British Journal of Pharmacology (2003) 139, 191–202 © 2003 Nature Publishing Group All rights reserved 0007–1188/03 $25.00
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REVIEW

Brain renin–angiotensin system dysfunction in hypertension: recent advances and perspectives

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This review focuses on the dysfunction of the intrinsic brain renin–angiotensin system (RAS) in the pathogenesis of hypertension. Hyperactivity of the brain RAS plays a critical role in mediating hypertension in both humans and animal models of hypertension, including the spontaneously hypertensive rat (SHR). The specific mechanisms by which increased brain RAS activity results in hypertension are not well understood but include increases in sympathetic vasomotor tone and impaired arterial baroreflex function. We discuss the contribution of endogenous angiotensin (Ang) II actions on presympathetic vasomotor rostral ventrolateral medulla neurons to enhance sympathetic activity and maintain hypertension. In addition, we discuss Ang II-induced attenuation of afferent baroreceptor feedback within the nucleus tractus solitarius and its relevance to the development of hypertension. We also outline the cellular and molecular mechanisms of Ang II signal transduction that may be critical for the initiation and establishment of hypertension. In particular, we present evidence for a phosphoinositide-3-kinase-dependent signaling pathway that appears to contribute to hypertension in the SHR, possibly via augmented Ang II-induced increases in neuronal firing rate and enhanced transcriptional noradrenaline neuromodulation. Finally, we outline future directions in utilizing our understanding of the brain RAS dysfunction in hypertension for the development of improved therapeutic intervention in hypertension.
British Journal of Pharmacology (2003) **139,** 191–202. doi:10.1038/sj.bjp.0705262

Keywords: Brain renin–angiotensin system; angiotensin receptor signal transduction; spontaneously hypertensive rat; sympathetic activity; Baroreflex; phosphoinositide-3-kinase; noradrenaline neuromodulation

Abbreviations: ACE, angiotensin converting enzyme; Ang, angiotensin; AP1, activating protein 1; AT_1 and AT_2, angiotensin type 1 and type 2; BP, blood pressure; CaMKII, calcium/calmodulin-dependent protein kinase II; D-βH, dopamine β-hydroxylase; DOCA, deoxycorticosterone acetate; FRK, Fos-regulating kinase; I_A, transient A type K^+ current; i.c.v., intracerebroventricular; I_{Kv}, delayed rectifier K^+ current; JNK, c-Jun NH_2-terminal kinase; MAP, mitogen-activated protein; MnPO, median preoptic nucleus; NA, noradrenaline; NAT, noradrenaline transporter; NTS, nucleus tractus solitarius; PI, phosphoinositide; PI3K, phosphoinositide-3-kinase; PKB and PKC, protein kinase B and C; PLC, phospholipase C; PVN, paraventricular nucleus; RAS, renin–angiotensin system; SFO, subfornical organ; SHR, spontaneously hypertensive rat(s); TGR mRen2, renin transgenic rat(s); TH, tyrosine hydroxylase

Introduction

Essential hypertension is one of the most prevalent cardiovascular disorders, afflicting approximately 15% of the population. It is a major risk factor for other cardiovascular diseases such as peripheral vascular disease and ischemic heart disease, as well as stroke and end-stage renal disease, thereby resulting in considerable morbidity and mortality. The etiology of this disease is multifactorial, resulting from the interaction of a number of genetic and environmental factors. Despite decades of research efforts, the specific mechanisms involved in mediating the elevation in blood pressure (BP) that characterizes hypertension are poorly understood. BP is regulated by sympathetic and parasympathetic nerve activity, circulating hormones and local autoregulatory mechanisms that interact to control cardiac output and vascular resistance. In addition, BP is modulated by the central integration of afferent neural and humoral inputs from the periphery. An increasing number of studies have demonstrated a critical role for the central nervous system in the development and maintenance of hypertension. In particular, increases in sympathetic nerve activity and alterations in arterial baroreflex function appear to contribute to the pathogenesis of this disease.

The development of hypertension in various animal models of hypertension, such as the spontaneously hypertensive rat (SHR), the renin transgenic (TGR mRen2) rat, the Dahl salt-sensitive rat and the deoxycorticosterone acetate (DOCA)-salt rat, is associated with increases in sympathetic activity (Takeda & Bunag, 1980; Arribas *et al*., 1996; Cabassi *et al*., 2002; Leenen *et al*., 2002). Increased sympathetic nerve activity would cause an elevation in BP via a direct vasoconstriction, and by increasing the force and rate of contraction of the heart. In addition, renal sympathetic nerve activity causes renin secretion that activates the systemic renin–angiotensin system (RAS) leading to angiotensin (Ang) II-induced vasoconstriction and antinatriuresis. Sustained increases in

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sympathetic outflow would also contribute to elevation of BP by causing trophic effects on vascular smooth muscle leading to increases in vascular resistance and enhanced responses to vasoconstrictor stimuli.

Alteration in arterial baroreflex function has also been implicated in the development of hypertension. The arterial baroreflex responds to changes in BP detected by carotid sinus and aortic arch baroreceptors, by modulating parasympathetic and sympathetic nerve activity and, hence, heart rate and vascular tone. This minimizes fluctuations in BP and maintains it close to a particular set point. In response to a static increase in BP, the baroreflex rapidly resets towards a higher pressure (Andresen & Yang, 1989). In hypertensive conditions, resetting of the operational point of the arterial baroreflex may therefore contribute to maintaining an increased BP rather than opposing it. In SHR, Lyon hypertensive rats, TGR mRen2 rats, DOCA-salt rats and Dahl salt-sensitive rats, the gain of the baroreflex, a measure of the baroreceptor reflex sensitivity, is decreased compared to normotensive controls resulting in blunted baroreflex control of heart rate and sympathetic nerve activity (Miyajima & Bunag, 1986; Hayashi *et al*., 1988; Nakamura *et al*., 1988; Lantelme *et al*., 1998; Borgonio *et al*., 2001). In many of these models, the impairment in baroreflex function precedes the development of hypertension and may therefore contribute to the development and maintenance of hypertension.

Similar to animal models of hypertension, primary human hypertension is also associated with increases in sympathetic activity and blunted arterial baroreflexes. Patients in the early phase of hypertension exhibit elevated plasma noradrenaline (NA) levels (Goldstein, 1981) and increased cardiac and renal NA spillover (Esler *et al*., 1988). While noncentral mechanisms such as facilitation of NA release (Floras, 1992) and impairment of neuronal NA reuptake (Rumantir *et al*., 2000) contribute to increased NA spillover, centrally mediated increases in sympathetic nerve outflow appear to play an important role. Microneurograph recordings of the peroneal nerve (Anderson *et al*., 1989; Grassi *et al*., 1998) and single-unit recordings (Grassi *et al*., 1998) in hypertensive patients indicate increased sympathetic nerve discharge, supporting the concept of a central neural mechanism for sympathetic activation in hypertension. In addition, in untreated hypertensive patients, spectral analysis of heart rate variability reveals an enhanced low-frequency component (0.1 Hz), which is considered a marker of sympathetic activity (Guzzetti *et al*., 1988). Furthermore, the efficacy of centrally acting antihypertensive agents such as clonidine also implicates a central mechanism in primary human hypertension. In both borderline hypertensives and hypertensive patients, the baroreflex control of heart rate and muscle sympathetic nerve activity is blunted and the gain of the baroreflex is decreased (Matsukawa *et al*., 1991a, b). While these studies implicate central mechanisms of sympathetic activation in the pathogenesis of hypertension, particular areas/nuclei involved are yet to be identified.

In hypertensive animals, functional changes within the central nervous system have been detected largely in hypothalamic and medullary areas that modulate sympathetic outflow (Colombari *et al*., 2001; De Wardener, 2001). A large number of neurotransmitters and neuromodulators contribute to regulating sympathetic outflow. Our research efforts have focused on a neuropeptide, Ang II, as increased expression and activity of components of the intrinsic brain RAS play a key role in many forms of experimental hypertension including the SHR, the TGR mRen2 rat, the Dahl salt-sensitive rat, the DOCA-salt rat and renal hypertensive rats (Itaya *et al*., 1986; Berecek *et al*., 1987; Huang & Leenen, 1998; Fontes *et al*., 2000; De Wardener, 2001; Kagiyama *et al*., 2001; Park & Leenen, 2001). Some of these models, including the SHR, exhibit both a hyperactive endocrine RAS, characterized by elevated circulating Ang II, as well as a hyperactive tissue RAS, characterized by elevated tissue Ang II. Within the brain, Ang II contributes to cardiovascular regulation *via* its action at various hypothalamic and medullary areas to enhance sympathetic outflow, blunt the sensitivity of the baroreflex and stimulate secretion of vasopressin (Culman *et al*., 1995; Averill & Diz, 2000; McKinley *et al*., 2001; Dampney *et al*., 2002). This article provides a brief overview of the brain RAS with emphasis on recent developments in this field. We then critically review recent studies that have advanced our understanding of the dysfunction of the brain RAS that underlies altered central BP regulation during the development and maintenance of hypertension. We will also discuss the cellular and molecular basis of Ang II signal transduction that may contribute to hypertension. Finally, we outline future directions that one must consider in advancing the field of central BP regulation from the experimental to the therapeutic level.

Altered regulation of the brain RAS in hypertension

Overview of the brain RAS

The RAS is an enzymatic cascade by which angiotensinogen is cleaved by renin and then by angiotensin converting enzyme (ACE) to produce Ang II, and subsequently cleaved by aminopeptidases to form other Angs. The brain expresses genes that encode all components of the RAS including angiotensin type I (AT_1) and type II (AT_2) receptors (Lenkei *et al*., 1997; Phillips & Sumners, 1998). A notable difference between the brain RAS and the systemic RAS is that the renin transcript predominantly expressed in the brain contains an alternate first exon (Lee-Kirsch *et al*., 1999; Clausmeyer *et al*., 2000; Sinn & Sigmund, 2000). This transcript encodes a truncated renin isoform that is predicted to be intracellular as it lacks the prefragment that targets to the secretory pathway. Recently, a receptor for prorenin/renin that appears to increase the efficiency of angiotensinogen cleavage to Ang I has been identified (Nguyen *et al*., 2002). It is highly expressed in tissues in which an intrinsic RAS has been described, including the brain, and may play an important physiological role in these tissues where angiotensinogen and renin concentrations are much lower than in plasma. Although Ang II actions are the best characterized, a role for Ang III (Ang 2 – 8) in cardiovascular regulation is emerging and it has been suggested that conversion of Ang II to Ang III is required for central Ang II actions (Reaux *et al*., 2001). Irrespective of the ligand (Ang II or Ang III), almost all the central actions on BP regulation and fluid homeostasis are mediated by the AT_1 receptor (Llorens-Cortes & Mendelsohn, 2002). Accordingly, within the brain, high densities of the AT_1 receptor are

distributed in cardiovascular regulatory areas including the lamina terminalis, that is, the subfornical organ (SFO), median preoptic nucleus (MnPO) and organum vasculosum laminae terminalis, the paraventricular nucleus (PVN), lateral parabrachial nucleus, ventrolateral medulla and the nucleus tractus solitarius (NTS) (Obermuller *et al*., 1991; Tsutsumi & Saveedra, 1991; Phillips *et al*., 1993; Allen *et al*., 1999). In humans, a single gene on chromosome 3 encodes the AT_1 receptor (Curnow *et al*., 1992) whereas two AT_1 receptor subtypes, AT_{1A} and AT_{1B}, encoded by distinct genes on different chromosomes have been identified in rodents (Murphy *et al*., 1991; Elton *et al*., 1992; Sandberg *et al*., 1992; Sasamura *et al*., 1992; Lewis *et al*., 1993). The AT_{1A} receptor is the predominantly expressed subtype in central cardiovascular regulatory areas in rodents (Lenkei *et al*., 1995), contributes to maintaining resting BP, and mediates pressor responses to central administration of Ang II (Davisson *et al*., 2000; Li *et al*., 2003). Although the AT_2 receptor is moderately expressed in certain nuclei involved in cardiovascular regulation such as the locus coeruleus, lateral septum and the medial amygdala (Song *et al*., 1992; Lenkei *et al*., 1997), its contribution to BP regulation in these areas remains elusive. However, functional evidence implicates a role for an AT_2 receptor-mediated contribution to baroreflex regulation (Lin *et al*., 1997; 2001; Luoh & Chan, 1998). Vasopressin release elicited by central administration of Ang II is mediated mainly by AT_1 receptors, but is also mediated by AT_2 receptors (Hogarty *et al*., 1992; Veltmar *et al*., 1992). An increase in endogenous Ang III also causes vasopressin release but the receptor that mediates this effect has not yet been identified (Zini *et al*., 1996). A receptor that is pharmacologically distinct from either AT_1 or AT_2 receptors appears to mediate Ang 1 – 7 actions on BP regulation, which in most cases oppose Ang II actions (Fontes *et al*., 1994; Couto *et al*., 2002). Thus, the broad function of the brain RAS in cardiovascular regulation is fairly well understood but its contribution to BP regulation in different areas/nuclei, and its altered regulation in hypertension is still a focus of much investigation.

Dysfunction of the brain RAS is implicated in the development of hypertension

The SHR has been the most widely used animal model for investigation of brain RAS dysfunction in hypertension. Increases in brain angiotensinogen expression precede the development of hypertension in SHR (Tamura *et al*., 1996), especially in the preoptic area where increases in angiotensinogen were apparent in 4-week-old SHR and increased with age (Shibata *et al*., 1993). In addition, renin-like activity in the anterior hypothalamus and NTS was higher in SHR compared to WKY rats during the development of hypertension (Ruiz *et al*., 1990). Ang II content and turnover within the hypothalamus, and Ang II immunoreactivity within the PVN and supraoptic nucleus, is also increased in adult SHR compared to their normotensive WKY control rats (Weyhenmeyer & Phillips, 1982; Ganten *et al*., 1983; Hermann *et al*., 1984; Phillips & Kimura, 1988). SHR also exhibit increased density of Ang II binding sites within the MnPO, SFO, PVN and NTS, and AT_{1A} receptor mRNA within the preoptic area compared to WKY rats (Gutkind *et al*., 1988; Komatus *et al*., 1996). Furthermore, a recent study demonstrated increased cellular AT_1 receptor density within the RVLM in SHR *versus* WKY rats (Hu *et al*., 2002). Pressor responses to intracerebroventricular (i.c.v.) injection of Ang II, or microinjections of Ang II within the preoptic area, NTS and RVLM, and depressor effects within the CVLM, are also correspondingly greater in SHR *versus* WKY rats, indicating increased receptor density and/or sensitivity in the SHR (Casto & Phillips, 1985; Matsuda *et al*., 1987; Wright *et al*., 1987; Muratani *et al*., 1991; Zhu *et al*., 1998). Thus, it can be concluded from these studies that a hyperactivity of the brain RAS precedes and/or parallels the development of hypertension in the SHR.

Studies utilizing pharmacological agents have provided evidence that the hyperactivity of the brain RAS mediates hypertension. Blockade of activity of the brain RAS utilizing injections of ACE inhibitors such as captopril, or an AT_1 receptor antagonist, losartan, into the lateral ventricle, decreases BP in SHR and TGR mRen2 rats as well as other models of hypertension, but does not affect BP in their normotensive controls (Hutchinson *et al*., 1980; Faber & Brody, 1984; Itaya *et al*., 1986; Berecek *et al*., 1987; Teruya *et al*., 1995; Szczepanska-Sadowska *et al*., 1998; Park & Leenen, 2001). Furthermore, microinjections of losartan into the anterior hypothalamic area also result in a decrease in BP in SHR (Yang *et al*., 1992). The depressor effect following inhibition of brain RAS activity in hypertensive rats appears to be mainly due to a decrease in sympathetic activity (Berecek *et al*., 1987; Huang & Leenen, 1998). These studies indicate that a hyperactivity of the brain RAS mediates hypertension in SHR and other hypertensive models. The role of the brain RAS in maintaining BP in normotensive rats is less clear as there are discrepancies in the literature, with some reports suggesting a tonic role for Ang peptides in maintaining resting BP (Ito & Sved, 1996; Tagawa *et al*., 1999; Fontes *et al*., 2000).

Antisense gene targeting of components of the RAS within the brain has provided further support for the view that a hyperactive brain RAS mediates hypertension. Inhibition of brain RAS activity by i.c.v. administration of antisense oligonucleotides to angiotensinogen or AT_1 receptor mRNA resulted in a short-term decrease in BP in SHR, whereas it had no effect in WKY rats (Wielbo *et al*., 1995; Kagiyama *et al*., 1999). Adeno-associated virus vector-mediated delivery of AT_1 receptor antisense oligonucleotides, either i.c.v. or directly into the hypothalamus, extended the magnitude and duration of the depressor effect up to 9 weeks in SHR (Phillips *et al*., 1997). In addition, cross-breeding of transgenic rats that exhibit decreased glial angiotensinogen concentrations with TGR mRen2 rats results in a marked reduction in brain Ang content and BP possibly via a vasopressinergic mechanism (Schinke *et al*., 1999). Although studies employing PVN lesions or GABAergic blockade have implicated this nucleus in mediating hypertension via sympathetic activation (Ciriello *et al*., 1984; Takeda *et al*., 1991; Allen, 2002), antisense oligonucleotide inhibition of angiotensinogen mRNA within the PVN did not affect BP in SHR up to 24 h after injection despite decreased plasma catecholamine and vasopressin levels (Kagiyama *et al*., 1999). This suggests that Ang II within the PVN contributes to maintaining resting sympathetic activity and vasopressin release consistent with inhibition of Ang II effects mediated by parvocellular and magnocellular subdivisions of the PVN, but does not contribute significantly to maintaining hypertension in adult SHR. In TGR mRen2 rats,

AT_1 receptor antisense oligonucleotide administration into the PVN did not significantly alter BP, but abolished salt-induced exacerbation of hypertension in these rats (Li *et al*., 1996). Overall, these findings have confirmed that hyperactivity of the brain RAS contributes to maintaining hypertension. Overactivity of the RAS within the PVN alone does not account for this effect but contributes to salt-induced exacerbation of hypertension.

Studies employing gene transfer or transgenic techniques to overexpress RAS components in a tissue-specific or cell type-specific manner in normotensive rats or mice have provided further insight into brain RAS dysfunction in hypertension, and a means to distinguish the functional contributions of the brain RAS from those of the systemic RAS. In normotensive rats, brain-specific *in vivo* gene transfer of the human ACE gene utilizing the hemagglutinating virus of Japan complexed with liposomes led to the widespread distribution of the transgene including expression in hypothalamic nuclei and in the ventral medulla (Nakamura *et al*., 1999). These rats exhibited elevated central Ang II levels and developed hypertension. In mice, overexpression of both the human angiotensinogen and the human renin genes under the control of either a glial fibrillary acidic protein promoter or a synapsin I promoter resulted in glia- or neuron-specific expression of the transgenes respectively (Morimoto *et al*., 2002). Both glia- and neuron-targeted double transgenic mice developed moderate hypertension, indicating that increased conversion of angiotensinogen to its active peptides within either glia or neurons results in hypertension. In the glia-targeted transgenic mice, AT_1 receptor activation and sympathetic activity but not vasopressin mechanisms mediated the hypertensive effect. Neuron-specific overexpression of the AT_{1A} receptor driven by a neuron-specific enolase promoter resulted in expression of the AT_{1A} transgene in neurons within cardiovascular as well as noncardiovascular regulatory areas in the brain with lower levels of expression in the adrenal medulla (Lazartigues *et al*., 2002). Resting BP was normal in the AT_{1A} transgenic mice, indicating that an increase in AT_{1A} receptors by itself does not result in hypertension. In these transgenic mice, central AT_1 receptor blockade decreased BP whereas it did not affect BP in nontransgenic mice, indicating that AT_1 receptors contribute to resting BP only in mice with overexpression of AT_{1A} receptors. This study therefore suggests that an increase in the production of Ang II is required for the development of hypertension. Alternatively, effective baroreceptor buffering may prevent the development of hypertension in the AT_{1A} transgenic mice. While these studies were able to limit expression of the transgene to a particular cell type within the CNS, further studies that limit transgene expression to particular areas/nuclei would be required to dissect the contribution of the brain RAS in different cardiovascular regulatory areas in the development of hypertension.

The precise mechanisms by which increased brain RAS activity results in hypertension is not yet known, but appear to involve increased sympathetic vasomotor tone. AT_1 receptors are associated with presympathetic vasomotor neurons in the RVLM (Dampney *et al*., 2002), the major source of excitatory supraspinal input to sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord. Bilateral microinjections of nonselective peptide Ang II receptor antagonists, (Sar^1, Thr^8)Ang II (sarthran) or (Sar^1, Ile^8)Ang II (sarile), into the RVLM result in profound decreases in sympathetic nerve activity and BP in normotensive rats (Ito & Sved, 1996; Tagawa *et al*., 1999). However, Lin et al. (1997, 2001) did not observe a decrease in BP in response to sarile microinjections into the RVLM for reasons that are unclear but may relate to differences in experimental conditions. Similar to findings in normotensive rats, microinjections of sarthran in SHR decreased BP to levels expected with total autonomic blockade (Ito *et al*., 2002). The depressor effect of sarthran does not appear to be mediated by AT_1, AT_2 or Ang 1 – 7 receptors (Hirooka *et al*., 1997; Ito & Sved, 2000; Potts *et al*., 2000), raising questions regarding the endogenous ligand and receptor(s) that mediate this action. In anesthetized normotensive rats, AT_1 receptor blockade within the RVLM does not significantly alter sympathetic nerve activity or BP (Averill *et al*., 1994; Fontes *et al*., 1994; Potts *et al*., 2000). However, a minor role for AT_1 receptors in contributing to resting activity of RVLM neurons is unmasked following blockade of tonic GABAergic inhibition (Tagawa *et al*., 2000). In contrast, in anesthetized SHR, bilateral RVLM microinjections of an AT_1 receptor antagonist, candersartan, resulted in large decreases in BP and sympathetic nerve activity that were not observed in their normotensive controls (Allen, 2001). A similar decrease in BP was elicited by microinjections of valsartan, another AT_1 receptor antagonist, into the RVLM in SHR, but not WKY rats (Ito *et al*., 2002). Furthermore, in conscious TGR mRen2 rats, bilateral RVLM microinjections of an AT_1 receptor antagonist resulted in a decrease in BP (Fontes *et al*., 2000). Thus, in contrast to normotensive rats, AT_1 receptor stimulation within the RVLM contributes to the enhanced tonic sympathoexcitatory activity of RVLM vasomotor neurons in hypertensive rats. This finding is consistent with a study that demonstrated an upregulation of AT_1 receptors within the RVLM in SHR (Hu *et al*., 2002). The input to AT_1 receptors in the RVLM appears to be from the PVN as disinhibition of the PVN resulted in an augmented decrease in BP in SHR compared to WKY rats, and this effect was eliminated by AT_1 receptor blockade within the RVLM (Ito *et al*., 2002). In SHR, RVLM vasomotor neurons are subject to decreased tonic GABAergic inhibition and enhanced excitatory amino-acid input compared to normotensive controls (Smith & Barron, 1990; Ito *et al*., 2000). Combined blockade of RVLM ionotropic excitatory amino-acid receptors and AT_1 receptors in SHR resulted in an additive effect (Ito *et al*., 2002), suggesting actions via independent mechanisms. Alternatively, a single mechanism that augments both excitatory amino-acid input and AT_1 receptor stimulation of RVLM neurons and results in sympathoexcitation and hypertension cannot be ruled out.

There is functional evidence for the presence of Ang 1 – 7 receptors in the RVLM as RVLM microinjections of Ang 1 – 7 increase BP (Fontes *et al*., 1994). Conversely, blockade of endogenous Ang 1 – 7 actions within the RVLM resulted in a decrease in BP in both anesthetized and conscious normotensive rats, suggesting a role for Ang 1 – 7 in maintaining BP (Fontes *et al*., 1994; 1997). In conscious TGR mRen2 rats, RVLM microinjections of an Ang 1 – 7 antagonist resulted in augmented decreases in BP compared to normotensive rats (Fontes *et al*., 2000). Thus, in TGR mRen2 rats, endogenous Ang 1 – 7 actions within the RVLM contribute to maintaining hypertension.

Increased brain RAS activity may also contribute to alterations in baroreflex function. Afferent baroreceptor input to the NTS inhibits sympathetic activity via a multisynaptic pathway involving an excitatory projection from the NTS to the CVLM, a subsequent inhibitory projection to the RVLM, and, lastly, an excitatory projection from the RVLM to sympathetic preganglionic neurons in the IML. Blockade of central AT_1 receptors utilizing i.c.v. infusions of losartan facilitated baroreflex control of heart rate in both normotensive rats and SHR, whereas i.c.v. infusion of an Ang 1–7 antagonist blunted baroreflex sensitivity of normotensive rats, but did not significantly alter the depressed baroreflex sensitivity of SHR (Oliveira *et al*., 1996). This study suggests that stimulation of AT_1 receptors and Ang 1–7 actions have opposing effects on the baroreflex response, causing a decrease or increase in baroreflex gain respectively. The blunted baroreflex sensitivity in SHR may relate in part to diminished central actions of Ang 1–7. Within the NTS, AT_1 receptor blockade facilitates baroreflex control of heart rate and, conversely, AT_1 receptor activation by exogenous Ang II depresses both sympathetic and vagal components of baroreflex-induced bradycardia, indicating that Ang II decreases baroreflex gain (Casto & Phillips, 1985; Campagnole-Santos *et al*., 1988; Kasparov *et al*., 1998; Matsumura *et al*., 1998; Boscan *et al*., 2001). Ang II-induced suppression of the baroreflex response was attenuated by inhibition of conversion of Ang II to Ang III, suggesting that part of the action of Ang II may result from conversion to Ang III (Luoh & Chan, 1998). While Ang II mediates its effects via AT_1 receptors, it appears that Ang III acts on both AT_1 and AT_2 receptors to depress the baroreflex response (Luoh & Chan, 1998). SHR and TGR mRen2 rats exhibit high medullary Ang II content and increased Ang II binding densities within the NTS (Gutkind *et al*., 1988; Senanayake *et al*., 1994; Morishita *et al*., 1995). Baroreflex control of heart rate in these hypertensive models is blunted compared to control rats and may be reversed by AT_1 receptor blockade within the NTS (Matsumura *et al*., 1998; Diz *et al*., 2002), indicating that increased AT_1 receptor stimulation within the NTS results in blunting of the baroreflex in these hypertensive models. Ang 1–7 actions in the NTS also appear to contribute to baroreflex modulation exerting a tonic facilitatory role on the bradycardic component (Chaves *et al*., 2000). In SHR, sensitivity to Ang 1–7 within the NTS is decreased and may therefore contribute to the blunted baroreflex control in this model (Chaves *et al*., 2000). In addition, Colombari *et al*. (2001) have suggested that enhanced chemoreceptor afferent input to the commissural NTS in SHR contributes to enhanced RVLM vasomotor neuron activity and may therefore contribute to hypertension in this model.

In summary, studies utilizing different approaches and animal models have verified that a hyperactive brain RAS mediates hypertension. Increased brain RAS activity may result in the development and maintenance of hypertension via both AT_1 receptor activation and endogenous Ang 1–7 actions on RVLM vasomotor neurons. In addition, AT_1 receptor-mediated suppression of afferent baroreceptor feedback as well as a reduced sensitivity to Ang 1–7 within the NTS may also contribute to the development of hypertension.

Cellular and molecular basis for brain RAS dysfunction in hypertension

In spite of identification and characterization of mechanisms that contribute to dysfunction of the brain RAS in hypertension, little is known about the cellular and molecular basis of this dysfunction. Central AT_1 receptor-mediated actions of Ang II are mediated by facilitation of excitatory transmission via catecholamines (Jenkins *et al*., 1996; Raizada *et al*., 1999), glutamate (Ferguson *et al*., 2001) and substance P (Paton & Kasparov, 1999; Diz *et al*., 2002), as well as enhanced inhibitory GABAergic neurotransmission (Paton *et al*., 2001). A number of studies have provided evidence that the pressor effects of Ang II are mediated by noradrenergic neurotransmission. AT_1 receptors are localized on noradrenergic neurons in specific brain nuclei/areas (Yang *et al*., 1997). Central Ang II administration enhances NA release in the PVN in parallel to the pressor response (Stadler *et al*., 1992), and increases tyrosine hydroxylase (TH)mRNA and activity in the hypothalamus and brainstem (Yu *et al*., 1996). In addition, central catecholaminergic depletion or noradrenergic antagonist administration prevents the pressor response to central Ang II, indicating that the Ang II-induced pressor response is mediated by NA (Camacho & Phillips, 1981; Jones, 1984; Bellin *et al*., 1987). In hypertensive SHR, Ang II-induced increases in TH activity and mRNA within the hypothalamus and brainstem are enhanced compared to WKY rats (Yu *et al*., 1996). Enhanced Ang II-induced NA neuromodulation and alterations in AT_1 signal transduction mechanisms that mediate the NA neuromodulation may therefore contribute to hypertension in the SHR.

AT_1 receptor signal transduction and alterations in hypertension

We have utilized primary neuronal cultures derived from the hypothalamus and brainstem of newborn prehypertensive SHR and normotensive WKY rats to elucidate Ang II-mediated signal transduction pathways and identify differences that could contribute to the overactive brain RAS in the SHR. Similar to Ang receptor subtype expression *in vivo*, SHR neuronal cultures exhibit increased AT_1 receptor gene expression and functional receptors compared to WKY cultures (Raizada *et al*., 1984; 1993). A large percentage of the AT_1 receptors are localized on catecholaminergic neurons (Raizada *et al*., 1999). Ang II, via AT_1 receptor-mediated signaling events, rapidly results in release of NA in these neuronal cultures, which we have termed an evoked response (Raizada *et al*., 1999). Persistent AT_1 receptor stimulation results in increased activity and transcription of catecholamine synthesizing enzymes such as TH and dopamine β-hydroxylase (D-βH), and the NA transporter (NAT), that is, enhanced responses to Ang II (Raizada *et al*., 1999). These observations are consistent with Ang II-induced NA synthesis and release observed following central injections *in vivo* (Sumners & Phillips, 1983; Stadler *et al*., 1992; Yu *et al*., 1996). In SHR neuronal cultures, Ang II-induced NA release and increases in NAT and TH transcription, and TH activity are enhanced (Lu *et al*., 1996b; Raizada *et al*., 1996), consistent with augmented *in vivo* Ang II stimulation of TH activity and mRNA in adult (hypertensive) SHR (Yu *et al*., 1996). These neuronal cultures therefore provide a simple *in vitro* system in which we are able

to mimic augmented Ang II actions on NA modulation in adult SHR, and preclude nonspecific influences that would confound results obtained from studies in the intact brain.

Similar to AT_1 receptor signaling in peripheral tissue (Inagami & Eguchi, 2000; Touyz & Schiffrin, 2000; Sayeski & Bernstein, 2001), brain AT_1 receptors appear to signal via multiple pathways. In neuronal cultures, Ang II, *via* the AT_1 receptor, activates Gq, increases phospholipase C (PLC) activity and phosphoinositide (PI) hydrolysis, resulting in increased cytosolic free Ca^{2+} and subsequent activation of Ca^{2+}-dependent enzymes such as protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII) (Raizada *et al*., 1999). Seltzer *et al.* (1995) reported an AT_1 receptor-mediated increase in PI turnover in the median eminence providing support for this pathway *in vivo*. Neuronal AT_1 receptors also signal *via* a PLC-dependent Ras – Raf-mitogen-activated protein (MAP) kinase pathway (Raizada *et al*., 1999). Activation of this pathway is essential for Ang II-induced increases in transcription of TH, D-βH and the NAT, and TH activity (Yang *et al*., 1996; Yang & Raizada, 1998; Figure 1a). Similar to Ang II, Ang III also increases TH activity and NA release *via* an AT_1 receptor – PLC-dependent pathway in rat hypothalamic tissue (Rodriguez-Campos *et al*., 2000).

Furthermore, Ang II induces transcription factors such as Fos and Jun by increasing *c-fos* and *c-jun* gene expression respectively (Yang *et al*., 1996; Sumners *et al*., 2002), and increases activity of Fos-regulating kinase (FRK) and c-Jun NH_2-terminal kinase (JNK) (Huang *et al*., 1998) which phosphorylate Fos and Jun prior to the formation of a fully active activating protein 1 (AP1) complex and translocation to the nucleus. In addition, AT_1 receptor activation results in increased binding activity at AP1 binding sites (Lu *et al*., 1996a; Figure 1a). We have therefore proposed that Ang II-induced transcription of TH, D-βH and NAT results from the interaction of the active AP1 transcription complex with AP1 binding sites in the promotor regions of these genes (Raizada *et al*., 1999). The production of Fos and Jun is mediated by MAP kinase activity, whereas the activity of FRK and JNK is dependent on PKC and phosphoinositide-3-kinase (PI3K) respectively (Yang *et al*., 1996; Huang *et al*., 1998; Sumners

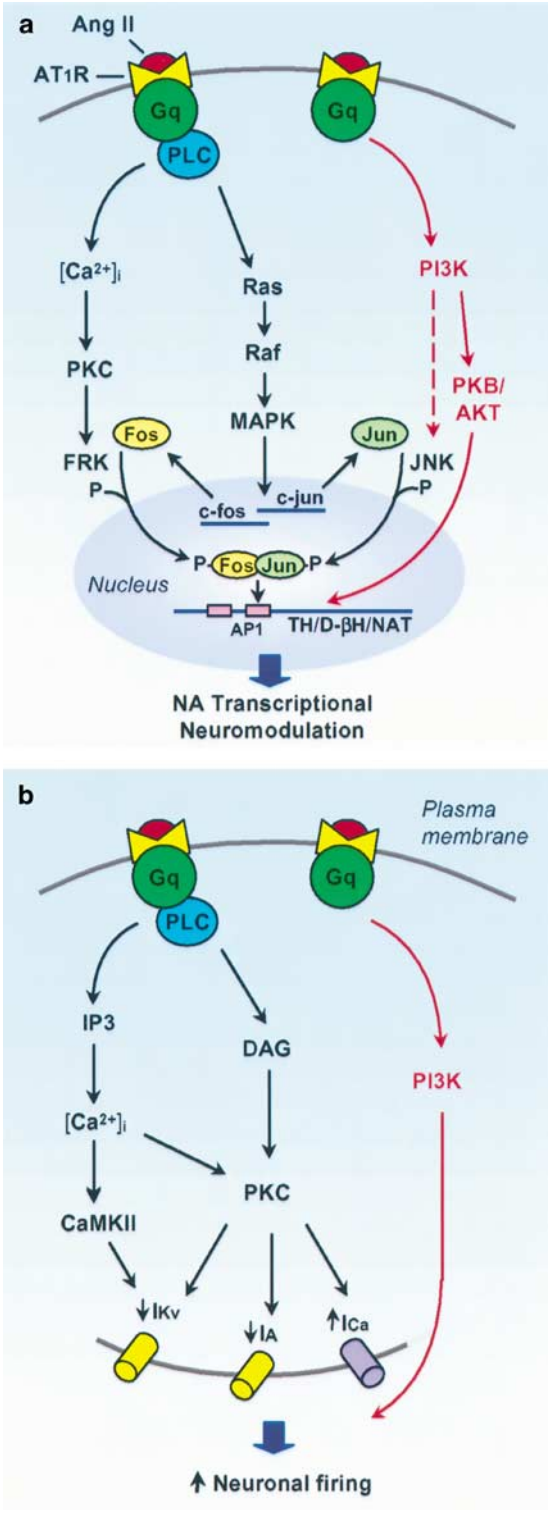

Figure 1 AT_1 receptor signal transduction pathways that result in transcriptional NA neuromodulation (a) and increased firing rate (b) in neuronal cultures. (a) AT_1 receptor activation increases the transcription of TH, D-βH and the NAT via a PLC-dependent Ras – Raf – MAP kinase pathway. This induces Fos and Jun by increasing *c-fos* and *c-jun* gene expression. PKC and PI3K mediate FRK and JNK activity, respectively, which in turn phosphorylate Fos and Jun, resulting in transactivation of the AP1 transcription complex. Interaction of the AP1 complex with AP1 binding sites in the promotor regions of TH, D-βH and the NAT results in transcription of these genes. A PI3K – PKB/Akt pathway (highlighted in red) uniquely mediates the augmented transcriptional NA neuromodulation in SHR neurons. Increased AP1 nuclear binding in SHR neurons may be due to enhanced PI3K-mediated JNK activity, resulting in subsequent increases in Jun phosphorylation and AP1 binding. (b) AT_1 receptor activation inhibits I_{Kv} and I_A, and stimulates I_{Ca} resulting in increased neuronal firing rate via PLC-mediated activation of PKC. PLC also activates CaMKII, which contributes to I_{Kv} inhibition. An additional signaling pathway that utilizes PI3K (pathway highlighted in red) mediates the enhanced Ang II-induced neuronal firing response in SHR neurons. Ang, angiotensin; AP1, activating protein 1; AT_1R, angiotensin type 1 receptor; $[Ca^{2+}]_i$, intracellular calcium; CaMKII, calcium/calmodulin-dependent protein kinase II; DAG, diacylglycerol; D-βH, dopamine β-hydroxylase; FRK, Fos-regulating kinase; I_A, transient A type K^+ current; I_{Ca}, total Ca^{2+} current; I_{Kv}, delayed rectifier K^+ current; IP_3, inositol 1,4,5-triphosphate; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; NA, noradrenaline; NAT, NA transporter; PI3K, phosphoinositide-3-kinase; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; TH, tyrosine hydroxylase.

et al., 2002). It therefore appears that diverse signaling pathways are involved in the regulation of Ang II-induced NA neuromodulation, and altered regulation of one or more of these pathways may result in the augmented NA neuromodulation observed in the SHR.

Despite augmented Ang II-mediated NA neuromodulation in SHR neuronal cultures, AT_1 receptor-mediated regulation of the MAP kinase signaling pathway (Yang & Raizada, 1998) and induction of Fos and Jun are comparable in SHR and WKY neuronal cultures (Lu *et al*., 1996a). Similarly, i.c.v. injections of Ang II caused a comparable stimulation of MAP kinase phosphorylation in the hypothalamus and brainstem of adult WKY and SHR (Yang & Raizada, 1998). In addition, bilateral microinjection of a MAP kinase inhibitor into the RVLM caused a similar percentage decrease in BP in SHR and WKY rats (Seyedabadi *et al*., 2001). These studies indicate that the MAP kinase pathway contributes to NA neuromodulation and BP regulation to a similar extent in both strains. However, Ang II-mediated AT_1 receptor phosphorylation is greater in SHR compared to WKY neurons and was demonstrated to be MAP kinase dependent (Yang *et al*., 1997). The reason for this discrepancy is not clear. Despite similar Ang II induction of Fos and Jun in WKY and SHR neuronal cultures, i.c.v. Ang II injections cause a greater increase in expression of Fos, Jun and Krox-24 in areas involved in cardiovascular homeostasis and osmoregulation such as the SFO, OVLT, PVN and the NTS, in hypertensive SHR compared to WKY rats (Blume *et al*., 1997). Whether enhanced Ang II-induced expression of transcription factors in the adult SHR relates to the enhanced transcriptional NA neuromodulation in response to Ang II in this model of hypertension is yet to be determined.

In SHR neurons, in addition to the Ras – Raf MAP kinase pathway, a MAP kinase-independent signaling pathway that involves PI3 K appears to modulate enhanced Ang II-mediated NA neuromodulation (Figure 1a, highlighted in red). Although AT_1 receptor activation results in comparable ($\sim$3.6-fold) stimulation of PI3 K activity in WKY and SHR neuronal cultures, its effect in SHR neurons is more persistent (Yang & Raizada, 1999). In WKY cultures PI3K activity returns to control values within 30 min following Ang II stimulation whereas in SHR cultures a two-fold stimulation in activity was detected even an hour after Ang II treatment. In addition, the rate of nuclear translocation of PI3 K is greater in SHR *versus* WKY neuronal cultures.

Consistent with Ang II stimulation of PI3 K activity in neuronal cultures, central Ang II injections in adult SHR caused more persistent increases in hypothalamic PI3K activity compared to WKY rats (Yang & Raizada, 1999). PI3K inhibition resulted in an $\sim$50% attenuation of Ang II-induced stimulation of TH and NAT mRNA and (^{3}H)NA uptake in SHR neuronal cultures whereas it had no effect in WKY neurons (Yang & Raizada, 1999). Furthermore, combined inhibition of MAP kinase and PI3K activity abolished Ang II-induced increases in TH and NAT mRNA in the SHR cultures. Ang II causes a five-fold greater induction of nuclear AP1 binding activity in SHR *versus* WKY neurons. PI3 K inhibition decreased AP1 binding activity in nuclear fractions of SHR neurons to levels similar to that in WKY neurons, whereas it had no effect on AP1 binding activity in WKY neurons (Yang & Raizada, 1999). Enhanced AP1 binding activity in SHR neuronal nuclear fractions may possibly be due to enhanced PI3K-mediated JNK activity, which would increase phosphorylation of Jun and may therefore result in increased AP1 binding. Consistent with enhanced PI3K signaling in SHR neurons, Ang II also causes an enhanced and more persistent stimulation of protein kinase B (PKB/Akt) activity (Yang & Raizada, 1999; Figure 1a, highlighted in red), an established downstream kinase in the PI3K signaling pathway. AT_1 receptor inhibition and PI3K inhibition abolished PKB/Akt stimulation, suggesting that the AT_1 receptor – PI3K pathway mediates PKB/Akt simulation (Yang & Raizada, 1999). These studies indicate that AT_1 receptor activation in SHR neurons causes enhanced PI3K-mediated AP1 binding activity and PKB/Akt activity, resulting in the augmented NA neuromodulation in SHR neurons. This PI3K pathway does not appear to mediate NA neuromodulation in WKY neurons, but has been implicated in Ang II-induced neuritogenesis (Yang *et al*., 2001).

Ang II-evoked NA release results from modulation of neuronal electrical activity via effects on membrane ion channels and their dependent currents. Ang II inhibits delayed rectifier K^+ current (I_{Kv}) and transient A type K^+ current (I_A), stimulates total Ca^{2+} current, and results in increases in neuronal firing rate in neuronal cultures (Sumners *et al*., 2002; Figure 1b). These effects are mediated by the AT_1 receptor as they occur in the presence of an AT_2 receptor antagonist and are abolished by losartan, an AT_1 receptor antagonist. These effects are consistent with the effects of Ang II on I_A and neuronal firing in SFO, PVN and SON neurons as well as on neuronal firing rate in MnPO and RVLM neurons in brain slices or *in situ* (Felix & Schlegel, 1978; Suga & Suzuki, 1979; Tasker & Dudek, 1991; Yang *et al*., 1992; Armstrong, 1995; Ferguson & Li, 1996; Li & Ferguson, 1996; Bai & Renaud, 1998). PKC inhibition attenuates Ang II-induced decreases in I_A and I_{Kv} and abolishes Ang II-evoked increases in total Ca^{2+} current, indicating that Ang II effects on these ion channel currents are either partly or wholly mediated by PKC (Sumners *et al*., 1996; Wang *et al*., 1997; Zhu *et al*., 1999). Studies utilizing anti-PKCα antibodies and PKCα antisense oligonucleotides implicate this isozyme in mediating Ang II-induced changes in I_{Kv} (Sumners *et al*., 2002).

Concurrent inhibition of PKC and CaMKII completely abolished Ang II effect on I_{Kv}, indicating that PKC and CaMKII mediate this effect (Zhu *et al*., 1999). Both PKC and CaMKII also mediate Ang II-induced increases in neuronal firing rate (Sun *et al*., 2002a, b).

In SHR cultures, AT_1 receptor activation results in an augmented increase in neuronal firing rate ($\sim$65%) compared to WKY neurons under the same conditions (Sun *et al*., 2002a, b). This is consistent with enhanced Ang II-induced depolarization and firing of RVLM neurons in neonatal SHR compared to WKY rats (Matsuura *et al*., 2002). PKC and CaMKII inhibitors completely blocked Ang II-induced firing in WKY neuronal cultures whereas it only attenuated it by $\sim$50% in SHR cultures, suggesting that an additional signaling pathway mediates the enhanced Ang II neuronal firing response in SHR neurons. Inhibition of PI3K attenuated Ang II-mediated neuronal firing in SHR but not in WKY neurons, indicating that a signaling pathway utilizing PI3K may mediate the augmented Ang II-induced response in SHR neurons (Sun *et al*., 2002a, b; Figure 1b, highlighted in red).

From the preceding discussion, it is apparent that both AT_1 receptor-mediated NA neuromodulation and increases in neuronal firing rate are partly mediated by PI3K in SHR but

not WKY neurons. It therefore appears that a signaling pathway involving PI3K may mediate the augmented Ang II-induced NA neuromodulation and neuronal firing in the SHR. PI3K-dependent NA neuromodulation and neuronal firing occurs in neurons from prehypertensive rats and is therefore due to an intrinsic difference in the SHR rather than a compensatory change in response to an increase in BP. The relevance of this pathway in BP regulation, and hence hypertension in this model, was demonstrated by Seyedabadi *et al*. (2001). Bilateral microinjections of a PI3K inhibitor into the RVLM of adult SHR resulted in an $\sim$35% decrease in BP, resulting in pressures similar to that in untreated or vehicle-treated WKY rats, but had no effect on BP in WKY rats. The decrease in BP in SHR was gradual (requiring hours), suggesting that PI3K mediates the elevated BP in SHR possibly via transcriptional or translational effects.

The PI3K inhibitor also blunted enhanced Ang II-induced pressor responses in SHR to levels comparable to responses in WKY rats. These studies therefore implicate a PI3K-dependent pathway in mediating the hypertension in SHR possibly via Ang II-evoked increases in neuronal firing and enhanced NA transcriptional neuromodulation.

Perspectives and future directions

In recent years, clear progress has been made in our understanding of the role of the brain RAS in the pathogenesis of hypertension. Genetic models of hypertension, such as the SHR, exhibit a hyperactive endocrine and tissue RAS, making it difficult to distinguish between the relative contributions of the two systems in functional studies. Earlier transgenic models have also been plagued by the same limitation. Recent advances in antisense strategies, *in vivo* gene transfer and transgenic techniques have enabled the development of models that exhibit brain- or nuclei/area-specific gain or loss of function. Studies utilizing these techniques have helped to clarify the role of specific components of the brain RAS in mediating hypertension. Progress has also been made in understanding the altered cellular and molecular basis for brain RAS dysfunction in hypertension. Studies have demonstrated that augmented Ang II-induced NA neuromodulation and hypertension is uniquely mediated by a PI3K-dependent signaling pathway. One of our challenges is to utilize our understanding of the brain RAS dysfunction in hypertension for the development of improved therapeutic intervention in hypertension. For example, the PI3K-mediated NA neuromodulation may present a potential target for brain/cell type-specific targeted genetic regulation using gene transfer techniques. Another challenge is to identify other genes whose expression is also uniquely linked to the development of hypertension. The development of high-throughput microarray gene profiling technology has enabled the assessment of the expression of a large number of genes within a single experiment. This technology provides tremendous potential for the elucidation of genetic regulation of signaling pathways and identification of target genes that are activated or repressed by the brain RAS. We are currently using this approach to identify genes that encode intracellular signaling proteins and target genes that are regulated by the brain RAS and contribute to the altered responsiveness to Ang II in hypertensive models (Francis *et al*., 2001; Veerasingham *et al*., 2002). The combination of microarray gene profiling with validation of the altered expression of identified genes, cellular and *in vivo* physiological approaches will further our understanding of brain RAS dysfunction in hypertension. Using this approach we have identified a decrease in central γ-adducin expression in models of hypertension that express a hyperactive brain RAS (Yang *et al*., 2002). Further studies will be required to determine whether this decrease in γ-adducin contributes to the development of hypertension in these models. The identification of genes that are uniquely linked to the development or maintenance of hypertension would provide novel targets of potential therapeutic value for the control, and possibly the cure of hypertension.

This work was supported by the National Heart, Lung, and Blood Institute Grant 33610. S.J.V. was supported by a fellowship from the Canadian Institutes of Health Research.

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(Received February 18, 2003
Accepted February 27, 2003)