. In the vasculature 20-HETE has direct effects on endothelial and vascular smooth muscle reactivity, but also counteracts natriuresis in the kidney(4; 13). 20-HETE also activates TRPV1 (Transient Receptor Potential Cation Channel, Subfamily V, Member 1) to promote myogenic constriction(1; 23). Regardless of the ultimate effector, in the study by Dordea et al. the 20-HETE antagonist 20-HEDE had little or no effect on blood pressure in WT animals suggesting that under basal conditions NO-sGC signalling completely suppresses the activity of 20-HETE from a physiological standpoint. Whether activation of particulate guanylyl cyclases (i.e. GC-A and GC-B), by the natriuretic peptide family of cardiovascular hormones, also suppresses 20-HETE signalling remains to be evaluated. The fact that sGC deletion leads to raised blood pressure suggests this alternate cGMP-generating pathway is unable or insufficient to counteract the elevated 20-HETE levels (i.e. the effect is exclusive to sGC). This observation might also hint at a more local interaction between sGC and 20-HETE, perhaps in the blood vessel wall, rather than an effect on electrolyte balance in the kidney (with the caveat that the prominent role of 20-HETE in autoregulation of renal blood flow may alter kidney function indirectly(25)).

Single nucleotide polymorphisms in the human 20-HETE-synthesising Cyp isoforms, Cyp4A11 and Cyp4F2, reduce enzyme activity and associate with hypertension and other cardiovascular diseases (though this isn’t a universal outcome)(7; 24). From a functional perspective, in hypertensive patients blood pressure correlates with urinary excretion of 20-HETE, whilst plasma 20-HETE levels are inversely correlated with brachial artery flow-mediated dilatation, which is at least in part NO-dependent(22). These observations imply that interactions between NO-sGC and 20-HETE are also likely to be important in regulating vascular tone and blood pressure in humans, although further investigation is required to substantiate this link. Such an interaction would have important implications for the clinical utility of sGC-targeted therapeutics in hypertension (and other cardiovascular disorders). Aberrant NO-sGC signalling is thought to be involved in the pathogenesis of many cardiovascular diseases(15) and therefore targeting sGC should reinstate the wide-ranging vasoprotective functions of NO (e.g. vasodilatation, anti-platelet & anti-leukocyte activity, anti-proliferative effects in vascular smooth muscle etc.). In addition, the study of Dordea et al. infers that therapeutic targeting of sGC is likely to have the added advantage of directly inhibiting pro-hypertensive pathways, including 20-HETE and the renin-angiotensin-aldosterone axis(2), thereby exerting a disease-
modifying pharmacodynamic impact that would offer a theoretical superiority over existing treatments (e.g. anti-hypertensives).

REFERENCES


