

Osmoregulatory Function in Health and Disease

A ROLE FOR BENZAMIL-SENSITIVE PROTEINS OF THE CENTRAL NERVOUS SYSTEM IN THE PATHOGENESIS OF SALT-DEPENDENT HYPERTENSION

Joanna M Abrams and John W Osborn

*Graduate Program in Neuroscience, Department of Integrative Biology and Physiology,
University of Minnesota, Minneapolis, Minnesota, USA*

SUMMARY

1. Although increasing evidence suggests that salt-sensitive hypertension is a disorder of the central nervous system (CNS), little is known about the critical proteins (e.g. ion channels or exchangers) that play a role in the pathogenesis of the disease.

2. Central pathways involved in the regulation of arterial pressure have been investigated. In addition, systems such as the renin–angiotensin–aldosterone axis, initially characterized in the periphery, are present in the CNS and seem to play a role in the regulation of arterial pressure.

3. Central administration of amiloride, or its analogue benzamil hydrochloride, has been shown to attenuate several forms of salt-sensitive hypertension. In addition, intracerebroventricular (i.c.v.) benzamil effectively blocks pressor responses to acute osmotic stimuli, such as i.c.v. hypertonic saline. Amiloride or its analogues have been shown to interact with the brain renin–angiotensin–aldosterone system (RAAS) and to effect the expression of endogenous ouabain-like compounds. Alterations of brain RAAS function and/or endobain expression could play a role in the interaction between amiloride compounds and arterial pressure. Peripheral treatments with benzamil, even at higher doses than those given centrally, have little or no effect on arterial pressure. These data provide strong evidence that benzamil-sensitive proteins (BSPs) of the CNS play a role in cardiovascular responsiveness to sodium.

4. Mineralocorticoids have been linked to human hypertension; many patients with essential hypertension respond well to pharmacological agents antagonizing the mineralocorticoid receptor and certain genetic forms of hypertension are caused by chronically elevated levels of aldosterone. The deoxycorticosterone acetate (DOCA)-salt model of hypertension is a benzamil-sensitive model that incorporates several factors implicated in the aetiology of human disease, including mineralocorticoid action and increased

dietary sodium. The DOCA-salt model is ideal for investigating the role of BSPs in the pathogenesis of hypertension, because mineralocorticoid action has been shown to modulate the activity of at least one benzamil-sensitive protein, namely the epithelial sodium channel.

5. Characterizing the BSPs involved in the pathogenesis of hypertension may provide a novel clinical target. Further studies are necessary to determine which BSPs are involved and where, in the nervous system, they are located.

Key words: acid-sensitive ion channel, aldosterone, benzamil, DOCA, epithelial sodium channel, salt-sensitive hypertension, sympathetic nervous system.

INTRODUCTION

Evidence suggests that, in humans, hypertension is a disorder of the central nervous system (CNS); specifically, sympathetic nervous activity (SNA) is increased in the human disease state.^{1,2} Furthermore, increasing dietary sodium has been shown to affect both arterial pressure and SNA.^{3–9} In addition, several animal models of salt-sensitive hypertension exhibit increased SNA or increased levels of plasma noradrenaline (an indicator of increased sympathetic drive).^{10–12} Although high sympathetic tone seems to be a hallmark of many salt-dependent models of hypertension, central administration of amiloride, or its analogue benzamil, attenuates both the level of hypertension reached and circulating levels of noradrenaline.¹³ These data suggest an interaction between benzamil-sensitive proteins (BSPs) and SNA; however, it remains to be shown where, within autonomic pathways, the BSPs mediating this interaction are located. The present review will address what is known about the neural pathways involved in the regulation of arterial pressure, as well as the characteristics of known BSPs. In addition, we will attempt to summarize possible interactions between the activity of BSPs and central neural control of cardiovascular function.

AETIOLOGY OF SALT-SENSITIVE HYPERTENSION

Central pathways

Some of what is currently understood about the neural pathways mediating salt-dependent forms of hypertension has come from

Correspondence: John W Osborn, 6-125 Jackson Hall, 321 Church St SE, Minneapolis, MN 55455, USA. Email: osbor003@umn.edu

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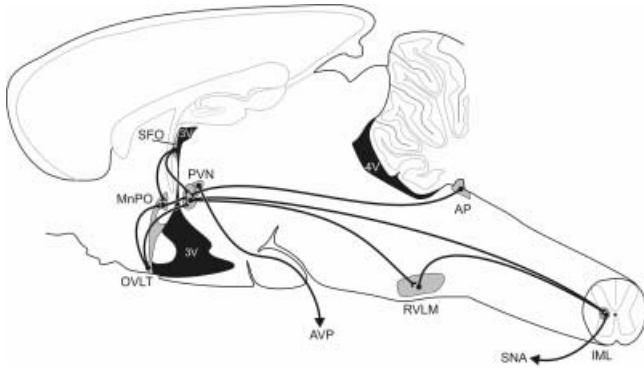


Fig. 1 Central pathways. Circumventricular organs (CVOs), including the area postrema (AP), organum vasculosum lamina terminalis (OVLT) and subfornical organ (SFO), have a weakened blood–brain barrier owing to their fenestrated capillaries. These central nervous system sites may be important for sensing circulating hormones and/or plasma osmolality.¹⁴ The CVOs send projections to integrative and regulatory sites, such as the median preoptic area (MnPO; involved in salt appetite and regulating drinking behaviour) and the paraventricular nucleus of the hypothalamus (PVN; critical for defence of homeostasis¹⁹). These pathways continue onto presympathetic areas, such as the rostral ventrolateral medulla (RVL) and the intermediolateral cell column (IML) of the spinal cord,³⁵ which allows for modulation of sympathetic activity and arterial pressure.^{94,95} Magnocellular neurons of the PVN also play a role in modulating arterial pressure through the adaptation of neuroendocrine responses, such as vasopressin release. SNA, sympathetic nerve activity; 3V, third ventricle; 4V, fourth ventricle.

studies using acute osmotic stressors and extrapolating those results to long-term salt sensitivity of arterial pressure. Drawing on these results, Fig. 1 summarizes the hypothetical central pathways involved in the pathogenesis of salt-dependent hypertension. The circumventricular organs (CVOs), with their fenestrated capillaries, exhibit a weakened blood–brain barrier; because of this, CVOs are uniquely situated in the CNS, able to sense changes in plasma osmolality or hormone levels and to drive subsequent neural activity¹⁴ (Fig. 1). Discrete lesions of the area postrema (AP), a hindbrain CVO, prevent increases in mean arterial pressure (MAP) in the DOCA-salt model of hypertension.¹⁵ Lesions of the anteroventral third ventricle (AV3V) region, which contains both the organum vasculosum lamina terminalis (OVLT) and projections from the subfornical organ (SFO) to downstream targets, decrease MAP in the DOCA-salt model¹⁶ and other salt-sensitive models of hypertension.^{17,18} The OVLT and SFO are forebrain CVOs.

The CVOs feed into midbrain sites and then to bulbospinal pathways that are critical for the control of sympathetic nervous activity.¹⁹ Activity of the median preoptic area (MnPO), a downstream target of the OVLT and SFO, seems to play a role in many models of hypertension,^{20–22} salt appetite²³ and drinking behaviour (of interest because drinking is increased in some forms of salt-sensitive hypertension).^{24,25} The MnPO, in turn, sends projections to the zona incerta (ZI) and to the paraventricular nucleus of the hypothalamus (PVN). The ZI plays a role in osmotically induced drinking. In single-cell electrophysiological studies, the ZI has been shown to be responsive to changes in osmolality in the AV3V region and at the MnPO;²⁶ firing rates of the ZI were affected by both hyperosmotic stimuli (i.c.v. hypertonic saline of varying concentrations) and a hypo-osmotic stimulus (distilled water) injected at upstream

connection sites. In addition, in electrophysiological recordings from the ZI of conscious animals, firing rates increase to the sight of water following osmotic stress (i.c.v. hypertonic saline administration), but not in control animals.²⁷ Finally, lesions of the ZI or ZI antagonism (through local injection of pharmaceutical agents) disrupts drinking behaviour in response to osmotic stressors.^{28–32}

The PVN, another downstream target of the CVOs and the MnPO,³³ has been implicated in the control of MAP. The PVN is a hypothalamic nucleus that plays a role in regulating neuroendocrine responses, such as modulating systemic vasopressin levels through activity of its magnocellular neurons. In addition, the PVN is a presympathetic site, sending projections from parvocellular neurons to the intermediolateral cell column of the spinal cord and to the rostral ventrolateral medulla (RVL).^{34–36} Lesions of the PVN attenuate several forms of hypertension.^{37,38} In addition, the OVLT, MnPO and PVN all show increased activity, as measured by c-Fos immunoreactivity, following acute DOCA-salt treatment.³⁹

Ultimately, integration of these upstream sites are thought to affect SNA by converging on sympathetic premotor neurons in the RVL. The RVL is believed to be the key brainstem site for the regulation of spinal sympathetic preganglionic neurons in the intermediolateral cell column (IML) of the spinal cord.⁴⁰

Role of central mineralocorticoids in salt-sensitive hypertension

Aldosterone, the primary mineralocorticoid, is a steroid hormone secreted by the adrenal gland that plays a major role in sodium balance and body fluid homeostasis. Unlike many other steroid hormones, aldosterone does not readily cross the blood–brain barrier^{41–43} and, so, it has been hypothesized that the CVOs may be critical for driving aldosterone-sensitive responses in the CNS.⁴⁴ Basic research implicates mineralocorticoids in the control of arterial pressure. Chronic i.c.v. infusion of aldosterone results in increased arterial pressure.^{45,46} Although systemic administration of aldosterone, or its precursor DOCA, also leads to increased arterial pressure, i.c.v. infusion of mineralocorticoid receptor (MR) antagonists blocks the hypertensive effect of systemic mineralocorticoids,^{45,47} indicating that central responses are necessary for the hypertensive effects seen in systemic aldosterone treatment. Furthermore, following i.c.v. administration of MR antagonists, Dahl salt-sensitive rats remain normotensive, even on a high-salt diet.^{48,49}

BENZAMIL-SENSITIVE PROTEINS

Degenerin/epithelial sodium channel superfamily

Benzamil and other amiloride analogues act on two large classes of proteins. The first are members of the degenerin/epithelial sodium channel (Deg/ENaC) superfamily of ion channels. All family members share a common topology, with subunits containing two membrane-spanning regions and a large, cysteine-rich extracellular loop. Mammalian family members include the ENaC and acid-sensitive ion channels (ASIC). In both groups, the proteins form multimers composed of four subunits. Two subunits, which are the main structural components of the channel, are repeated. The remaining subunits are modulatory or accessory components of the channel.^{50–55}

The ENaCs are sodium channels and are involved in salt homeostasis. The ENaCs play a critical role in sodium reabsorption in the

distal nephron, as well as at the lung and colon.^{55,56} In addition, ENaCs may play a role in the arterial baroreceptor reflex.⁵⁷ A δ -subunit, sharing much homology with the α -subunit, has been identified. Although the δ ENaC has a broad neural distribution, to date it has only been found in primates and so probably does not play a role in rodent sodium homeostasis.⁵⁸ At least one laboratory has shown the presence of a benzamil-blockable ENaC in many autonomic regulatory sites; however, these results remain to be replicated by other laboratories.⁵⁹ In attempts to replicate these findings, using primers published in that study,⁵⁹ we were unable to amplify a clear, single reverse transcription product.⁶⁰

The ASICs are proton gated, non-selective cation channels that are widely expressed in neural tissue. The ASICs play a role in such diverse functions as nociception, response to ischaemic events and ability to taste salt. Gating properties and channel function are markedly affected by the particular subunits present and so the specific multimer formed may dictate the physiological function of the channel.

Ion transport systems

The second large class of BSPs is comprised of ion transport systems. These include the Na^+/H^+ exchanger, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, the Na^+ pump and the Ca^{2+} pump. The Na^+/H^+ ion exchanger, or antiport, is a membrane-localized protein found in a variety of cell types. It relies on secondary active transport to facilitate the movement of ions (i.e. ion flux generated by the active transport of other proteins, such as by the Na^+/K^+ -ATPase, generates the gradients needed to run these ion exchangers). Increased activity of the Na^+/H^+ exchanger has been linked to primary hypertension in humans.⁶¹

The $\text{Na}^+/\text{Ca}^{2+}$ exchanger is a bidirectional transporter whose activity depends on the electrochemical gradients present at the membrane. The activity of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is coupled to that of the Na^+/H^+ antiport and may also play a role in hypertension; although benzamil treatment has been shown to reduce certain models of salt-sensitive hypertension, Keep *et al.*⁶² found that amiloride analogues with greater affinity for the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (and very low affinity for Na^+ channels) are equally effective in blocking the hypertensive effect of DOCA-salt treatment.

Although amiloride and benzamil are inhibitors of these proteins, the ion transport systems have higher affinity for other amiloride analogues, such as 3',4'-dichlorobenzamil, 2',4'-dimethylbenzamil, 5-(*N*-ethyl-*N*-isopropyl) amiloride and 5-(*N*-methyl-*N*-isobutyl) amiloride.

DOCA-SALT HYPERTENSION

DOCA-salt: A neural model

Although the peripheral effects of DOCA-salt hypertension (i.e. renal and vascular) are well documented,⁶³ the model is considered a neurogenic form of hypertension. Lesions of the AV3V region¹⁶ or of the AP¹⁵ attenuate DOCA-salt hypertension. In addition, following treatment with a sympatholytic agent, such as hexamethonium, DOCA animals show greater decreases in MAP than control animals.^{64,65} Furthermore, SNA and plasma noradrenaline levels are increased upon DOCA-salt treatment.⁶⁶ Treatment with DOCA⁶⁷ or other mineralocorticoids⁶⁸ has been shown to increase levels of

plasma vasopressin, as well as salt appetite,⁶⁹ further indication of increased CNS activity following mineralocorticoid treatment. Thirst may also be increased in DOCA-salt hypertension; however, this is often conflated with salt appetite, because animals are frequently given saline in place of drinking water. Finally, i.c.v. benzamil treatment attenuates DOCA-salt hypertension, whereas peripheral benzamil administration has no effect.^{13,70} Taken together, these data provide strong evidence that the DOCA-salt model is driven by neural activity.

DOCA-salt hypertension, MR and BSPs

The MR is a cytoplasmic receptor with broad neural distribution that is typically found in association with heat shock and other chaperone proteins. When the MR binds its ligand, the protein undergoes a conformational change that frees it from its chaperone proteins and exposes a nuclear translocation signal. In the nucleus, the MR acts as a transcription factor.⁷¹ Because of these properties of the MR, nuclear localization of the protein can be taken as an index of MR activity. Indeed, recent studies have shown that MR adjacent to the AP in neurons of the nucleus tractus solitarius (NTS) translocate to the cellular nucleus following aldosterone treatment.⁷² This unique population of neurons expresses 11 β -hydroxysteroid dehydrogenase type 2 (HSD2), an enzyme that allows the MR to be aldosterone selective. Because MR have a roughly equal affinity for aldosterone and the more prevalent glucocorticoids, under normal conditions MR are thought to be nearly completely occupied by glucocorticoid. However, HSD2 plays a permissive role in neuronal responsiveness to aldosterone: because HSD2 catalyses the dehydrogenation of active 11 β -hydroxycorticoids, it can confer aldosterone selectivity to the normally non-selective MR. Thus, cells expressing HSD2 are able to respond to changes in aldosterone levels, despite higher systemic levels of glucocorticoid. In fact, the HSD2 neurons of the NTS may play a role in modulating responses to DOCA treatment; following 1 week of DOCA, activity in these neurons is increased.⁷³

The DOCA-salt model is an ideal model for investigating the role of BSPs in the pathogenesis of hypertension. Figure 2 summarizes the putative relationship between mineralocorticoid action, high-salt diet and benzamil-blockable channels. Interactions between the MR and ENaCs have been well established.^{68,74} The MR is a transcription factor for the α -subunit of the ENaC, a BSP known to play a role in sodium transport in the kidneys.⁷⁵ Not only has MR activity been linked to the expression of BSPs, but it has also been linked to the enzymes that regulate the plasma membrane localization of these channels.⁷⁴ In addition to direct effects on ENaC transcription, the MR is a regulator of Sgk1, a kinase that plays a role in trafficking ENaCs to the cell membrane and in increasing sodium transport across ENaCs. An additional outcome of MR activation and subsequent Sgk1 activity is the inhibitory phosphorylation of Nedd-4. Nedd-4 plays a role in ubiquitin-mediated degradation of ENaC channels. Because of these effects, i.c.v. infusion of an MR antagonist will not only block the direct effects of aldosterone, but may also cause long-term changes in the sodium permeability of neurons. Although the relationship between MR and ENaCs has been demonstrated, this interaction remains to be established for ASICs or other BSPs.

Our model is based on the assumption that the BSPs involved in the model are ion channels. Because ENaCs have an established role in sodium homeostasis in the periphery, it has been hypothesized that they subserve this role in the CNS. In addition, benzamil has

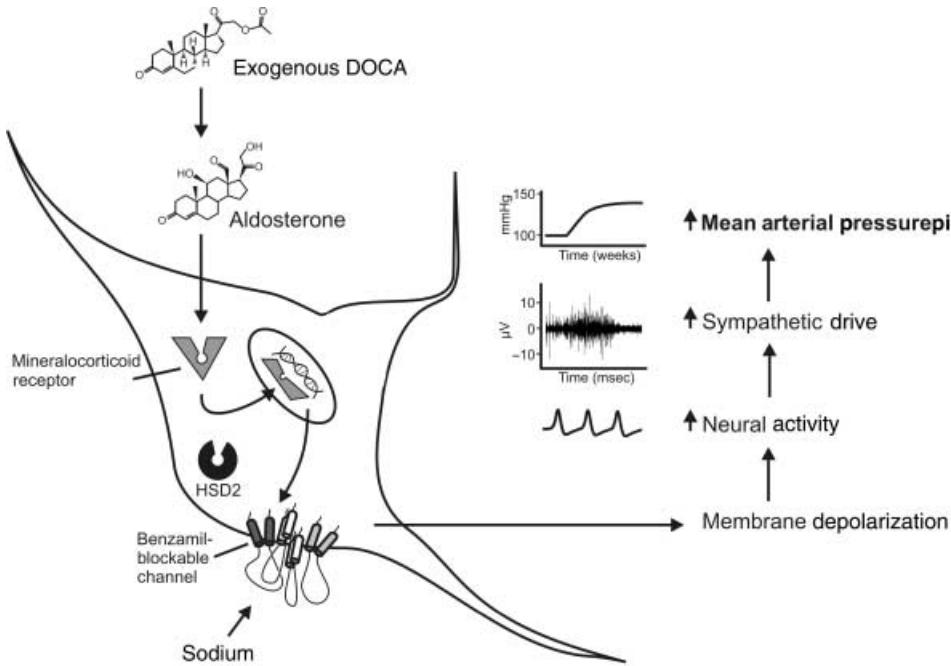


Fig. 2 Model of the role of benzamil-sensitive proteins (BSPs) in the pathogenesis of DOCA-salt hypertension. We propose that benzamil-sensitive ion channels play a role in DOCA-salt hypertension. Following DOCA treatment and subsequent increased mineralocorticoid receptor activity, there would be an increased localization of BSPs to the membrane of 11 β -hydroxysteroid dehydrogenase type 2 (HSD2)-expressing cells. We believe that this increase in membrane-localized BSPs is likely to occur at sodium-sensitive sites, such as the organum vasculosum lamina terminalis, because BSPs seem to play a role in both chronic models, such as DOCA-salt hypertension, and acute sensitivity to cerebrospinal fluid sodium levels. Membrane localization of BSPs would increase neural activity in the presence of high sodium levels; this, in turn, could modulate activity at downstream sites and thereby regulate sympathetic nerve activity and increases in arterial pressure.

high affinity for the ENaC and even low doses of benzamil can block acute pressor responses to osmotic stimuli. However, as noted earlier, amiloride analogues can also interact with ion transport systems and these systems have been shown to play a role in the regulation of arterial pressure.

Benzamil and DOCA-salt hypertension

DOCA-salt hypertension is attenuated by central treatment with amiloride or its analogue benzamil.^{13,70} Benzamil is a fairly specific blocker, targeting the ENaC and ASIC proteins and only at higher doses targeting certain ion transport systems. Although i.c.v. benzamil treatment attenuates DOCA-salt hypertension and decreases vasopressin and noradrenaline levels, systemic benzamil treatment has no significant effect (even when given at much higher doses), indicating a critical role for central BSPs.

Early studies of the effect of benzamil on DOCA-salt hypertension used tail-cuff measurements in animals with established hypertension. We have recently used radiotelemetry transmitters to record MAP in awake, behaving animals, 24 h/day. These studies suggest that DOCA-salt hypertension develops in several distinct phases (generally, there is an initial rapid rise in MAP, followed by a slower, more prolonged period of increasing MAP) and that benzamil plays a role in attenuating the later, more slowly developing phase of hypertension.⁷⁶

OTHER INTERACTIONS BETWEEN BENZAMIL AND SALT-SENSITIVE CARDIOVASCULAR RESPONSES

Sodium sensitivity and BSPs

Administration of benzamil will block not only DOCA-salt hypertension, but also genetic models of hypertension that are driven by a high-salt diet.^{77,78} Therefore, it is likely that the BSPs play a role

in sodium sensitivity; specifically, we hypothesize that when the MR binds its ligand, there is a subsequent upregulation of BSPs, which would increase membrane permeability to sodium. This response, in the face of transient increases in plasma sodium levels, would lead to membrane depolarization and an increase in neuronal activity, driving sympathetic outflow and increases in MAP (see Fig. 2).

In addition to a role in salt-sensitive forms of hypertension, BSPs seem to play a role in pressor responses to acute stimuli. The rapid rise in arterial pressure, typically seen following i.c.v. hypertonic saline, is blocked by i.c.v. benzamil, as shown in Fig. 3. In this experiment, anaesthetized rats ($n = 6$; 250–310 g) were instrumented for direct measurement of MAP and i.c.v. injection of hypertonic saline. After obtaining baseline MAP for 5 min, 50 μ L of 1.5% saline was injected over 30 s. Mean arterial pressure was recorded for an additional 20 min after injection. Then, 20 μ L of 20 nmol/L benzamil hydrochloride (e.g. 0.4 pmol benzamil) was delivered i.c.v. into the lateral ventricle. A second injection of saline was administered 5 min after the benzamil injection and MAP was recorded for 20 min. Note that i.c.v. benzamil abolished pressor responses to i.c.v. hypertonic saline (J Abrams, O Daramola and J Osborn, unpubl. obs., 2005).

Although not examined in our study, Brooks *et al.* have shown that acute pressor responses to central osmotic stimulation contain both a vasopressinergic and a non-vasopressinergic component.⁷⁹ Nishimura *et al.* have demonstrated that i.c.v. benzamil treatment not only eliminates the pressor response to an acute injection of 1.5 mol/L NaCl, but also attenuates levels of sympathetic discharge and plasma vasopressin compared with control animals.¹³ Central benzamil treatment blocks nearly all pressor response to acute hypertonic saline (Fig. 3), suggesting that both vasopressinergic and other mechanisms are sensitive to benzamil treatment. However, pressor responses to more chronic stimuli, such as DOCA-salt treatment, are merely attenuated by i.c.v. benzamil. The molecular substrate for this acute/chronic dichotomy remains to be elucidated; however, it is likely that in chronic protocols, the activity of vasopressinergic neurons is decreased following i.c.v. benzamil.⁷⁶

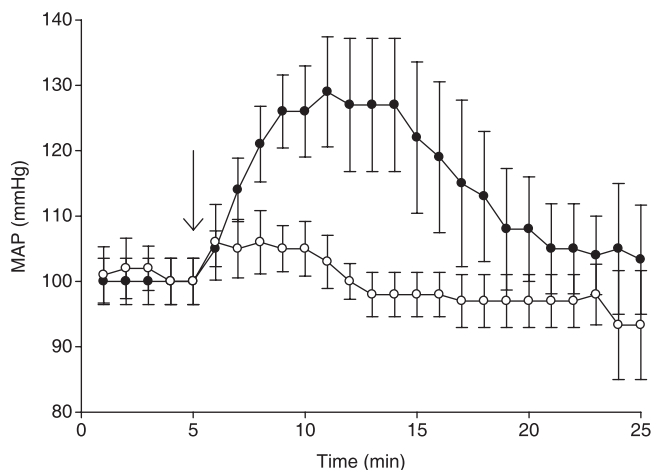


Fig. 3 Acute pressor response to i.c.v. saline is blocked by benzamil hydrochloride. Pentobarbital anesthetized rats ($n = 6$) were instrumented for direct measurement of mean arterial pressure (MAP) via a femoral arterial catheter. Mean arterial pressure was measured by connecting the catheter to a volume-displacement pressure transducer coupled to a Grass polygraph (Grass Instruments, West Warwick, RI, USA). Under stereotaxic guidance, the tip of a 23 gauge stainless steel cannula was placed in the lateral cerebral ventricle. After obtaining baseline MAP for 5 min, 50 μL of 1.5% saline was injected over 30 s and MAP was recorded for an additional 20 min after injection. Then, 20 mL of 20 nmol/L benzamil hydrochloride (e.g. 0.4 pmol) was delivered i.c.v. into the lateral ventricle. A second injection of saline was administered 5 min after the benzamil injection and MAP was recorded for 20 min. The arrow indicates the time of i.c.v. hypertonic saline injection. (●), before i.c.v. benzamil; (○), after i.c.v. benzamil.

The RAAS

The RAAS is a well-studied system that may serve as a 'sensor' to the CNS; plasma hormone concentrations of this system change in response to dietary sodium levels. The RAAS may be involved in the DOCA-salt model; changes in the RAAS have been measured, in both the brain and kidney, following DOCA-salt treatment. The mRNA for both renin and angiotensin-converting enzyme is down-regulated in the kidney following DOCA-salt treatment; however, mRNA levels for these transcripts do not change in the brain. Conversely, following i.c.v. benzamil treatment, the mRNA for critical members of the RAAS is downregulated in the brain, but not in the kidney. Peripheral benzamil treatment has no effect on the members of the RAAS, either in the brain or the kidney. These data indicate that BSPs of the CNS have the ability to modulate activity of the central RAAS and thereby modulate arterial pressure.⁷⁰

Endogenous ouabain-like compounds and hypertension

Endogenous *Digitalis*-like compounds (also termed ouabain-like compounds or endobains) were first found in human-derived tissues in 1991⁸⁰ and these compounds have been implicated in human essential hypertension.^{81–83} Endogenous ouabain-like compounds have since been characterized in bovine- and murine-derived tissues.⁸⁴ Ouabain is a well-known inhibitor of the Na^+/K^+ -ATPase, as well as the $\text{Na}^+/\text{Ca}^{2+}$ exchanger and sodium pump.

Using an immunoassay, Gottlieb *et al.* found increased endobains in the plasma of heart failure patients.⁸⁵ In addition, plasma endobains

have been shown to increase with sodium intake in humans;^{86,87} high sodium intake also leads to increases in endobains in a variety of tissues in rats.⁸⁸ Furthermore, chronic infusions of ouabain have been shown to produce hypertension in the rat.^{88–90} Increasing levels of ouabain-like compounds affect the central RAAS and could explain increased SNA.⁹¹

Administration of benzamil has been shown to block the effect of increasing ouabain levels.^{92,93} In addition, benzamil can act directly on several ouabain-sensitive proteins (e.g. $\text{Na}^+/\text{Ca}^{2+}$ exchanger, Na^+ pump). These data suggest a potential interaction between endobains and BSPs of the CNS.

CONCLUSION

Evidence from multiple animal models suggests a critical role for BSPs in the pathogenesis of salt-sensitive hypertension; however, the specific proteins involved have not been characterized. Although it has long been hypothesized that the ENaC may play a role in the central control of cardiovascular responses, finding ENaC in the CNS has proven difficult. Although at least one laboratory has shown the presence of ENaCs in the CNS,⁵⁹ other laboratories have been unable to replicate this result or have only found evidence for certain ENaC subunits.⁶⁰ Furthermore, in a study testing the efficacy of several amiloride analogues in blocking DOCA-salt hypertension, Keep *et al.* found that even amiloride analogues with low affinity for sodium channels were able to attenuate DOCA-salt hypertension. They concluded that the antihypertensive effects of amiloride analogues may be due to inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange in the brain.⁶² Investigations of the RAAS or of endobains provide further evidence that other proteins, either in the place of or in addition to the ion channels, may be the BSPs involved in salt-sensitive hypertension. Proper characterization of the BSPs involved in salt-sensitive hypertension is critical, both for our understanding of the disease and as an avenue for the development of novel clinical treatments.

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