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Angiotensin II Signalling in Bartter's and Gitelman's Syndromes

A Negative Human Model of Hypertension

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Abstract

In extensive studies of patients with Bartter's and Gitelman's syndromes, we have shown biochemical abnormalities in angiotensin II (Ang II) short- and long-term cell signalling, which depict a mirror image of those found in hypertension. The information obtained from the study of this human model of altered vascular tone regulation shows that it can be used to gather more general data and/or confirm mechanistic details of the cellular and biochemical events involved in the pathophysiology of vascular tone control, and to shed light on the multiplicity of the Ang II signalling-related mechanisms responsible for the pathophysiology of hypertension and its long-term complications, such as cardiovascular remodelling and atherogenesis.

1. Introduction

Functional and structural changes of the peripheral vasculature and environmental factors that influence cardiac, renal and vascular function are the main causative factors of arterial hypertension. Functional changes of peripheral vasculature include increased vascular reactivity to vasoconstrictors, reduced vasodilation and altered endothelial cell function, the latter being essentially determined by reduced nitric oxide (NO) production.[1] 'Vascular remodelling', which includes increased vascular smooth muscle cell growth, cell migration and fibrogenesis, results from structural alterations caused by the long-term effects of vasoconstrictors.[2] Another factor contributing to vascular dysfunction in hypertension is oxidative stress, which induces inflammation of the vascular wall associated with migration of proinflammatory cells, increased expression of redox-sensitive proinflammatory genes, protein accumulation and fibrosis.[3]

Angiotensin II (Ang II) is one of the most important humoral factors involved in the vascular alterations in hypertension.[4] The multiple actions of Ang II are mediated via specific, complex intracellular signalling pathways that are activated after binding of the peptide to its cell-surface receptors. Ang II has pleiotropic cellular effects mediated by the activation of short- and long-term signalling mechanisms.[5] The short-term signalling mechanisms involve monomeric and heterotrimeric G proteins, phospholipase C (PLC)-β, leading to most of the well known haemodynamic and

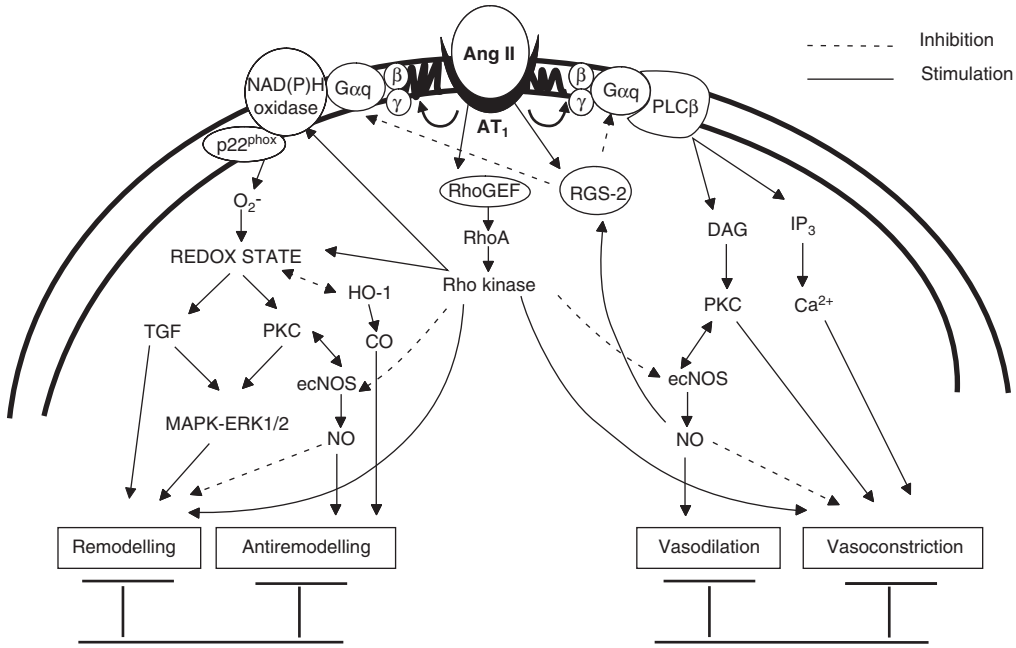

Fig. 1. Schematic representation of the relationship between short- and long-term angiotensin II (Ang II) signalling and regulator of G protein signalling (RGS)-2 as its regulatory element. RGS-2 acts as a negative regulator of the α subunit of Gq protein, the signalling mediator of Ang II, and regulates Ang II short-term (right) and long-term (left) signalling. The former leads to the haemodynamic and endocrine effects of the hormone, including vascular smooth muscle contraction; the latter causes remodelling of target organs through oxidative stress. The RhoA/Rho kinase pathway is part of Ang II signalling through the Ang II-mediated activation of RhoGEF, a RhoA regulator, contributing both to short- and long-term signalling of Ang II. In normal conditions, vasoconstriction and remodelling are balanced with vasodilation and antiremodelling, but partial or complete deletion of RGS-2 causes hypertension.[5] **AT_1** = angiotensin II type 1; **CO** = carbon monoxide; **DAG** = diacylglycerol; **ecNOS** = endothelial subunit of NO synthase; **HO-1** = heme oxygenase-1; **IP_3** = inositol trisphosphate; **MAPK-ERK** = mitogen-activated protein kinase; **NO** = nitric oxide; **PKC** = protein kinase C; **PLC** = phospholipase C; **TGF** = transforming growth factor.

endocrine effects of Ang II such as vascular smooth muscle contraction. This pathway involves release of intracellular messengers such as inositol trisphosphate (IP_3) and Ca^{2+} and activation of protein kinase C (PKC), leading to vascular smooth muscle contraction. This pathway is counterbalanced by the vasodilatory and antiproliferative activity of the NO system. In fact, the activity of the endothelial subunit of NO synthase (ecNOS) is negatively regulated by PKC. On the other hand, the cellular effects mediated by long-term signalling of Ang II cause cardiovascular remodelling (common to hypertension), atherosclerosis and heart failure, mostly through modulation of the cell oxidative state.[3,5] In fact, Ang II increases oxidative stress via upregulation of NADH/NADPH oxidase, the major superoxide (O_2^-)-generating enzyme, with consequent O_2^- overproduction.[6] Activation of $p22^{phox}$, a 22-kD α subunit of cytochrome b_{558} included in the NADH/NADPH oxidase, plays a key role in O_2^- production. It functions as an integral subunit of the final electron transport from NADPH to heme and molecular oxygen in generating O_2^- and is stimulated by Ang II.[6] This pathway also involves the induction of established oxidative stress-related effectors such as transforming growth factor-β (TGFβ)[7] and PKC, which activate oxidative stress-related kinases such as mitogen-activated protein kinase (MAPK/ERK),[8] finally leading to cardiovascular remodelling and atherogenesis. This pathway is counterbalanced by the activity of both NO and heme oxygenase-1 (HO-1) systems, which are protective towards oxidative stress[9] and regulated by redox dependent and independent stimuli,[10] some of which (such as the intracellular messenger cAMP and cGMP) lead to vasodilation through the HO-1-induced production of the vasodilatory carbon monoxide (CO) [figure 1].

The multiple interrelationships that link hypertension, Ang II, oxidative stress and NO systems suggest that Ang II, oxidative stress and NO comprise a homeostatic system that regulates both vascular function and structure.[5] A major link is via the effect of Ang II on NADH/NADPH oxidase. Ang II upregulates this oxidase[6] and the resulting increased free radical production reacts with NO to reduce its bioavailability, thereby impairing NO-dependent vasodilation and increasing vascular oxidative stress. These effects cause hypertension and cardiovascular remodelling.[5] The significance of Ang II in the vascular pathology associated with hypertension is supported by experimental and clinical studies demonstrating that angiotensin-converting enzyme

(ACE) inhibitors and Ang II type 1 (AT_1) receptor blockers not only lower blood pressure but also are able to correct arterial remodelling, improve endothelial function, reduce vasomotor tone, decrease inflammation and normalise abnormal signalling events in vascular smooth muscle cells. All these steps have been investigated in patients with Bartter's and Gitelman's syndromes with results opposite to those of human essential hypertension. Therefore, we propose these syndromes as a useful human negative clinical model for the study of the pathophysiological mechanisms of human hypertension.

1.1 The Model of Bartter's and Gitelman's Syndromes

The clinical picture of Bartter's/Gitelman's syndromes reflects genetically determined functional defects of kidney transporters and ion channels, leading to a puzzling clinical picture characterised by hypokalaemia, sodium depletion, activation of the renin-angiotensin-aldosterone system with increased plasma levels of Ang II, yet normo-hypotension, reduced peripheral resistance and hyporesponsiveness to pressor agents.[11,12] The identification of the genetic defects of Bartter's/Gitelman's syndromes clarified the pathogenesis of these diseases and established that the hyporesponsiveness to pressor agents and normo-hypotension are secondary to mutations in cotransporters and ion channels determining Na^+ and K^+ wasting, which, together with volume contraction, are the major consequences of the genetic abnormalities of Bartter's/Gitelman's syndromes.[11,12]

Bartter's/Gitelman's syndromes have been considered good human models to explore the mechanisms responsible for maintaining/controlling vascular tone.[13] Bartter's/Gitelman's syndromes have, in fact, attracted much attention for persistent normo-hypotension despite biochemical and hormonal abnormalities typical of hypertension. Therefore, understanding why patients with Bartter's/Gitelman's syndromes do not develop hypertension, in spite of high Ang II and activation of the renin-angiotensin-aldosterone system, could shed light on the cellular basis of hypertension.

In our extensive studies of patients with Bartter's/Gitelman's syndromes, we have shown that the short-term Ang II signalling pathway, which mediates most of the known haemodynamic and endocrine effects of the peptide including vasoconstriction, is blunted.[13] This is consistent with the reduced peripheral resistance, vascular hyporeactivity and normo-hypotension that is typical of patients with Bartter's/Gitelman's syndromes. We have also reported in these patients that the long-term signalling pathway of Ang II, which modulates the cell redox state to determine cardiovascular remodelling and atherosclerosis, is altered.[14,15] All of the biochemical abnormalities of Ang II short- and long-term signalling present in Bartter's/Gitelman's patients depict a mirror image of those found in hypertension, in which the cellular biochemical events induced by Ang II are upregulated (figure 2).[5]

In this review we survey our current understanding of the most important abnormalities of both Ang II short- and long-term signalling in patients with Bartter's/Gitelman's syndromes. The information obtained from this human model of altered vascular tone regulation, other than hypertension but more homogeneous, underscores its utility to gather more general data and/or confirm mechanistic details of the cellular biochemical events involved in the pathophysiology of vascular tone control and to shed light on the multiplicity of Ang II signalling-related mechanisms responsible for the pathophysiology of hypertension.

2. Mechanisms of Vascular Tone Control

2.1 Calcium, Phospholipase C, Protein Kinase C

This area has been the subject of intense research both in terms of its basic importance for physiology and its practical application to human health.[26-29] Removal of extracellular Ca^{2+} results in a rapid relaxation of arteries, while an intracellular $[Ca^{2+}]i$ increase produces smooth muscle cell contraction.[30,31] The source of Ca^{2+} can be either intrinsic, that is, intracellular stores, or extrinsic, that is, extracellular. Calcium entry into vascular smooth cells is mediated by two major types of calcium ion channels. One, the receptor-gated channel,[27] is dependent on the occupancy level of specific receptors. Noradrenaline[32] and Ang II[33] stimulate Ca^{2+} influx through a receptor-gated mechanism. The other, the voltage-gated channel, is modulated by the membrane potential.[27]

Vascular smooth muscle cell free Ca^{2+} levels and vascular tone are closely linked. Alterations in Ca^{2+} may, in fact, affect vasoconstriction.[30,31] IP_3 is an important stimulus for calcium release from the endoplasmic reticulum.[26,27] IP_3 is generated by the enzymatic hydrolysis of plasma membrane phospholipids containing inositol. The reaction is catalysed by PLC, a membrane-bound enzyme that is activated by the binding of agonists to cell membrane receptors.[27] At least ten isoenzymes of PLC have been identified, classified into three families: β, which is associated with the G protein system; γ, which is activated by tyrosine kinases; and δ, whose activation has not yet been completely elucidated.[34] The production of IP_3 is accompanied by the production of diacylglycerol (DAG), an activator of PKC. IP_3 is water soluble, diffuses into the cytoplasm and stimulates the release of calcium from intracellular calcium stores[27,35] while DAG remains in the membrane, where it activates PKC.[26]

The role of PKC in the regulation of vascular smooth muscle cell contraction is not well understood but probably encompasses

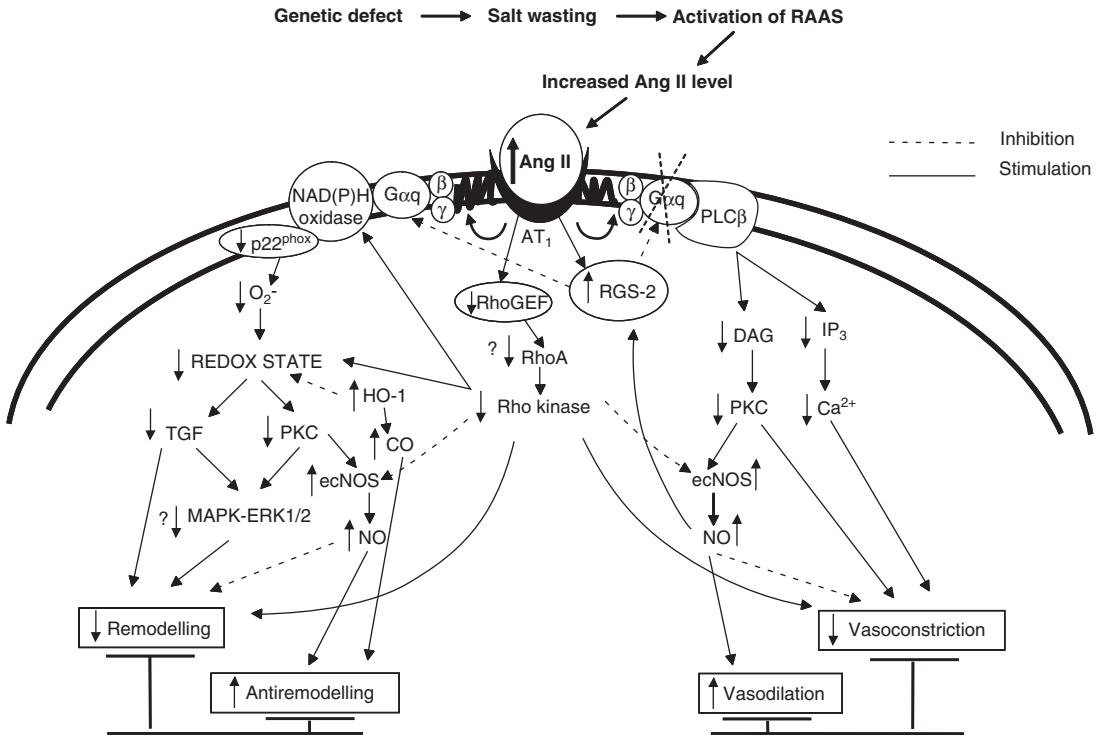

Fig. 2. Possible scenario of short- and long-term angiotensin II (Ang II) signalling in Bartter's/Gitelman's syndromes (BS/GS), as depicted by the results of our studies. In BS/GS, the increased level of Ang II induced by the genetically determined salt wasting increases the expression and production of regulator of G protein signalling (RGS)-2.[16] Increased RGS-2 desensitises Ang II-G protein-coupled receptor signalling, downregulating the expression of the α subunit of Gq protein,[17,18] which reduces phospholipase C (PLC)-β activity and leads to low inositol trisphosphate (IP_3)[19] and diacylglycerol (DAG) production. The first blunts intracellular Ca^{2+} release;[20] the latter reduces protein kinase C (PKC) activity,[17] vascular smooth muscle contraction and vascular tone and maintains normo-hypotension. Reduced activity of PKC upregulates gene expression of the endothelial nitric oxide synthase (ecNOS),[17,21] with upregulation of heme oxygenase-1 (HO-1)[14] and increased production of nitric oxide (NO)[22,23] and carbon monoxide (CO), both contributing factors of the reduced vascular tone of BS/GS. Increased NO production may further increase RGS-2 production. Increased RGS-2 downregulates the α subunit of Gq protein and reduces the Ang II-mediated expression of the NAD(P)H subunit $p22^{phox}$,[14] reducing O_2^- production, transforming growth factor-β (TGFβ) expression[14] and PKC activity,[24] and increasing expression of ecNOS[25] and HO-1,[14] and production of NO[23] and CO, all pointing towards an anomalous long-term signalling of Ang II, which leads to reduced fibrogenic activity and remodelling in BS/GS. In BS/GS, the RhoA/Rho kinase pathway, which contributes to vasoconstriction through inhibition of NO system and cardiovascular remodelling through activation of oxidative stress, is reduced.[15] The reduced gene and protein expression of the α subunit of Gq protein in BS/GS[17,18] is consistent with a primary defect that resides upstream of the RhoA/Rho kinase pathway and reduces the activity of RhoA/Rho kinase pathway, thereby contributing to the reduced vasoconstriction and cardiovascular remodelling of BS/GS patients. **MAPK-ERK** = mitogen-activated protein kinase.

several mechanisms. It has been suggested that PKC alters the vascular smooth muscle cell sensitivity to $[Ca^{2+}]i$.[26] Moreover, PKC modulates vascular tone directly by affecting voltage-gated channels[36] or Kv channels,[37] thereby enhancing myogenic contraction.

In our cohort of patients with Bartter's/Gitelman's syndromes, both genetically and biochemically characterised and with normo/hypotension in the face of hormonal abnormalities more typical of hypertension, we examined the role of calcium in the control of vascular tone.

In Bartter's/Gitelman's patients, we have shown that neutrophil resting $[Ca^{2+}]i$ did not differ from controls, but the response of their cells to fMetLeuPhe (fMLP), an agonist with activity that parallels that of Ang II on vascular smooth muscle cells, is significantly smaller than that shown by controls.[20] This suggests that patients with Bartter's/Gitelman's syndromes exhibit an intrinsic anomaly in the mechanism(s) responsible for intracellular Ca^{2+} mobilisation.[20] This is most likely due to a postreceptor defect as the receptor number and affinity for fLMP is unaffected.[19]

Since IP_3 is the most powerful signal for intracellular calcium release,[28] we have measured IP_3 levels at baseline and after fMLP stimulation in neutrophils from Bartter's/Gitelman's patients and found that, in keeping with the observations on intracellular calcium, it was about 50% reduced compared with controls.[19]

The decline in intracellular IP_3 production led us to propose that the defect along the cellular signal transduction system in Bartter's/Gitelman's syndromes is located upstream at a postre-

ceptor level. This was further supported by the observation that, in addition to IP_3 and Ca^{2+} defects, patients affected by Bartter's/Gitelman's syndromes show a reduced membrane PKC reactivity after stimulation.[17,19] The signalling sequence, in fact, begins after the receptor stimulation, with its coupling to PLC, which breaks down polyphosphoinositide producing the intracellular messengers IP_3 and DAG. IP_3 causes mobilisation of Ca^{2+} from intracellular stores, while DAG activates PKC, an effect mimicked by tumour-promoting phorbol esters.[26]

2.2 Nitric Oxide/Cyclic GMP System

The vascular endothelium controls vascular tone through the production of NO, a potent vasodilator.[24,38] NO is produced via the constitutive endothelial cell NO synthase (ecNOS or NOS III) that catalyses the conversion of L-arginine to L-citrulline.[39] This enzyme binds calmodulin in a calcium-dependent manner and can be activated by stimuli that increase the concentration of intracellular free calcium.[40] NO diffuses to the smooth muscle layer where it reduces vascular tone via its effects on $[Ca^{2+}]i$. As an initial step, NO activates the enzyme guanylate cyclase. The activation of guanylate cyclase produces cGMP, which activates G kinase, resulting in a decline of cell-free $[Ca^{2+}]i$ and vascular relaxation.[29] This effect is mediated via several different pathways. G kinase activity reduces IP_3 generation, phosphorylates the IP_3 receptor, which reduces its sensitivity to IP_3,[41] and stimulates ATPase-dependent Ca^{2+} extrusion.[24]

In patients with Bartter's/Gitelman's syndromes, we have reported an increased urinary excretion of NO metabolites, positively correlated with urinary excretion of cGMP, which was also increased compared with controls.[22] In addition, direct assessment of vascular tone in these patients, using plethysmographic evaluations that reflect NO activity such as forearm blood flow, suggested an increased production of NO.[23] These data were consistent with increased expression of ecNOS in patients with Bartter's/Gitelman's syndrome. This was confirmed in monocytes from patients in which we showed an increased ecNOS gene expression.[21] In addition, in another study we found indirect indications that NO levels in patients with Bartter's/Gitelman's syndromes are elevated.[25] We observed that in Bartter's/Gitelman's patients, low-density lipoproteins (LDLs) have a reduced susceptibility to oxidation, and the lag phase of conjugated diene formation, an oxidation related marker, was strongly correlated with urinary NO_2^-/NO_3^-.[25] These data suggest that NO is acting as an antioxidant and that plasma NO is increased in these patients.[25,42] Another indirect proof that NO is elevated in patients with Bartter's/Gitelman's syndromes came from experiments assessing the furosemide-sensitive lithium efflux (FSLE) from lithium-loaded erythrocytes of healthy subjects in the presence of plasma from controls and from Bartter's/Gitelman's patients, *in vitro*.[43] Plasma from patients with either Bartter's/Gitelman's syndrome inhibited FSLE, suggesting that plasma factor(s) that inhibit(s) this transport is/are present in Bartter's/Gitelman's patients. As NO inhibits Na^+-K^+-$2Cl^-$ cotransport,[44] our data are consistent with elevated plasma NO and further suggest that it contributes to K^+ wasting, hypokalaemia and hypotension by inhibiting renal tubule cotransport in patients affected by Bartter's/Gitelman's syndromes.[43]

2.3 G Proteins and Regulators of G-Protein Signalling

The heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins) transduce receptor-generated signals across the plasma membrane.[45,46] The stimulation of Ang II activates two G proteins of the Gq family,[47] Gq and G_{11}, leading to an increased cytoplasmic Ca^{2+} concentration. Only the α subunit of the Gq protein transduces the signal to PLCβ to generate IP_3 and DAG;[47] the former releases Ca^{2+} from the intracellular store, while the latter stimulates PKC, both leading to an arterial myogenic response.

In addition to trimeric G proteins, small molecular weight or monomeric G proteins may be involved in cell signal transduction affecting vascular tone. This could occur via changes of Ca^{2+} sensitivity of contractile proteins in vascular smooth muscle cells brought about by the activation of monomeric G proteins such as Ras, Rho, and Raf. These proteins, in a way similar to the α subunit of the trimeric G proteins, are activated by GTP binding, which enables them to influence effector proteins.[46] The activation of these proteins also leads to the stimulation of MAPKs and to an increase in Ca^{2+} sensitivity, directly or after agonist stimulation.[48]

Signal transduction by G protein-coupled receptors (GPCRs) is regulated by regulators of G protein signalling (RGS) proteins, which act as GTPase-activating proteins (GAPs) for Gα subunits and also competitively inhibit Gα binding to effectors such as PLC.[48-50] The GAP activity of RGS reduces the steady state level of GTP-bound Gα subunit, thus turning off the G protein signal.[49-51] The role of RGS-2 in GPCR-mediated blood pressure control and as a key control element of Ang II signalling (acting as a negative regulator of the α subunit of Gq protein, the signalling mediator common to Ang II and other physiological vasoconstrictors), has been investigated in an RGS-2 knockout mouse where partial or complete deletion of RGS-2 causes hypertension.[52] RGS-2 plays a major role in the desensitisation of GPCR signalling[49] through interaction and binding to GPCR.[50,51] These findings highlight the importance of GPCRs in blood pressure homeo-

stasis and suggest that a dysfunctional GPCR signalling contributes to the initiation and/or maintenance of essential hypertension.[5]

The hyporesponsiveness to Ang II present in Bartter's/Gitelman's syndromes suggested that abnormalities of Ang II-GPCRs complex and its regulator RGS-2 might be involved. In fact, the reduced intracellular IP_3 and Ca^{2+} release[19,20] and the reduced PKC activity[17] shown in Bartter's/Gitelman's syndromes despite normal Ang II receptor density and the absence of mutations of AT_1 receptors[53] pointed towards the presence of abnormality at the level of Gq protein and/or RGS-2, downstream of the coupling of Ang II with its receptor. mRNA expression and protein level of the α subunit of the Gq protein were significantly reduced in mononuclear cells of patients with Bartter's/Gitelman's syndromes,[17,18] while RGS-2 gene and protein expression was increased.[16] The same phenomenon in vascular smooth muscle cells may reduce vascular reactivity and cause hypotension. The increased mRNA and protein expression of RGS-2 in patients with Bartter's/Gitelman's syndromes is also confirmed by the failure of Ang II *in vitro* to increase RGS-2 expression in patients with Bartter's/Gitelman's syndromes while mononuclear cells from healthy controls responded with increased levels of RGS-2 protein upon incubation with exogenous Ang II, thus suggesting that RGS-2 expression is maximally stimulated.[16] These findings suggest that the interacting RGS-2-Ang II GPCR complex desensitises Ang II-GPCR signalling, reduces postreceptor Ang II signalling, alters vascular tone regulation and blunts Ang II induced vasoconstriction. It is reasonable, therefore, to hypothesise that RGS-2-Ang II GPCR complex and RGS-2-mediated desensitisation of Ang II-GPCR signalling are major determinants of the reduced content of Gα subunit of Gq protein mRNA and protein, reported in patients with Bartter's/Gitelman's syndromes.[17,18]

Our demonstration of the increased content of RGS-2 in patients with Bartter's/Gitelman's syndromes is the first obtained in humans and in a clinical condition characterised by altered vascular tone regulation other than hypertension. These data in humans, in agreement with those obtained in experimental models,[52] underline that the RGS-2 level has a major impact on blood pressure regulation.

2.4 Oxidative Stress and Redox Signalling

Oxidative stress has been implicated in the pathogenesis of hypertension and its long-term complications.[3] Cellular effects mediated by long-term signalling of Ang II play a causative role in the cardiovascular remodelling common to hypertension, atherosclerosis and heart failure, mostly through induction of oxidative stress.[5,6] Ang II, in fact, induces oxidative stress via upregulation of NADH/NADPH oxidase, the major superoxide (O_2^-)-generating enzyme, with consequent O_2^- overproduction.[6] Activation of $p22^{phox}$, a 22-kD α subunit of cytochrome b_{558} included in the NADH/NADPH oxidase, plays a key role in O_2^- production. It functions as an integral subunit of the final electron transport from NADPH to heme and molecular oxygen in generating O_2^- and is stimulated by Ang II.[6] The Ang II-mediated O_2^- overproduction reduces the bioavailability of NO because of its oxidative inactivation by excessive production of O_2^- in the vascular wall, thereby altering endothelial-dependent relaxation and inducing hypertension and remodelling.[54] This pathway also involves induction of established oxidative stress-related effectors, such as TGFβ and PKC, which activate oxidative stress-related kinases such as MAPK/ERK,[8] leading to cardiovascular remodelling and atherogenesis.

In patients with Bartter's/Gitelman's syndromes we have shown that the gene expression of oxidative stress-related proteins such as $p22^{phox}$ and TGFβ is reduced.[14] In addition, the blunted increase of $p22^{phox}$ and TGFβ gene expression upon incubation with Ang II *in vitro* in Bartter's/Gitelman's patients compared with controls strengthens the evidence of blunted response to Ang II in Bartter's/Gitelman's patients.[14] The contention that oxidative stress is reduced in Bartter's/Gitelman's patients is supported by the demonstration of increased HO-1 gene expression.[14] HO is a rate-limiting enzyme that catalyses the degradation of heme into biliverdin and CO.[9] Biliverdin is further metabolised to bilirubin, which is a very potent antioxidant,[9] while the vasodilatory CO may contribute to vasodilation and reduced peripheral resistance of Bartter's/Gitelman's syndromes. The increased plasma antioxidant power is also in keeping with low oxidative stress-related response in Bartter's/Gitelman's syndromes.[14] It could reflect sparing of other scavengers as a consequence of decreased reactive oxygen species production. This is supported by the finding of a low plasma level of peroxynitrite, a NO oxidised derivative of the chemical reaction with O_2^-, which was undetectable in plasma of Bartter's/Gitelman's patients as in the control plasma despite increased NO production in the former.[14] Finally, the reduced oxidative state present in Bartter's/Gitelman's patients is also confirmed by the observation that in Bartter's/Gitelman's syndromes, LDL has a reduced susceptibility to oxidation and that the lag phase of conjugated diene formation, an oxidation-related marker, was significantly increased compared with the lag phase of conjugated diene formation of healthy subjects.[25]

2.5 RhoA/Rho Kinase Pathway

It is becoming increasingly evident that the RhoA/Rho kinase pathway may play an important role in hypertension and vascular

remodelling.[55,56] RhoA is a monomeric G protein that is regulated by Ang II. Rho kinase is a serine/threonine protein kinase,[57,58] a downstream of effector of RhoA monomeric G protein. The RhoA/Rho kinase pathway modulates the phosphorylation state of the regulatory chain of myosin II, mainly through inhibition of myosin phosphatase, and contributes to agonist-induced Ca^{2+} sensitisation in smooth muscle contraction.[55,56] The ultimate effect is to increase smooth muscle cell contraction. This has stimulated interest in Rho kinase as a contributor to the pathogenesis of hypertension and atherosclerosis.[55,59] Rho kinase activation is also crucial for Ang II-induced plasminogen activator inhibitor-1 (PAI-1) gene expression,[60] a known profibrotic factor, and is involved in cytokinesis, cell migration and invasion.[55] The involvement of Rho kinase in these effects suggests that Rho kinase may represent a link between the short- and long-term signalling of Ang II, which mediates most of the known haemodynamic and endocrine effects of the peptide (short-term signalling of Ang II)[4,5,33] and determines cardiovascular remodelling and atherosclerosis essentially through the induction of oxidative stress (long-term signalling of Ang II).[4,5,33] Ang II stimulates RhoA/Rho kinase pathways through G protein-coupled neurotransmitter receptor signalling, also involving Gq protein.[54] RhoA/Rho kinase pathway is also activated by reactive oxygen species.[61]

We have recently shown that in Bartter's/Gitelman's patients both Rho kinase gene and Rho kinase protein expression are reduced[15] and that the stimulatory effect of Ang II on Rho kinase gene and protein expression is lacking.[15] These data are in agreement with the reduced vascular responsiveness to Ang II and other pressors that is typical of Bartter's/Gitelman's syndromes. The reduced gene and protein expression of the α subunit of Gq protein that we have shown in cells from Bartter's/Gitelman's patients[17,18] is consistent with a primary defect that resides upstream in the RhoA/Rho kinase pathway, which may determine the reduced Rho kinase gene and protein expression and the reduced response to Ang II challenge. This could, in fact, be located at the level of the activation of RhoA and/or abnormalities of factors involved in its activity, such as guanine nucleotide exchange factors (RhoGEFs) or guanine nucleotide dissociation inhibitor (RhoGDI), all of which are able to determine abnormalities downstream of agonist/receptor coupling along the RhoA/Rho kinase pathway.[55,56] In fact, activation of RhoGEF has been demonstrated to play a role in the pathophysiology of hypertension and remodelling,[62] as well as in the increased amount/activity of RhoGDI.[63]

In view of the direct modulation by Rho kinase of PAI-1 gene expression,[60] which is one of the major effectors of Ang II-mediated Rho kinase activation involved in proliferation and atherogenesis,[64] the decline in Rho kinase gene and protein expression found in cells from patients with Bartter's/Gitelman's syndrome[15] provides a ready explanation for reduced PAI-1 gene and protein expression.[15] In addition, since PAI-1 has been shown to be a profibrotic factor,[64] these data provide further support for altered long-term Ang II signalling in Bartter's/Gitelman's patients, specifically with respect to vascular remodelling processes.

2.6 Future Directions

Future directions for these studies include further elaboration of the effects on the pathways previously examined. A further confirmation of the essential role played by RGS-2 in the pathophysiology of hypertension in humans could come from studies silencing the gene encoding RGS-2 in Bartter's/Gitelman's syndromes, which is overexpressed.[16] Using siRNA for RGS-2 it could be possible to evaluate the effect of RGS-2 knockout on the signalling events downstream of the G protein-coupled receptors.[65] In particular, the evaluation of determinant steps of both short- and long-term signalling of Ang II downstream of its receptor binding (such as intracellular Ca^{2+} release and/or intracellular IP_3 production, PKC activity, evaluation of oxidative stress status) after RGS-2 mRNA silencing could give information on cellular and biochemical events involved in the abnormal vascular tone regulation and remodelling present in human hypertension. In addition, Bartter's/Gitelman's syndromes may also be used as a model to investigate the regulatory role of the monomeric G proteins (Ras, Rho and Raf) in the control of myogenic tone. In particular, the approach through Bartter's/Gitelman's syndromes will allow the study of the monomeric G protein RhoA, its regulators RhoAGEFs and RhoAGDI, and the RhoA/Rho kinase pathway in terms of smooth muscle Ca^{2+} sensitivity as well as a potential role in affecting the activity of kinases that are involved in the control of vascular tone and cardiovascular remodelling induced by Ang II.

3. Conclusions

The major abnormality of the vascular pathophysiology of hypertension is represented by increased constrictor and decreased dilator responses and altered structure represented by vascular remodelling. Ang II plays a pivotal role in the induction of these processes through its both short- and long-term signalling (stimulation of vascular smooth muscle cell contraction, inhibition of NO-mediated vasodilation, induction of cell growth, increase of extracellular matrix protein content, induction of cell migration and inflammation). Ang II mediates these cellular events in hypertension through mechanisms that occur at the postreceptor level, associated with upregulation of Ang II-stimulated G protein-coupled phospholipases, PKC, oxidative stress and RhoA/Rho kinase-dependent pathways. Dysregulation of the interaction be-

Table I. Cell abnormalities described in Bartter's/Gitelman's syndromes and essential hypertension

Cell abnormality	Bartter's/Gitelman's syndromes	Essential hypertension
Intracellular calcium release	Diminished[20]	Increased[66,67]
Intracellular IP_3 level	Diminished[19]	Increased[67]
PKC expression and activity	Diminished[17]	Increased[67]
NO metabolites	Increased[22]	Diminished[68]
Endothelium-dependent relaxation	Increased[23]	Diminished[69]
ecNOS expression	Increased[21]	Diminished[70]
Gαq expression	Diminished[17,18]	Increased[71]
RGS-2 expression	Increased[16]	Diminished[52,72]
Oxidative stress		
$p22^{phox}$ expression	Diminished[14]	Increased[6,73,74]
TGFβ expression	Diminished[14]	Increased[75-77]
HO-1 expression	Increased[14]	Diminished[78,79]
Rho kinase expression/activity	Diminished[15]	Increased[59,80,81]
PAI-1 expression	Diminished[15]	Increased[64,82]

ecNOS = endothelial nitric oxide synthase; **HO-1** = heme oxygenase-1; **IP_3** = inositol trisphosphate; **NO** = nitric oxide; **PAI-1** = plasminogen activator inhibitor-1; **PKC** = protein kinase C; **RGS-2** = regulators of G protein signalling-2; **TGF**β = transforming growth factor-β.

tween these pathways could result in the functional and structural vascular changes in hypertension. Although the elucidation of Ang II-mediated signalling in vascular smooth muscle cells has gained much information in the last years, the biochemical processes that mediate abnormal cell signalling in hypertension still remain to be fully elucidated.

The model of Bartter's/Gitelman's syndromes can be of use for gaining insight into the mechanisms responsible for maintaining/controlling vascular tone. In fact, by studying patients affected by these syndromes, we have developed data, summarised in table I, that have revealed alterations in the mechanisms that play major roles in regulating vascular tone and structure. In particular, the results obtained with respect to the upregulation of RGS-2 in patients with Bartter's/Gitelman's syndromes, which is the first demonstration of an altered level of this regulator of Gq protein in a human clinical condition characterised by altered tone regulation, suggest a critical involvement of an altered level of RGS-2 in a Gq-mediated Ang II intracellular signalling system in human hypertension and in the pathophysiology of essential hypertension.

In summary, this review has described insights into the mechanisms responsible for the control of vascular tone and structure, gained by using Bartter's/Gitelman's syndromes as a human model. These results are of particular interest as Bartter's/Gitelman's syndromes represent the mirror image of the derangements involved in the pathophysiology of arterial hypertension and atherosclerosis.[5] Nature has provided a clinical model for evaluating and understanding mechanisms involved in Ang II signalling in hypertension and our studies in Bartter's/Gitelman's syndromes provide some insight into these mechanisms, the loss/alteration of which can lead to conditions such as hypertension and vascular remodelling on one hand, or to conditions of vascular hyporeactivity such as Bartter's/Gitelman's syndromes, on the other.

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