Central neuromodulatory pathways regulating sympathetic activity in hypertension

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Gabor A, Leenen FH. Central neuromodulatory pathways regulating sympathetic activity in hypertension. J Appl Physiol 113: 1294–1303, 2012. First published July 5, 2012; doi:10.1152/japplphysiol.00553.2012.—The classical neurotransmitters, glutamate and GABA, mediate fast (milliseconds) synaptic transmission and modulate its effectiveness through slow (seconds to minutes) signaling processes. Angiotensinergic pathways, from the lamina terminalis to the paraventricular nucleus (PVN)/supraoptic nucleus and rostral ventrolateral medulla (RVLM), are activated by stimuli such as circulating angiotensin type II (Ang II), cerebrospinal fluid (CSF) sodium ion concentration ([Na⁺]), and possibly plasma aldosterone, leading to sympathoexcitation, largely by decreasing GABA and increasing glutamate release. The aldosterone-endogenous ouabain (EO) pathway is a much slower neuromodulatory pathway. Aldosterone enhances EO release, and the latter increases chronic activity in angiotensinergic pathways by, e.g., increasing expression for Ang I receptor (AT1R) and NADPH oxidase subunits in the PVN. Blockade of this pathway does not affect the initial sympathoexcitatory and pressor responses but to a large extent, prevents chronic responses to CSF [Na⁺] or Ang II. Recruitment of these two neuromodulatory pathways allows the central nervous system (CNS) to shift gears to rapidly cause and sustain sympathetic hyperactivity in an efficient manner. Decreased GABA release, increased glutamate release, and enhanced AT1R activation in, e.g., the PVN and RVLM contribute to the elevated blood pressure in a number of hypertension models. In Dahl S rats and spontaneous hypertensive rats, high salt activates the CNS aldosterone-EO pathway, and the salt-induced hypertension can be prevented/reversed by specific CNS blockade of any of the steps in the cascade from aldosterone synthase to AT1R. Further studies are needed to advance our understanding of how and where in the brain these rapid, slow, and very slow CNS pathways are activated and interact in models of hypertension and other disease states associated with chronic sympathetic hyperactivity.

IT IS WELL ESTABLISHED THAT the classical neurotransmitters, glutamate and GABA, are the primary neurotransmitters by which neurons in the brain communicate. In fast synaptic transmission, nerve cells use these neurotransmitters to rapidly (milliseconds) send information between nerve cells. Paul Greengard was awarded the Nobel prize for, among others, showing that nerve cells also communicate with each other using slow synaptic transmission. Slow transmission is mediated through more complicated, slower signaling, involving secondary messengers, protein kinases, and phosphatases (36). This neuromodulation modulates fast synaptic transmission by increasing the efficacy of neurotransmitter release from the nerve terminal or the extent of the responses in the postsynaptic neuron (Fig. 1). In this review, we address two major neuromodulatory pathways involved in long-term regulation of sympathetic activity by altering classical neurotransmitter and neuropeptide synaptic transmission and review their role in the pathophysiology of hypertension.

FAST AND SLOW NEUROTRANSMISSION BY GABA AND GLUTAMATE

An action potential arriving at a nerve terminal releases glutamate or GABA into the synaptic cleft, which then binds to ligand-gated, fast-acting ionotropic receptors, changing their conformation and causing their ion channel to open in <1/1,000 of a second. Upon opening, ionotropic AMPA receptors allow sodium ion (Na⁺) to flow into the cell rapidly, thereby initiating an increase in membrane potential in the postsyn-
activity of voltage-gated Ca\textsuperscript{2+} channels, a slower intracellular signaling pathway that inhibits the increase in chloride ion (Cl\textsuperscript{−}). GABA binding to ionotropic GABA receptors, AMPA receptors, pushes Mg\textsuperscript{2+} out of the pore, further increasing Na\textsuperscript{+} conductance and strengthening the rise in membrane potential (20). GABA binding to ionotropic GABA receptors, i.e., GABA\textsubscript{A}, also rapidly opens their ion pore, causing an increase in chloride ion (Cl\textsuperscript{−}) conductance, thereby hyperpolarizing the neuron and inhibiting action potentials. Greengard first demonstrated that these classical neurotransmitters also activate secondary messengers that 1) modulate the quantity of neurotransmitter release from the presynaptic terminal or 2) regulate the responsiveness of postsynaptic neurons to neurotransmitters by modulating the sensitivity or quantity of receptors on the postsynaptic neuron (35). For example, ionotropic NMDA receptors are also permeable to calcium ion (Ca\textsuperscript{2+}). A transient increase in Ca\textsuperscript{2+} stimulates a slower pathway, i.e., seconds to minutes, which activates protein kinases, leading to phosphorylation of AMPA receptor subunits (62a), thereby increasing their open probability. Both glutamate and GABA metabotropic receptors also activate slower intracellular signaling pathways. Glutamate binding to metabotropic glutamate receptors stimulates a number of intracellular pathways that enhance synaptic transmission (86). Similarly, GABA binding to the GABA\textsubscript{B} metabotropic G-protein-coupled receptor activates G\textsubscript{B\gamma} subunits, thereby stimulating a slower intracellular signaling pathway that inhibits the activity of voltage-gated Ca\textsuperscript{2+} channels, decreasing Ca\textsuperscript{2+} influx and release of excitatory neurotransmitters from presynaptic terminals (21, 107).

Both glutamate and GABA as well as their receptors are localized in nuclei involved in the neural control of sympathetic drive, particularly the lamina terminalis (LT), i.e., subfornical organ (SFO), organum vasculosum of the LT (OVLT), and median preoptic nucleus (MnPO), as well as the anterior hypothalamic area, paraventricular nucleus (PVN), and rostral ventrolateral medulla (RVLM). Microinjection of glutamate or an analog into the MnPO (70), PVN (57), or RVLM (55) within seconds increases sympathetic nerve activity (SNA), blood pressure (BP), and heart rate (HR). Conversely, microinjection of a GABA receptor agonist into the PVN (2) or RVLM (55) within seconds inhibits SNA, BP, and HR. A number of studies indicate that sympathoexcitatory glutamatergic neurons in these nuclei are tonically inhibited by GABA. Microinjection of a glutamate receptor blocker in the PVN (26, 67) or RVLM (55) of normotensive rats does not affect SNA, BP, or HR, whereas a GABA\textsubscript{A} receptor blocker in the SFO, MnPO (70), PVN (68), or RVLM (96) increases SNA, BP, and HR. A GABA\textsubscript{A} receptor blocker in the PVN increases local glutamate release (68), and pressor and sympathetic responses from a GABA\textsubscript{A} receptor blocker in the PVN are prevented by a glutamate receptor blocker (17, 68), suggesting that in physiological conditions, GABA release in the PVN tonically inhibits local glutamate release. From a regulatory perspective, it appears that a sympathoexcitatory glutamatergic pathway projects from nuclei of the LT to the PVN, from where it may increase neuronal activity of spinally projecting neurons directly or indirectly via the RVLM. Glutamatergic neurons project from the SFO to the MnPO (59) and enter the PVN from regions of the LT (18). Pressor and sympathetic responses to a glutamate analog in the MnPO or a GABA receptor blocker in the SFO or MnPO can be prevented by a glutamate receptor blocker in the PVN (70), suggesting that an increase in neuronal activity in the SFO or MnPO can be relayed to the PVN via glutamatergic projections. Microinjection of a NMDA receptor agonist in the PVN increases neuronal activity in RVLM neurons and raises BP, and these effects are attenuated by a glutamate receptor blocker in the RVLM (112), indicating that sympathoexcitatory glutamatergic projections from the PVN to the RVLM contribute to the pressor responses to increased glutamate release in the PVN.

**ANGIOTENSINERGIC PATHWAYS**

An extensive network of angiotensin type II (Ang II) as well as Ang I receptor (AT\textsubscript{1}R) containing cell bodies and nerve terminals exists in cardiovascular regulatory nuclei, such as the SFO, PVN, supraoptic nucleus (SON), and RVLM (69, 80, 102). An action potential may trigger the release of Ang II from nerve terminals into the synaptic cleft. Ang II binding to AT\textsubscript{1}Rs stimulates a slow, i.e., seconds to minutes, G-protein...
signaling pathway associated with protein kinase activation and an increase in reactive oxygen species (ROS) (98, 122). An increase in intracellular ROS and superoxide production inhibits the activity of voltage-gated potassium channels (123), thereby enhancing action potential propagation (97, 105) and increasing neuronal activity to, e.g., presympathetic neurons (122). Ang II may activate presympathetic neurons through a mixed current by AT1R activation, either directly or on presympathetic neurons or indirectly on their presynaptic terminals (13). Effects of Ang II on the release of the primary neurotransmitters, GABA and glutamate, appear to play a major role. In the PVN, Ang II-induced activation of AT1R on end-terminals of GABAergic interneurons stimulates an intracellular G_{i/o} protein signaling pathway leading to activation of NADPH oxidase (15). The resulting increase in ROS inhibits GABA release onto presympathetic neurons projecting to the RVLM (66) or intermedialateral cell column (IML) (65). Ang II in the RVLM also increases glutamate release from glutamate interneurons to activate adjacent neurosecretory magnocellular neurons (62). Similar mechanisms appear to be operative in the RVLM (77, 121).

A number of studies provide evidence for an angiotensinergic sympathoexcitatory pathway among the LT-PVN-RVLM. Regions of the SFO, OVLT, and MnPO, known to project to parvocellular subdivisions of the PVN (pPVN) (73, 90, 104), are densely filled with Ang II-containing neurons (69, 80). Connections between the SFO and PVN appear to use Ang II as a neurotransmitter, since stimulation of neurons in the SFO by Ang II or glutamatic acid increases Ang II release in the PVN (106). Electrical stimulation of the SFO results in AT1R-mediated excitation of neurons of the PVN antidromically identified as projecting to the IML (6) or the posterior pituitary (24). Microinjection of Ang II in the SFO increases BP, and this effect of Ang II is prevented by an AT1R blocker in the PVN (61). Llewellyn et al. (70) did not confirm this, possibly attributable to the low dose of losartan injected in the PVN, which was ~10- to 20-fold lower than doses used in other studies to block the BP and HR responses to Ang II in the PVN (14, 27). Considering that a glutamate receptor blocker in the PVN also abolishes the sympathetic and pressor responses from Ang II in the SFO (70), the Ang II-induced increase in neuronal activity in the SFO may be relayed to the PVN via angiotensinergic projections, activating local glutamatergic interneurons (23) that relay the increased neuronal activity to presympathetic neurons. Bathing neurons of the PVN with Ang II in vitro excites retrogradely labeled neurons of the PVN terminating in the RVLM (13). Disinhibiting the PVN with a GABA receptor antagonist increases renal sympathetic nerve activity (RSNA) and BP, and these effects are attenuated by 40-50% with an AT1R blocker in the RVLM (91). Similarly, the increase in BP from microinjection of Ang II in the PVN can be prevented by an AT1R blocker in the RVLM (61), and Ang II in the RVLM increases local glutamate release, BP, and HR (121). These findings suggest that an increase in Ang II release and decrease in GABA receptor activation in the PVN activate an angiotensinergic pathway between the PVN and RVLM, causing AT1R activation in the RVLM, thereby increasing local glutamate release, SNA, and BP.

**ALDOSTERONE-MINERALCORTICOID RECEPTOR-EPITHELIAL NA^+ CHANNEL-OUABAIN PATHWAY IN THE CENTRAL NERVOUS SYSTEM**

Central nervous system pathways mediating sympathetic and pressor responses to aldosterone. Forebrain areas, such as the LT, SON, and PVN, coexpress mineralcorticoid receptor (MR) (5, 36, 101) and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) (74, 118), which confers aldosterone specificity to MR (29). These brain regions are also aldosterone sensitive. A brief intracerebroventricular (icv) infusion of aldosterone in artificial cerebrospinal fluid (aCSF; 145 mM) does not change RSNA, BP, or HR but combined with modestly higher Na\(^+\) concentration ([Na\(^+\)]) (160 mM), causes sympathetic and pressor responses (27, 99). Infusion of aldosterone into the SFO (unpublished observations) or PVN (27) also enhances the pressor responses to local infusion of Na\(^+\)-rich aCSF. Chronic icv infusion of aldosterone raises sympathetic activity and BP, more when combined with slightly elevated [Na\(^+\)] (150–152 mM) in the vehicle (31, 47, 99, 119). An increase in CSF [Na\(^+\)] therefore facilitates the pressor effects of central aldosterone. Both short-term and chronic effects of aldosterone appear to depend on activation of a MR-epithelial Na\(^+\) channel (ENaC)-endogenous ouabain (EO) pathway (63). Aldosterone, by binding to MR, appears to increase ENaC activity, and enhanced Na\(^+\) entry through ENaC may increase release of neuromodulators such as EO (27, 99), enhancing the excitability of presympathetic neurons involved in BP regulation. Our group was the first to show mRNA and protein expression of all three ENaC subunits (α, β, γ) in the hypothalamus of rats, including the LT, PVN, and SON. Both MR and ENaC exhibit greater expression in magnocellular neurons of the PVN and SON compared with parvocellular neurons of the PVN (5, 101). Teruyama et al. (93) recently confirmed these findings. All three subunits are also expressed in the hypothalamus of mice (54). In vasopressinergic magnocellular neurons of the PVN, ENaC currents contribute to the resting membrane potential and modulate neuronal activity (93). EO immunoreactivity is present in the PVN and SON and scattered around the nuclei of the LT (110, 111). Immortalized hypothalamic N1 cells derived from mouse magnocellular neurons release EO in the supernatant. Aldosterone increases EO release by N1 cells, and a MR blocker prevents this effect of aldosterone (115). Magnocellular neurons of the PVN and SON may therefore release EO to nuclei of the LT and parvocellular neurons of the PVN. This EO release could be analogous to volume transmission, as shown for peptides (25), with diffusion from magnocellular terminals through extracellular fluid across distances many times larger than the synaptic cleft. Chronic icv infusion of aldosterone increases hypothalamic EO content, and the ENaC blocker benzamil prevents this increase (99). Moreover, the enhanced sympathetic and pressor responses to aldosterone and Na\(^+\), either icv (99) or in the PVN (27), can be prevented by benzamil or EO-binding fragment antigens-binding (Fab) fragments. icv infusion of a MR blocker (31, 52, 119), ENaC blocker (99), or the Fab fragments (52) prevents sympathoexcitation and hypertension from chronic icv infusion of aldosterone. Altogether, these findings indicate that an increase in aldosterone via binding to MR and ENaC activation triggers the local synthesis and secretion of EO in the hypothalamus. Acutely, ouabain en-
An AT1R blocker also prevents all responses to aldosterone (27, 119), indicating that responses to aldosterone-induced release of EO also depend on AT1R activation. Chronically, aldosterone via EO also appears to change protein expression, further enhancing AT1R-mediated responses. Chronic icv infusion of aldosterone increases angiotensin-converting enzyme (ACE) and AT1Rs, as well as NADPH oxidase subunits (52) and superoxide levels (119) in the PVN, and these effects are prevented by a MR blocker (52, 119), ENaC blocker, or the Fab fragments (52). In parallel, chronic icv infusion of aldosterone decreases neuronal nitric oxide synthase in the PVN (52), thereby diminishing the sympathoinhibitory effects of tonic NO release in the PVN (28, 117). These findings are consistent with the concept that in the hypothalamus, aldosterone activates a MR-ENaC-EO pathway, which enhances—by both acute and more chronic mechanisms—the activity of angiotensinergic pathways. The resultant increased responses to AT1R activation will enhance activity in glutamatergic sympathoexcitatory pathways, causing sympathetic hyperactivity and hypertension.

**Stimuli for the aldosterone-MR-ENaC-EO pathway.** Aldosterone is present in a wide variety of brain regions and is highly abundant in the hypothalamus (30, 38, 114). Most of the aldosterone in the brain of normal rats appears to originate from the hypothalamus (30, 38, 114). Most of the aldosterone is present in a wide variety of brain regions and is highly abundant in the hypothalamus (30, 38, 114). Most of the aldosterone in the hypothalamus, including early-stage enzymes, such as steroidogenic acute regulatory protein, cytochrome P450 enzyme side-chain cleavage, 3β-HSD, and late-stage enzymes, i.e., CYP11B1 (11β-hydroxylase) and CYP11B2 (aldosterone synthase) (33, 60), but not surprisingly, at much lower levels compared with the adrenal gland (58, 113). Both CYP11B1 and CYP11B2 mRNA are expressed in the SFO, PVN, and SON (101, 109). Recent studies suggest that these low mRNA expression levels do translate into enzymatic activity that locally synthesizes aldosterone. Both an increase in plasma Ang II and in CSF [Na+] appear to increase local synthesis of aldosterone in the hypothalamus, activating the MR-ENaC-EO pathway. This pathway may also be activated by an increase in circulating aldosterone.

**Activation by circulating Ang II.** A chronic increase in circulating Ang II increases Fos-related antigen (Fra)-like immunoreactivity transiently in the SFO in the first few days and more progressively and persistently in the SON and both the magnocellular subdivision of the PVN (mPVN) and pPVN of rabbits (19) and rats (37). In Wistar rats, chronic subcutaneous (sc) infusion of Ang II increases CYP11B2 mRNA expression in the LT (109) as well as the SFO, SON (1a), and PVN (109) and raises aldosterone content in the hypothalamus (37). icv infusion of an aldosterone synthase inhibitor prevents this increase in aldosterone, and both a MR blocker and aldosterone synthase inhibitor markedly attenuate Fra expression in the mPVN and pPVN but not in the SFO and SON (37). We postulated (37) that an increase in circulating Ang II activates neurons in the SFO and OVLT, and this increased neuronal activity is relayed to the PVN. More chronically, increased neuronal activity in the SFO also appears to be relayed to magnocellular neurons of the SON or PVN, leading to increased local production of aldosterone in magnocellular neurons. How this increased neuronal activity may signal aldosterone production in magnocellular neurons has not yet been studied. An increase in Ang II release in the SON may decrease local GABA release and GABAA receptor activation, increasing in magnocellular neurons the activity of steroidogenic enzymes such as 3β-HSD (22). Aldosterone release and MR stimulation, possibly in the SON, may, via ENaC, enhance local EO production in magnocellular neurons in SON and/or PVN (115) and thereby, the activity of angiotensinergic pathways in the pPVN (Fig. 2). Consistent with this dual activation of pPVN and sympathetic activity, inhibition of this neuromodulatory pathway does not affect the initial increase in BP by Ang II but largely prevents the further increase in BP (37, 108). Chronic sc infusion of Ang II also increases mRNA and protein expression of AT1R (103) and increases microglia activation and mRNA expression of proinflammatory cytokines in the PVN (87). Whether these Ang II-induced increases in the PVN depend on the aldosterone-EO pathway has not yet been studied.

**Activation by CSF [Na+].** Pressor responses to an acute icv infusion of Na+-rich aCSF are attenuated by 40–50% by an AT1R blocker in the SFO (unpublished observation) or lesions of the OVLT and ventral part of the MnPO (95) and fully prevented by an AT1R blocker in the MnPO (10). These findings suggest that an increase in CSF [Na+] causes Ang II release and AT1R stimulation in the SFO and OVLT, and increased neuronal activity from both sites is likely relayed to the MnPO. An AT1R blocker in the PVN fully blocks the pressor responses to CSF [Na+] (27), suggesting that this increase in neuronal activity in the MnPO is likely relayed to the PVN by angiotensinergic projections causing Ang II release in the PVN. Chronic icv infusion of Na+-rich aCSF increases hypothalamic aldosterone and EO content and ACE and AT1R densities in nuclei of the LT and PVN (38, 46). icv infusion of an aldosterone synthase inhibitor prevents the increase in hypothalamic aldosterone but not corticosterone (49). Moreover, the increase in hypothalamic EO is prevented by icv infusion of an aldosterone synthase inhibitor (49), MR blocker (38), or ENaC blocker (41). The Fab fragments also prevent the increase in ACE and AT1R densities in cardiovascular nuclei from chronic icv infusion of Na+-rich aCSF (38). How and where an increase in CSF [Na+] leads to steroidogenesis have not yet been studied. Considering that CSF [Na+] increases AT1R activation in the SFO and MnPO, increased neuronal activity in the SFO by a chronic increase in CSF [Na+] may also raise aldosterone production in magnocellular neurons of the SON and PVN (Fig. 1). icv infusion of a MR blocker (unpublished observations) or an aldosterone synthase inhibitor (49) does not affect the acute (hours) rise in BP but prevents the chronic (>2 days) increases in sympathetic activity and BP from icv infusion of Na+-rich aCSF. In contrast, icv infusion of an AT1R blocker abolishes the increases in sympathetic activity and BP from both a short-term (44) and chronic (43) increase in CSF [Na+]. These findings suggest that an increase in CSF [Na+] acutely increases activity of...
angiotensinergic pathways, SNA, and BP and more chronically activates the aldosterone-MR pathway that enhances these responses to Na⁺.

**Activation by circulating aldosterone.** Chronic sc infusion of aldosterone in rats drinking 1% NaCl gradually increases BP, and this effect of aldosterone is prevented by icv infusion of a MR or AT₁R blocker (108), suggesting that circulating aldosterone activates a MR-AT₁R pathway in the CNS, leading to the increase in BP. The actual CNS pathways activated by circulating aldosterone have not yet been studied. Due to its high reflection coefficient at the blood brain barrier, circulating aldosterone poorly penetrates most brain areas (81, 83). Indeed, we showed that chronic sc infusion of aldosterone in intact rats raises plasma aldosterone nearly 10-fold but causes only a minimal, nonsignificant increase in hypothalamic aldosterone (37). Electrolytic lesioning of the anteroventral part of the third ventricle (includes the OVLT and ventral MnPO) (88) or PVN (76) prevents the development of hypertension from deoxycorticosterone acetate (DOCA)-salt treatment. Intracarotid infusion of a hypotonic solution, estimated to lower the osmolality of blood perfusing the forebrain by 2–3%, decreases SNA and BP in DOCA-salt rats (9, 79). These studies suggest that nuclei in the LT and the PVN mediate DOCA-salt-induced sympathetic hyperactivity and hypertension. Chronic icv infusion of an ENaC blocker prevents (78) or attenuates by 50% (1) the development of DOCA-salt hypertension. icv infusion of an AT₁R blocker reverses the BP increase from DOCA-salt (82). These studies indicate that ENaC and AT₁R activation in the CNS contribute to the DOCA-salt hypertension. In contrast to DOCA, effects of circulating aldosterone may be limited to the circumventricular organs, i.e., SFO and OVLT, which express MR, ENaC (5), and 11β-HSD-2 (101). One may speculate that circulating aldosterone may enhance signaling from, e.g., the SFO to the PVN and SON. Increased aldosterone production and release may then activate the MR-ENaC-EO pathway, enhancing the activity of angiotensinergic pathways.

**CHANGES IN FAST AND SLOW TRANSMISSION IN HYPERTENSION**

**Glutamate and GABA.** Changes in glutamate and GABA signaling in the CNS contribute to the elevated SNA and BP in a number of models of hypertension. icv injection of GABA or a GABA receptor agonist causes larger decreases in BP and HR in spontaneous hypertensive rats (SHR) (8) or DOCA-salt rats (71) compared with control rats. Similarly, the decreases in SNA and BP from a GABA receptor agonist in the PVN are enhanced in SHR (3) or renal-wrapped hypertensive rats (72) or Dahl S rats on a high-salt diet (55) compared with normotensive control rats. In contrast, the increases in SNA, BP, and HR by a GABA receptor blocker in the PVN are attenuated in renal-wrapped hypertensive rats (72) and in SHR (67). These findings suggest that there is a decrease in GABA release and GABA receptor activation in brain nuclei such as the PVN in these models of hypertension. Infusion of a glutamate blocker into the PVN (26) or RVLM (55) does not affect BP in Dahl S and R rats on a regular salt diet but lowers the elevated BP in Dahl S rats on a high-salt diet. Similarly, infusion of a glutamate receptor blocker in the PVN decreases SNA, BP, and HR in hypertensive SHR but not Wistar-Kyoto (WKY) rats (67) and in the RVLM, decreases BP in Goldblatt hypertensive rats (12). These findings indicate that there is decreased GABA
receptor activation and increased glutamate receptor activation in the PVN and RVLM in these models of hypertension.

Angiotensinergic pathways. Increased activity of angiotensinergic pathways in the CNS contributes to the sympathetic hyperactivity and hypertension in a number of models of hypertension. icv infusion of an AT1R blocker prevents/reverses the increase in BP from cold exposure (89), DOCA-salt (82), increased plasma aldosterone (108), and a high-salt diet in Dahl S rats and SHR (40, 42). icv infusion of a renin inhibitor (82), increased plasma aldosterone (108), and a high-salt diet in Dahl S rats (50), indicating that renin in the brain plays an essential role in the salt-induced hypertension. A number of brain regions contributing to the enhanced AT1R activation have been identified. Injection of an AT1R blocker into the MnPO does not affect BP in SHR and WKY rats on a regular salt diet but reverses the BP increase from a high-salt diet in SHR (10), indicating that AT1R activation in the MnPO contributes to the elevated BP in SHR on high but not regular salt intake. Injection of an AT1R blocker into the PVN (26) or RVLM (55) also does not affect BP in Dahl S and R rats on a regular salt diet but reverses the BP increase in Dahl S rats from a high-salt diet, indicating that enhanced AT1R activation in the PVN and RVLM contributes to the elevated BP in hypertensive Dahl S rats on a high-salt intake. This enhanced AT1R activation may reflect both increased production and release of Ang II as well as increased responsiveness to Ang II, since both ACE and AT1R densities substantially increase in the PVN of Dahl S rats on high salt (102). At the peak BP decrease by an AT1R blocker, a glutamate receptor blocker in the PVN of Dahl S rats on a high-salt diet further decreases BP by ~50%, whereas the AT1R blocker does not further decrease BP at the peak BP response to the glutamate receptor blocker (26). These findings suggest that the effects of increased AT1R activation in the PVN of hypertensive Dahl S rats are fully mediated by local glutamate release.

Aldosterone-MR-ENaC-EO pathway. High-salt intake increases CSF [Na+] in salt-sensitive rats, i.e., Dahl S rats and SHR, but not salt-resistant rats, i.e., Dahl R and WKY rats (45, 75). This increase in CSF [Na+] appears to be determined genetically (4) and is not observed in nongenetic models of salt hypertension such as DOCA-salt rats (92). The aldosterone-E0 neumodulatory pathway in the CNS plays an essential role in the development of hypertension in Dahl S rats and SHR on a high-salt diet. In Dahl S rats, a high-salt diet also increases hypothalamic content of aldosterone, corticosterone (51), and EO (100) and ACE and AT1R densities in, e.g., the PVN (102). icv infusion of an aldosterone synthase inhibitor prevents the increase in hypothalamic aldosterone and attenuates the hypertension by ~60% in Dahl S rats on a high-salt intake (33, 51). icv infusion of the steroid synthase 3β-HSD inhibitor trilostane also does not affect BP in Dahl S and R rats on a regular salt diet but reverses the BP increase in Dahl S rats from a high-salt diet, indicating that enhanced AT1R activation in the MnPO contributes to the elevated BP in SHR on high but not regular salt intake. Injection of an AT1R blocker into the MnPO does not affect BP in SHR and WKY rats on a regular salt diet but reverses the BP increase in Dahl S rats from a high-salt diet, indicating that AT1R activation in the MnPO contributes to the elevated BP in SHR on high but not regular salt intake. Injection of an AT1R blocker into the PVN (26) or RVLM (55) also does not affect BP in Dahl S and R rats on a regular salt diet but reverses the BP increase in Dahl S rats from a high-salt diet, indicating that enhanced AT1R activation in the PVN and RVLM contributes to the elevated BP in hypertensive Dahl S rats on a high-salt intake. This enhanced AT1R activation may reflect both increased production and release of Ang II as well as increased responsiveness to Ang II, since both ACE and AT1R densities substantially increase in the PVN of Dahl S rats on high salt (102). At the peak BP decrease by an AT1R blocker, a glutamate receptor blocker in the PVN of Dahl S rats on a high-salt diet further decreases BP by ~50%, whereas the AT1R blocker does not further decrease BP at the peak BP response to the glutamate receptor blocker (26). These findings suggest that the effects of increased AT1R activation in the PVN of hypertensive Dahl S rats are fully mediated by local glutamate release.

Fig. 3. Schematic sagittal section of the brain indicating proposed pathways mediating responses to plasma Ang II and CSF [Na+]. An increase in plasma Ang II or CSF [Na+] enhances neuronal activity in the SFO and OVLT, and this increased activity is relayed to the MnPO and subsequently, to the PVN, leading to increased Ang II release in the PVN (top panel). Enhanced angiotensinergic signaling in the PVN decreases local GABA release and increases local glutamate release. Increased neuronal activity in the PVN is relayed to the rostral ventrolateral medulla (RVLM), activating a similar angiotensinergic signaling pathway. Chronically, increased neuronal activity in the SFO and OVLT is also relayed to the SON, enhancing aldosterone production in magnocellular neurons (bottom panel). Aldosterone release increases production of EO by magnocellular neurons in the SON/mPVN. EO in the PVN enhances angiotensinergic signaling, further increasing and maintaining sympathetic hyperactivity. The clear area represents the 3rd ventricle filled with CSF. Dotted arrows represent angiotensinergic or glutamatergic excitatory projections. IML, intermedio/lateral cell column; SNA, sympathetic nerve activity; BP, blood pressure.
rhone synthase inhibitor, MR blocker (51), or ENaC blocker (100) prevents the increase in hypothalamic EO content (100) from a high-salt diet in Dahl S rats. In Dahl S rats on high salt, central infusion of the ENaC blocker (100) or the Fab fragments (45) also prevents the sympathetic hyperactivity and hypertension. These findings indicate that in Dahl S rats, a high-salt diet activates the aldosterone-MR-ENaC-EO pathway in the CNS that is essential for the sympathoexcitation and hypertension.

A high-salt diet also increases hypothalamic EO (64) and BP (10, 64) in SHR. icv injection of a MR blocker in SHR on a high-salt diet reverses the salt-induced increase in BP (85). Chronic icv infusion of the Fab fragments does not change BP in SHR on a regular salt diet but prevents the exacerbation of hypertension by a high-salt diet (39, 42). These findings suggest that the high-salt-induced increase in BP in SHR is also mediated by increased activation of the central MR-EO neuromodulatory pathway. No studies in Dahl S rats and only one in SHR has, so far, assessed where this neuromodulatory pathway in the CNS contributes to the hypertension from high salt. Injection of the Fab fragments into the MnPO does not affect BP in SHR and WKY rats on a regular salt diet but normalizes the BP increase from a high-salt diet in SHR (10), indicating that EO action in the MnPO contributes to the elevated BP in SHR on high but not regular salt intake. icv infusion of the Fab fragments blocks the increase in hypothalamic ACE activity in Dahl S rats on a high-salt diet (120). However, whether the aldosterone-EO neuromodulatory pathway contributes to the enhanced AT1R activation in the MnPO (10), PVN (26), or RVLM (55) of hypertensive Dahl S rats or SHR still needs to be assessed. In mice with aortic banding-induced pressure overload, central infusion of a MR blocker prevents the increases in hypothalamic aENaC and AT1R. Central infusion of a MR or ENaC blocker prevents the high-salt-induced increase in urinary excretion of norepinephrine, suggesting that a central aldosterone-ENaC pathway mediates the increase in sympathetic activity (53, 54). Further studies are needed to evaluate the contribution of these pathways in other models of sympathetic hyperactivity and hypertension. Whether these pathways modulate the neuronal plasticity, e.g., input resistance of presympathetic neurons of the hypothalamus that enhances the activity of angiotensinergic pathways in, e.g., the PVN, thereby sustaining increased fast synaptic neurotransmission (Fig. 3). In several models of hypertension, enhanced AT1R activation, decreased GABA receptor activation, and increased glutamate receptor activation in the brain, i.e., PVN and RVLM, contribute to the elevated SNA and BP. The high-salt-induced sympathetic hyperactivity and hypertension in Dahl S rats and SHR are mediated by activation of the central MR-ENaC-EO neuromodulatory pathway, apparently leading, e.g., in the PVN, to increased AT1R activation and enhanced local glutamate release. Integration of rapid, slow, and very slow CNS pathways regulating sympathoexcitation provides a framework to understand how different stimuli and mechanisms may interact and how to proceed for new, specific therapeutic strategies. Further studies are needed to clarify where in the hypothalamus these pathways interact to contribute to the elevated SNA and BP in different models of hypertension. Adeno-associated virus–small interfering RNA against components of these pathways, e.g., MR and AT1R in specific hypothalamic nuclei to achieve long-term inhibition (108), represents one such approach.

**GRANTS**

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**DISCLOSURES**

No potential conflicts of interest are relevant to this article.

**AUTHOR CONTRIBUTIONS**

Author contributions: A.G. prepared figures; A.G. drafted manuscript; A.G. and F.H.H. edited and revised manuscript; A.G. and F.H.H. approved final version of manuscript.

**REFERENCES**


