

## REVIEW

# The central mechanism underlying hypertension: a review of the roles of sodium ions, epithelial sodium channels, the renin–angiotensin–aldosterone system, oxidative stress and endogenous digitalis in the brain

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The central nervous system has a key role in regulating the circulatory system by modulating the sympathetic and parasympathetic nervous systems, pituitary hormone release, and the baroreceptor reflex. Digoxin- and ouabain-like immunoreactive materials were found > 20 years ago in the hypothalamic nuclei. These factors appeared to localize to the paraventricular and supraoptic nuclei and the nerve fibers at the circumventricular organs and supposed to affect electrolyte balance and blood pressure. The turnover rate of these materials increases with increasing sodium intake. As intracerebroventricular injection of ouabain increases blood pressure via sympathetic activation, an endogenous digitalis-like factor (EDLF) was thought to regulate cardiovascular system-related functions in the brain, particularly after sodium loading. Experiments conducted mainly in rats revealed that the mechanism of action of ouabain in the brain involves sodium ions, epithelial sodium channels (ENaCs) and the renin–angiotensin–aldosterone system (RAAS), all of which are affected by sodium loading. Rats fed a high-sodium diet develop elevated sodium levels in their cerebrospinal fluid, which activates ENaCs. Activated ENaCs and/or increased intracellular sodium in neurons activate the RAAS; this releases EDLF in the brain, activating the sympathetic nervous system. The RAAS promotes oxidative stress in the brain, further activating the RAAS and augmenting sympathetic outflow. Angiotensin II and aldosterone of peripheral origin act in the brain to activate this cascade, increasing sympathetic outflow and leading to hypertension. Thus, the brain Na<sup>+</sup>–ENaC–RAAS–EDLF axis activates sympathetic outflow and has a crucial role in essential and secondary hypertension. This report provides an overview of the central mechanism underlying hypertension and discusses the use of antihypertensive agents.

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## INTRODUCTION

Epidemiological studies show that hypertension onset is strongly associated with salt consumption:<sup>1–5</sup> there is a close relationship between average sodium salt intake and the incidence of hypertension,<sup>6,7</sup> and restriction of sodium intake substantially decreases blood pressure.<sup>8,9</sup> When sodium salt is loaded, factors that inhibit Na<sup>+</sup>, K<sup>+</sup>-ATPase activity increase in the circulating blood<sup>10</sup> and in some tissues.<sup>11</sup> As inhibitors of Na<sup>+</sup>, K<sup>+</sup>-ATPase activity were identified as digitalis glycosides, they are termed ‘endogenous digitalis-like factors’ (EDLFs).<sup>12–15</sup> Despite extensive efforts over 40 years, the salt–EDLF–hypertension cascade has been elucidated only recently. Interestingly, there are several EDLFs in the circulating blood, even in subjects who have never ingested any digitalis glycoside either as medicine or in food containing digitalis-like substances.<sup>16–20</sup> EDLFs include ouabain,

digoxin, marinobufagenin (MBG), marinobufotoxin (MBT), telocinobufagin, proscillaridin A, bufalin and others. There are two types of EDLFs: cardenolides derived from plants and bufadienolides derived from toads. As these molecules contain a steroid nucleus and increase cardiac and vascular contractility by inhibiting Na<sup>+</sup>, K<sup>+</sup>-ATPases in cell membranes, they are called ‘cardiotonic steroids’ (CTSs).

Pharmacological evidence indicates that the circulating level of CTSs may not be high enough to exert physiological effects<sup>21,22</sup> because the main Na<sup>+</sup>, K<sup>+</sup>-ATPase subunit, the  $\alpha_1$ -subunit, is resistant to ouabain. This apparent resistance was a major reason why CTSs were not researched extensively in terms of possible pathophysiological roles in cardiovascular disease. However, in recent years, EDLF has emerged as a key player, at least locally in the brain, in the onset of sodium-induced hypertension.<sup>23–25</sup>

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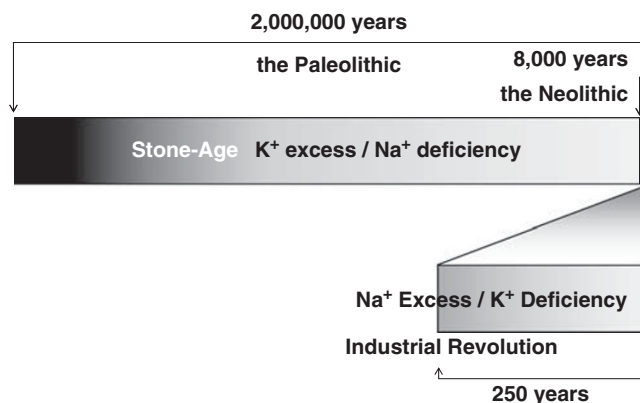
In terms of blood pressure regulation, both a central nervous system (CNS) EDLF and the renin–angiotensin–aldosterone system (RAAS) in the CNS are important.<sup>26,27</sup> Current antihypertensive agents may act at the sites responsible for blood pressure control in the CNS to decrease sympathetic outflow.<sup>28</sup> Regardless of whether the CNS is involved in the genesis of hypertension in an individual patient, it is a major determinant of the response to antihypertensive therapy once a treatment strategy is adopted as commented by Esler.<sup>29</sup> Sympathetic and parasympathetic nervous system activity and/or vasopressin release are the major mechanisms by which the CNS influences blood pressure, although other minor mechanisms may also be involved. When vasodilators are used, for example, the reactive increase in plasma catecholamine helps limit the decrease in blood pressure. Sympathetic activation may lead to a reactive increase in plasma renin activity (PRA) and to sodium retention, which also has an important role in limiting antihypertensive activity. Thus, the CNS is constantly regulating blood pressure toward its set point, and blood pressure is neither increased nor decreased unless the blood pressure set point is changed by the CNS. Among antihypertensive agents, the effectiveness of calcium channel blockers (CCBs) and RAAS inhibitors could reflect their specific actions in the CNS, which help reduce reactive vasopressor responses. In other words, current antihypertensive agents must act by affecting central hypertensive mechanisms and thus suppressing sympathetic outflow. Treatment strategies that address the implications of the CNS response are more likely to be effective than approaches that avoid or ignore CNS involvement.

This review presents recent advances in our understanding of the central mechanism of hypertension, including EDLF research. We also discuss the implications of this central mechanism of action in terms of the clinical treatment of hypertension.

## SODIUM AND HYPERTENSION

Historically, humans in the Stone Age who lived inland on continents consumed minimal amounts of sodium salt in their diets. Until recently, Yanomamo Indians in the Brazilian Amazon region lived like Stone Age people, and their 24-h urinary excretion of sodium was 0.9 mmol (0.53 g of NaCl).<sup>30</sup> Notably, the amount of sodium they consumed was roughly one-twentieth of that consumed by people living in developed countries,<sup>31</sup> suggesting that many humans today consume about 20 times more sodium salt than the minimum requirement. The average blood pressure of the Yanomamo Indians was 96.0/60.6 mm Hg and did not increase with ageing.<sup>32</sup> In addition, no hypertension was observed in this community, in contrast to other modern communities. In the Stone Age, the average life span was approximately 30 years. During this time, traits that worked to increase blood pressure with increasing stress would be favorable for survival: people who could easily elevate their blood pressure to provide sufficient blood to skeletal muscles and major organs would have a survival advantage when attacked by enemies or wild animals. Atherosclerosis, which is the greatest public health concern in modern society, would have no impact on Stone Age society, as there were few, if any, elderly people. Thus, the ability to easily increase blood pressure is a trait that might have conferred a survival advantage until modern times (Figure 1).

Sodium is the most essential mineral in mammalian physiology. In particular, ingestion of high amounts of sodium salt may be required to keep blood pressure high. As sodium intake is limited in natural foods, a physiological mechanism to prevent sodium loss into urine would have been established early in human evolution. The most powerful mechanism is the RAAS,<sup>33–35</sup> which is maximally activated



**Figure 1** Humans have lived with a minimal intake of sodium for several million years. Accordingly, they have developed highly regulated physiological system mechanisms to retain sodium and maintain blood pressure at an appropriate level. The diet of humans has changed drastically and rapidly in recent years, with increased consumption of processed foods with high levels of sodium. Excessive dietary sodium may have adverse effects that lead to elevated blood pressure. A full color version of this figure is available at the *Hypertension Research* journal online.

in people with a minimal sodium intake. In addition, sodium is reabsorbed via the activated sympathetic nervous system, and reabsorption is specifically controlled by renal nerves.<sup>36–38</sup> Insulin, which increases in metabolic syndrome (obesity) and in the initial stages of type 2 diabetes, also acts to retain sodium by suppressing sodium loss into urine via renal tubules.<sup>39–41</sup> Obese people who are metabolically resistant to insulin are not resistant to renal tubular reabsorption of sodium by insulin.<sup>42</sup> Although prevention of sodium loss may once have conferred a selective advantage, ingestion of excessive amounts of sodium now results in chronic hypertension, a major cause of atherosclerosis in modern society. Current efforts focus on preventing hypertension, and this historical perspective indicates that life style modification, and especially a diet that includes a minimum level of sodium salt, is very important for treating hypertension in the clinical setting.<sup>43,44</sup>

In contrast to sodium, potassium was abundant in the fresh foods that made up the Stone Age diet. There are few physiological mechanisms that control potassium retention, and potassium loss in urine is dependent on urine volume.<sup>45–47</sup> In modern times, diets have shifted drastically from fresh to processed foods, reducing potassium intake.<sup>48</sup> As potassium supplementation leads to natriuresis,<sup>49</sup> potassium deficiency may aggravate sodium overloading. Results of the Dietary Approaches to Stop Hypertension trial<sup>50</sup> showed clearly that changes in diet, including both sodium restriction and potassium supplementation, are important for lowering of blood pressure.<sup>51</sup>

Epidemiological studies worldwide suggest that the optimal daily intake of sodium salt (NaCl) is 6–7 g,<sup>52</sup> roughly half of the current average intake of salt. However, because humans have lived with sodium deficiency for a long time, we have developed a powerful salt appetite.<sup>53</sup> This innate desire for salty foods makes it very hard to drastically reduce sodium intake. In fact, when sodium salt is reduced in foods, older people in particular lose their appetites. Ideally, therapy to control hypertension would control pressor mechanisms induced by excess sodium intake even when a significant amount of sodium was consumed. Thus, ‘hypertension and sodium’ has been a major target of hypertension research for a long time,<sup>54–56</sup> and there is increasing attention on endogenous digitalis as a key player in the hypertension–salt relationship.

## THE ROLE OF ENDOGENOUS DIGITALIS IN HYPERTENSION

### Is third factor endogenous digitalis?

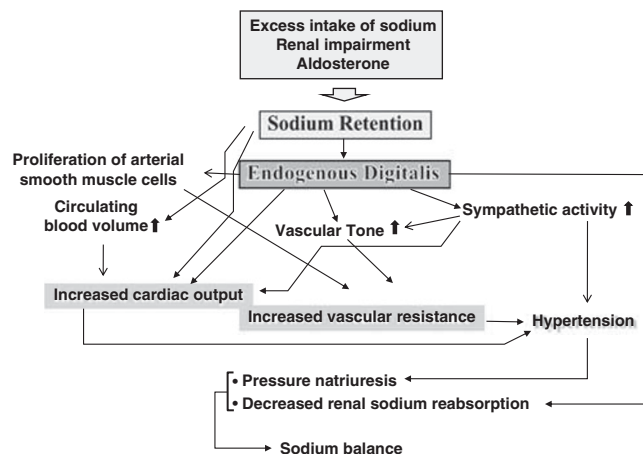
Continuous administration of mineralocorticoids leads to sodium retention, which in turn leads to natriuresis when the sodium level exceeds a threshold.<sup>57,58</sup> This phenomenon is known as ‘mineralocorticoid escape.’ The two major causes of natriuresis are increased glomerular filtration rate and decreased aldosterone levels, but neither is involved in mineralocorticoid escape. The factor involved in this phenomenon is thus referred to as ‘the third factor,’<sup>59</sup> and the most likely candidate for this third factor is an EDLF: suppression of renal tubular  $\text{Na}^+$ , $\text{K}^+$ -ATPase activity markedly increases sodium excretion,<sup>60,61</sup> and EDLFs suppress this enzyme. Supporting this, a  $\text{Na}^+$ , $\text{K}^+$ -ATPase inhibitor is increased in the circulation and tissue  $\text{Na}^+$ , $\text{K}^+$ -ATPase activity is suppressed when animals are fed high-sodium diets.<sup>10,11</sup> An extensive search for the third factor began a few decades ago. Of note, pigs treated with subcutaneous administration of deoxycorticosterone acetate plus 1% sodium chloride as drinking water develop antinatriuresis in the initial 2 days; at this point, digitalis-like  $\text{Na}^+$ , $\text{K}^+$ -ATPase inhibitory activity increases at least 30-fold compared with baseline and natriuresis occurs (Figure 2).<sup>62</sup>

### Endogenous digoxin was first explored as an EDLF

Digoxin is used clinically as a CTS to treat arrhythmia and cardiac failure.<sup>63</sup> As administration of excessive doses of digoxin leads to serious arrhythmias, the circulating levels of immunoreactive digoxin are monitored during digoxin therapy. Even when digoxin has not been given to patients, digoxin-like immunoreactivity (DLI) is sometimes detected in the plasma,<sup>64,65</sup> possibly due to an EDLF that cross-reacts with the anti-digoxin antibody. In fact, DLI is higher in deoxycorticosterone acetate–salt hypertensive rats than in control rats,<sup>66</sup> and circulating DLI increases with sodium loading in rats.<sup>67</sup> In humans, urinary DLI correlates with blood pressure and with urinary sodium.<sup>68</sup> In 2000, our group identified circulating DLI as digoxin using liquid chromatography and mass spectrometry.<sup>69</sup>

### Ouabain is another candidate for an EDLF

A hydrophilic digitalis, ouabain, has also been considered a potential EDLF.<sup>70–72</sup> Hamlyn *et al.*<sup>73</sup> isolated ouabain or its isomer from a very large volume of human serum in 1991. Our group used liquid chromatography and mass spectrometry<sup>69</sup> and nuclear magnetic resonance<sup>74</sup> to demonstrate that there is ouabain in circulating human blood and in the culture supernatant of PC-12 cells. Although there are very low concentrations of digoxin and ouabain in circulating blood in rodents and humans,<sup>75</sup> their physiological roles are unclear because the  $\text{Na}^+$ , $\text{K}^+$ -ATPase in rodents is resistant to these digitalis glycosides.<sup>76</sup> However, a low dose of ouabain induces hypertension in rats,<sup>77</sup> probably because suppression of  $\text{Na}^+$ , $\text{K}^+$ -ATPase activity increases contraction of vascular smooth muscle and myocardium more or less. The  $\alpha_2$ -isoform is thought to be targeted by ouabain.<sup>78</sup> Another explanation is that a low concentration of ouabain increases renal tubular  $\text{Na}^+$ , $\text{K}^+$ -ATPase activity to augment sodium reabsorption, similar to aldosterone.<sup>79</sup> This is not in accordance with the working hypothesis that endogenous digitalis is released and causes natriuresis to restore the sodium balance in response to excessive sodium accumulation.<sup>80</sup> Molecules considered to act as EDLFs show exclusively hypertensive effects,<sup>81,82</sup> but the underlying mechanisms of action seems complex. For example, nanomolar levels of ouabain increase the synthesis and release of angiotensin II (Ang II) from the endothelium of the tail vascular beds of spontaneously hypertensive rats,<sup>83</sup> but also increase nitric oxide release from



**Figure 2** A working hypothesis for the development of hypertension associated with excess accumulation of sodium salt. Sodium retention stimulates production of endogenous digitalis, which elevates vascular tone and cardiac output. This increases blood pressure and directly inhibits the renal tubular  $\text{Na}^+$ , $\text{K}^+$ -ATPase, resulting in reduced sodium reabsorption. Sodium balance is then achieved concurrently with the development of hypertension. A full color version of this figure is available at the *Hypertension Research* journal online.

endothelial cells.<sup>84</sup> Ang II and nitric oxide have opposing effect on vascular smooth muscle as known well.

### The Milan hypertensive rat strain is the ideal animal model for endogenous ouabain-induced hypertension

The Milan hypertensive rat has a point mutation in a gene that encodes adducin,<sup>85</sup> which leads to the stimulation of  $\text{Na}^+$ , $\text{K}^+$ -ATPase activity. As a result, renal tubular reabsorption of sodium increases, and sodium retention results in hypertension.<sup>86</sup> In this animal model, plasma levels of ouabain-like immunoreactivity (OLI) are elevated,<sup>87</sup> which is the principal cause of hypertension. PST2238 is an analogue of digitoxin to block the action of ouabain, which lowers blood pressure in the Milan hypertensive rats.<sup>88</sup> PST2238 also suppresses hypertension caused by low-dose ouabain.<sup>89</sup> Therefore, the cause of this type of hypertension is believed to be ouabain. It is thought that those having adducing polymorphism like similar to the one in Milan hypertensive rats,<sup>90,91</sup> and PST2238 has potential for treating these patients.<sup>92</sup> However, a recent large-scale clinical trial failed to find a significant reduction of blood pressure with PST2238.<sup>93</sup>

### Sites of production of endogenous digitalis: the hypothalamus and/or the adrenal gland?

Digoxin and ouabain are of plant origin. As humans eat plants, we may be ingesting these substances. In fact, when isotope-labelled digoxin is fed to animals, it accumulates in the adrenal glands, brain and pituitary gland, which may be where digitalis is produced.<sup>94,95</sup> For a long time, the question of whether digitalis detected in those organs and in plasma was of endogenous or exogenous origin was debated.<sup>55,96</sup> However, it is now known that ouabain is absorbed poorly (3–5%) in the intestine, indicating that it may be endogenous.<sup>97</sup>

There is an interesting case report of an ouabain-producing adrenocortical tumor.<sup>98</sup> The patient’s hypertension returned to normal with extirpation of the tumor. We found that the plasma level of OLI was elevated in a patient with pheochromocytoma originated from the adrenal medulla. The level of OLI also decreased in this patient after extirpation of the tumor.<sup>99</sup>

When we investigated the tissue localization of OLI using a specific anti-ouabain antibody, OLI was detected in the adrenal medulla,<sup>100</sup> paraventricular nucleus (PVN) and supraoptic nucleus in the hypothalamus, as well as in the pituitary gland.<sup>101,102</sup> OLI-positive neurons were in the magnocellular region of the PVN. Their nerve fibers and varicosities also contain OLI, indicating that, similar to vasopressin, OLI is secreted from those neurons. The nerve fibers were distributed densely to the subfornical organ, organum vasculosum of the laminae terminalis and median eminence, which are implicated in water-electrolyte metabolism.<sup>103</sup> OLI was also found at the posterior lobe of the pituitary gland. The exact same immunoreactivity pattern was found using an anti-digoxin antibody.<sup>104</sup>

The plasma levels of DLI increase and the hypothalamus levels decrease with sodium loading in rats.<sup>67</sup> In contrast, when microtubules are destroyed with intracerebroventricular (ICV) injections of colchicine, the hypothalamic DLI content increases while the plasma levels decrease.<sup>67</sup> We therefore thought that the turnover rate of DLI in the hypothalamus increased with sodium loading, suggesting that DLI was produced in the hypothalamus and possibly released from the pituitary. ICV treatments with 6-hydroxydopamine elicit decreases in OLI contents in the pituitary, the hypothalamus and the plasma.<sup>105</sup> These results suggest that the production and release of OLI are closely associated with the brain, particularly the hypothalamus–pituitary axis, and that noradrenergic or dopaminergic neurons, or both, have a key role in this mechanism. Although we did not examine the turnover rate of OLI, it might be similar to that of DLI because OLI was observed in the same region of the hypothalamus.

Cultured immortalized cell lines have been used to study the mechanisms underlying the production of endogenous digitalis. When bovine adrenocortical cells are cultured in medium without serum, the level of OLI released in the medium is 10-fold higher than the OLI content in the cells.<sup>106</sup> Y-1 cells of adrenocortical origin produce ouabain in culture too.<sup>107</sup>

N1 cells, which are an immortalized cell line of hypothalamic origin, were determined to be of PVN or supraoptic nucleus origin because they produce vasopressin and oxytocin.<sup>108</sup> We recently found that ouabain is released into the serum-free culture medium of N1 cells in a time-dependent manner.<sup>109</sup> Therefore, the hypothalamus as well as the adrenal gland produces ouabain.

Milan hypertensive rats show a roughly 10-fold increase in ouabain content in their brains compared with control rats.<sup>87</sup> The hypothalamus, but not the adrenal gland, of this animal model shows marked upregulation of genes coding for the P450 side chain cleavage enzyme and for the delta5-3beta-hydroxysteroid dehydrogenase/delta5-delta4-isomerase enzymes.<sup>110</sup> Knockdown of the gene coding for these enzymes decreases production of ouabain-like factor from neural tissue.<sup>110</sup> Therefore, ouabain may be produced in the rat hypothalamus. We also found that rat PC-12 cells, which are of adrenomedullary origin, produce ouabain.<sup>73</sup> As the adrenal medulla is of neural crest origin, this may suggest that neural tissues produce ouabain ubiquitously. As noted, OLI is also detected in the rat adrenal medulla by immunohistochemistry.<sup>99</sup> To summarize, ouabain and digoxin are produced by neuronal cells in the hypothalamus and by adrenocortical and -medullary cells.

#### **Bufadienolides are acting as endogenous digitalis**

Bufadienolides are molecules with a six-membered lactone ring in the C17 position of the steroid nucleus (there is a five-membered lactone ring in this position in cardenolides of plant origin). Bufadienolides can be isolated from cataractous eye lenses<sup>111</sup> and from toad skin and salivary glands.<sup>112</sup> They have been used in traditional Chinese

medicine.<sup>113</sup> Similar to cardenolides, bufadienolides inhibit Na<sup>+</sup>,K<sup>+</sup>-ATPase activity<sup>114,115</sup> and are considered to be EDLFs and CTSs. Bufadienolides include MBG, MBT, telocinobufagin, telocinobufotoxin, bufalin, 19-norbufalin, proscillaridin A and others. Bufalin has positive inotropic and pressor responses associated with robust natriuretic activity, which are stronger than equimolar concentrations of ouabain.<sup>116</sup> Of the bufadienolides, MBG has been studied most extensively.<sup>117–120</sup> MBG has great affinity for and inhibits the activity of the  $\alpha_1$ -subunit of the Na<sup>+</sup>,K<sup>+</sup>-ATPase,<sup>80</sup> which is the main Na<sup>+</sup> pump isoform in vascular sarcolemma and renal tubules.

We originally isolated MBT,<sup>20</sup> telocinobufagin<sup>121</sup> and telocinobufotoxin<sup>122</sup> from human plasma and from cultured Y-1 and PC-12 cells. Some reports indicate that these bufadienolides are of adrenocortical origin.<sup>123,124</sup> However, because the CNS has been implicated in electrolyte balance and blood pressure regulation, bufadienolides may also be produced in the nervous system. As the adrenal medulla is of neural crest origin, we investigated whether PC-12 cells produce these bufadienolides. Like ouabain, MBT, telocinobufagin and telocinobufotoxin were all found in cultured PC-12 cells.<sup>122</sup> However, using the anti-MBG antibody, which cross-reacts with MBT, to perform immunohistochemical staining of the hypothalamus, we were unable to detect significant immunoreactivity in the hypothalamic nuclei. This may be due to technical reasons, because bufadienolides are lipophilic and may have been extracted into the organic solvent during sample tissue fixation.

In summary, bufadienolides seem to be produced mainly in the adrenal cortex. This contrasts with ouabain, which is produced mainly in the hypothalamus. These substances may work separately to regulate electrolyte balance and cardiovascular functions.

#### **Stimulation of CTS secretion**

On sodium loading, ouabain and digoxin levels increase not only in plasma but also in urine,<sup>10,117,125–128</sup> suggesting that sodium loading triggers their production. Furthermore, OLI levels increase during exercise<sup>129</sup> and are elevated in patients with acute myocardial infarction.<sup>130,131</sup> Thus, the role of these CTSs might be different from that of other CTSs that increase with sodium loading, particularly in the CNS.

In terms of long-term regulation of CTS secretion, OLI is increased in patients with chronic renal failure,<sup>121,132</sup> primary aldosteronism,<sup>133,134</sup> congestive heart failure<sup>135,136</sup> and pre-eclampsia,<sup>137,138</sup> and, to a lesser extent, in most patients with essential hypertension.<sup>132,139–142</sup> Patients with essential hypertension have cardiac hypertrophy, bradycardia and increased ejection fraction, which are known pharmacological effects of digitalis glycosides.<sup>143</sup> Plasma OLI concentrations decreased in obese subjects after 3 months of supervised exercise.<sup>144</sup> As regular exercise decreases sympathetic activity and blood pressure, OLI may be involved in this mechanism.

Although plasma levels of OLI and DLI can increase or decrease in response to physiological and pathophysiological environmental factors, the magnitude of the changes is so small that it is hard to imagine that they have vasoconstrictive or cardiotoxic effects *in vivo*. On the other hand, subtle local changes in levels in the brain may have significant biological effects, similar to those of a neurotransmitter or neuromodulator. Supporting this idea, microinjection of ouabain into the hypothalamus or lateral ventricle elevates blood pressure by increasing peripheral sympathetic activity.<sup>145–148</sup> Therefore, plasma concentrations of these CTSs may originate in CNS tissue.

Thus, investigation of EDLFs led to the discovery of many other factors that act in concert with EDLF in response to sodium loading in the CNS. The proposed theory that sodium metabolism is influenced

by EDLFs and other factors in the CNS, and that this is essential in the genesis of hypertension has now been confirmed.

#### ACTIVATION OF THE BRAIN RAAS BY SODIUM LOADING

Antihypertensive agents such as CCBs, angiotensin I-converting enzyme inhibitor (ACEI), angiotensin type-1 receptor blocker (ARB) and mineralocorticoid receptor blocker (MRB) are very useful in controlling hypertension of any cause in the clinical setting.<sup>149–153</sup> RAAS blockers in particular may be superior to other agents because they can prevent the onset of diabetes mellitus<sup>154,155</sup> and protect against cardiovascular complications.<sup>156–159</sup> ACEI completely restores normal blood pressure levels in rats with spontaneous hypertension.<sup>160,161</sup>

Sodium loading suppresses PRA and serum aldosterone concentration.<sup>162</sup> Therefore, the RAAS was not thought to be essential in the pathogenesis of hypertension on sodium loading. In patients with essential hypertension who may have high-sodium consumption, roughly one-third have lower PRA.<sup>163,164</sup> RAAS blockers were still effective in these patients.<sup>165,166</sup> Unexpectedly, MRB is more effective in patients with low-renin essential hypertension than in high-renin patients.<sup>167</sup>

PRA mainly reflects the activity of renal renin, but there is renin not only in salivary glands<sup>168</sup> but also in the brain.<sup>169</sup> Expression of renal renin mRNA markedly increases with sodium depletion and captopril treatment, whereas brain renin mRNA decreases.<sup>170</sup> Brain renin may differ from that produced elsewhere as the end product, Ang II in the CNS, causes sympathetic activation and increases blood pressure.<sup>171–175</sup> When we examined renin mRNA in the hypothalamus, expression was higher in rats fed a high-salt diet compared with control rats with a normal diet.<sup>176</sup> Similarly, expression of ACE mRNA and angiotensin type-1 receptor mRNA was higher in deoxycorticosterone acetate–salt hypertensive rats,<sup>177</sup> and ICV injection of Ang II causes far greater rises in blood pressure in those rats than in control rats. So, in contrast to its effect on PRA, sodium loading increases the activity of the brain renin–angiotensin system. There is aldosterone in the hypothalamus,<sup>178</sup> and its levels increase with sodium loading in rats,<sup>179</sup> suggesting that sodium loading activates the brain RAAS. Both Ang II and aldosterone injected ICV cause centrally induced increases in blood pressure.<sup>180,181</sup> Therefore, activation of the brain RAAS may be an essential cause of hypertension. If RAAS blockers affect central sites, it makes sense that they are effective even in low-renin essential hypertensives.<sup>164,165</sup>

In our experience, the hypertensive response to ICV injection of hypertonic saline is accompanied by an increase in the plasma DLI concentration, which is blocked by ICV pretreatment with ARB.<sup>182</sup> On the basis of this, we proposed that sodium loading could activate the brain renin–angiotensin system, with EDLFs implicated in this series of responses. Blood pressure increases after ICV injection of hypertonic saline are accompanied by increased peripheral sympathetic tone.<sup>183,184</sup> In particular, renal nerve activity markedly increases with ICV injection of hypertonic saline,<sup>185</sup> and renal arterial blood flow, as measured with radioactive microspheres, decreases.<sup>186</sup> As this response is reversed by ICV pretreatment with atrial natriuretic peptide or C-type natriuretic peptide, it appears that these peptides compete with the RAAS in the brain. There are natriuretic peptides in the brain,<sup>187,188</sup> and brain natriuretic peptide and C-type natriuretic peptide were originally isolated from the brain. Elevated sympathetic activity acts as a powerful anti-natriuretic factor.<sup>189</sup> Therefore, sodium loading may lead to sodium retention via the central mechanism (that is, positive feedback). However, lower concentrations of ICV sodium suppress renal sympathetic nerves<sup>190</sup> and cause natriuresis in rats<sup>191</sup> and sheep<sup>192</sup> (that is, negative feedback). It is hard to understand which mechanism is actually working *in vivo*. There may be a sodium

level threshold in the cerebrospinal fluid that triggers the positive feedback mechanism.

#### IS THE EPITHELIAL SODIUM CHANNEL (ENAC) A SENSOR FOR SODIUM IONS IN THE BRAIN?

Vasopressor responses to ICV injections of hypertonic saline may not be triggered by nonspecific stimuli like osmotic pressure, because equimolar amounts of urea injected ICV do not cause the same response.<sup>193</sup> As we taste sodium salt via ENaCs on our tongues,<sup>194,195</sup> we proposed that ENaC also works as a sensor for sodium ions in the CNS. The increases in blood pressure and sympathetic hyperactivity caused by ICV injection of hypertonic saline are abolished with ICV pretreatment with benzamil, a selective ENaC blocker.<sup>196</sup> Wang *et al.*<sup>197</sup> studied the relationship between ENaC and ouabain in the CNS and found that ICV injection of low-dose aldosterone in artificial cerebrospinal fluid with elevated sodium content markedly elevated blood pressure and sympathetic activity; these effects were abolished by ICV pretreatment with benzamil. Furthermore, ICV pretreatment with digibind, the Fc fragment of the anti-digoxin antibody that blocks ouabain's inhibition of the Na<sup>+</sup>,K<sup>+</sup>-ATPase, also significantly suppressed the hypertensive activity of aldosterone and hypertonic artificial cerebrospinal fluid. This group conducted a series of studies of these relationships,<sup>198–202</sup> and others reported similar findings.<sup>203</sup>

Taken together, these findings indicate that ENaC may sense Na<sup>+</sup> in CSF and stimulate aldosterone production. This triggers the release of ouabain, which in turn activates sympathetic outflow and causes hypertension.

#### THE RELATIONSHIP BETWEEN THE RAAS AND OUABAIN IN THE BRAIN

As noted, pressor responses and increases in plasma DLI after ICV injections of hypertonic saline are abolished by ICV pretreatment with ARB.<sup>182</sup> The renin–angiotensin system is upregulated in rats fed a high-salt diet, as shown by increased expression of renin,<sup>176</sup> and ACE and angiotensin type-1 receptor mRNA.<sup>177</sup> ICV injection of Ang II causes greater pressor responses in rats fed a high-salt diet than in rats with normal sodium levels. Augmented central pressor responses to Ang II in sodium-loaded rats are supported by the work of Houghton *et al.*<sup>26</sup> Increased production of Ang II in the hypothalamus in response to sodium loading has been shown using microdialysis,<sup>204</sup> and there is sodium retention in rats with renal failure, indicating that the brain renin–angiotensin system is upregulated.<sup>205</sup> Pressor responses to ICV infusion of high-sodium artificial cerebrospinal fluid are blocked by ICV pretreatment with spironolactone, an MRB.<sup>197</sup> Thereby, hypothalamic aldosterone is increased and OLI content is decreased in both the hypothalamus and pituitary gland by spironolactone. This indicates that the higher levels of sodium ion in the artificial cerebrospinal fluid stimulated production of aldosterone and ouabain. The enzymes required for aldosterone synthesis from cholesterol are expressed in the brains of both rats<sup>206,207</sup> and humans,<sup>208</sup> and there are mineralocorticoid receptors (MRs) in the brain.<sup>207</sup> Dahl salt-sensitive rats have higher hypothalamic aldosterone levels than in Sprague–Dawley rats.<sup>209</sup> These findings suggest that increased sodium ion levels trigger activation of the RAAS, releasing ouabain and leading to sympathetic activation as indicated by Huang *et al.*<sup>210</sup> However, ICV treatment with aldosterone elevates renin–angiotensin system activity in the brain.<sup>211</sup> Therefore, there are complex interactions involving renin, angiotensin, aldosterone and ouabain in the brain. Ouabain acts downstream of aldosterone in this scenario, because spironolactone blocks the sodium ion-dependent increase in brain OLI.<sup>197</sup>

To summarize, the RAAS in the brain is activated by sodium ions, which causes pressor responses via activation of the sympathetic nervous system.

### CENTRAL ACTIONS OF ANG II AND ALDOSTERONE OF PERIPHERAL ORIGIN

It has been known for at least 40 years that Ang II injected into vertebral arteries causes pressor responses and sympathetic activation.<sup>27,212</sup> ICV injection of Ang II also elicits pressor responses along with sympathetic overactivity.<sup>171–175</sup> These findings show that Ang II of peripheral or central origin directly affects the central vasomotor center to cause sympathetic activation. In fact, peripheral administration of Ang II causes sympathetic activation irrespective of the pressor response.<sup>213,214</sup> However, generally speaking, pressor responses to subcutaneous or intravenous injections of Ang II were long thought to be due to Ang II's direct vasoconstrictive activity and inotropic actions on the heart because Ang II is a very potent vasoconstrictor *in vivo*. The finding in 2010 that pressor responses to systemic administration of Ang II are mediated exclusively via the CNS was thus surprising.<sup>215</sup> In that study, ICV pretreatment with an aldosterone synthase inhibitor abolished pressor responses to subcutaneous infusion of low-dose Ang II. When the dose of Ang II was high, the pressor response was only partially inhibited because the direct peripheral effects overcame the central effects. Moreover, the inhibitory effects could be reproduced by ICV pretreatment with either eplerenone or digibind.<sup>215</sup> Another research group reported similar findings: ICV infusion of an MRB, RU28318, almost completely blocked the pressor responses to subcutaneous infusions of Ang II.<sup>216</sup> Furthermore, chronic intravenous infusion of Ang II gradually increased blood pressure, reaching a plateau level after about 2 weeks.<sup>217</sup> Fra-like activity, an indicator of chronic neuronal activation, was increased in the PVN in that study. ICV treatment with losartan (an ARB), tempol (a reactive oxygen scavenger) or pyrrolidine dithiocarbamate (an NF- $\kappa$ B inhibitor), all abolished hypertensive responses to intravenously infused Ang II.

These mechanisms of actions of Ang II are very different from its established roles in constricting arteries and increasing cardiac muscle contractility. However, these studies show that aldosterone production may be induced by Ang II via angiotensin type-1 receptors in the brain, once again suggesting that the brain RAAS is acting to regulate systemic circulation. We may have to change our concept of aldosterone's mechanism of action as well. Aldosterone acts on renal tubules, inducing sodium retention, and also exerts inotropic effects on the heart.<sup>218–220</sup> However, these effects may not underlie hypertension induced by aldosterone; instead, aldosterone's site of action may be the hypothalamus.

The finding that the pressor responses to Ang II are abolished by tempol and pyrrolidine dithiocarbamate indicates that Ang II leads to oxidative stress in the brain.<sup>217</sup> Similar findings showing that oxidative stress in the brain is reduced by olmesartan, an ARB, have been reported in stroke-prone spontaneously hypertensive rats.<sup>221</sup> Renal sympathetic discharge caused by ICV injection of Ang II is suppressed by ICV treatment with tempol, supporting the idea that Ang II elicits an oxidative stress reaction in the brain to cause hypertension.<sup>222</sup> When aldosterone is infused subcutaneously in rats with supplementation of 1% saline as drinking water for 4 weeks, blood pressure gradually increases by about 30 mm Hg with concomitant increases in (salty) water drinking.<sup>216</sup> In that study, when irbesartan (an ARB), RU28318 or spironolactone were infused ICV with osmotic minipumps, the pressor responses caused by subcutaneous aldosterone plus salt loading were abolished in all three pretreatment groups,<sup>216</sup> and the increase in

saline intake was lower. As these pressor responses were inhibited by nicotinamide adenine dinucleotide phosphate inhibitors, that is, apocynin or tempol, aldosterone must be acting as an oxidative stressor.<sup>216</sup> However, the drinking behavior was not blocked by apocynin or tempol, in contrast to the actions of ARB and MRB. Therefore, salt appetite and pressor mechanism may be regulated independently in the brain. Further, drinking salty water is not directly related to aldosterone's pressor activity, although it may affect the long-term control of blood pressure. These findings are supported by a report that showed that blood pressure increases accompanied by sympathetic activation were ameliorated by RU28318, losartan or tempol.<sup>211</sup>

Both RAAS and sympathetic nervous system activity are increased in patients with heart failure. Specifically, plasma and hypothalamic aldosterone levels are increased in a rat ischemia-induced heart failure model.<sup>223</sup> Further, the rats with heart failure showed higher mRNA and protein expression levels of ACE and Ang II type-1R, and expression was suppressed by ICV treatment with RU28318.<sup>223</sup> RU28318 also suppressed the excitation of PVN neurons in the hypothalamus, and the plasma level of norepinephrine was lower. Thus, aldosterone of adrenocortical origin appears to reach the hypothalamus, triggering a series of events accompanied by the progression of heart failure. As systemic administration of aldosterone causes hypertension, which is abolished by a small dose of ICV-infused MRB,<sup>216</sup> these observations show that aldosterone is actually acting at MRs in the CNS to cause sympathetic excitation and hypertension. The renin-angiotensin system may be downstream of the MR, because aldosterone elicits oxidative stress to activate the renin-angiotensin system (because MRB decreases nicotinamide adenine dinucleotide phosphate-mediated superoxide production in the hypothalamus). An excellent review article describing oxidative stress in the CNS and sympathetic activation is appeared recently.<sup>224</sup>

These findings indicate that Ang II and aldosterone independently cause oxidative stress in the brain, increasing sympathetic activation and leading to increases in blood pressure. They further indicate that aldosterone upregulates the renin-angiotensin system. These novel findings regarding the central actions of Ang II and aldosterone are exciting for people working in the field of hypertension research.

### CENTRAL MECHANISMS OF HYPERTENSION

The CNS regulates blood pressure, and the baroreceptor reflex mechanism acts as a homeostatic mechanism for stabilizing blood pressure.<sup>225</sup> However, baroreceptors only stabilize blood pressure at the set point rather than actually determining the set point.<sup>226–228</sup> Regulation occurs via the medulla oblongata, and the set point is thought to be determined by the higher center of the central vasomotor control, possibly in the hypothalamic nuclei, that is, the PVN and supraoptic nucleus.<sup>229</sup> The baroreceptor set point in hypertensive individuals is shifted to a higher level, and their baroreceptor sensitivity is lowered.<sup>230</sup> When ACEI is administered systemically to decrease circulating Ang II, the set point shifts to a normal level as does the baroreceptor sensitivity.<sup>230</sup> Even when blood pressure is increased to hypertensive levels by phenylephrine infusion, the set point remains in the normal range. This indicates that Ang II is essential for determining the blood pressure set point. Consequently, blockade of the renin-angiotensin system restores blood pressure to normal. Although it seems logical that ACEI decreases Ang II in the peripheral circulation, it may act on the CNS. In either case, because the CNS is the control center for circulation, Ang II acts at the central site to shift the blood pressure set point.

As the brain RAAS is activated when there is excessive intake of sodium salt, the effect is opposite that of ACEI administration: the blood pressure set point is raised, that is, hypertension develops. Needless to say, EDLF is important in the central mechanism of action of the sodium–RAAS cascade.

### CENTRAL EFFECTS OF OTHER STEROID HORMONES

Although centrally induced vasopressor responses have been documented for aldosterone,<sup>209,210</sup> the central effects of glucocorticoids and sex hormones are less well established. Our group showed that cortisol acts at a central site to increase sympathetic outflow and cause vasopressor responses that are abolished by ICV pretreatment with ACEI or [1-Sar, 8-Ileu] Ang II.<sup>231</sup> Cortisol is converted to cortisone by 11 $\beta$ -hydroxy steroid dehydrogenase-2 locally at the affected site.<sup>232</sup> MRs have equal affinity for aldosterone and cortisol,<sup>233</sup> but because cortisol is converted to cortisone by 11 $\beta$ -hydroxy steroid dehydrogenase-2 before acting at MRs, cortisol usually does not affect MRs. However, if 11 $\beta$ -hydroxy steroid dehydrogenase-2 is absent in tissues where MRs are present, cortisol can bind to MRs and have the same effects as aldosterone. It is clear that MRs are expressed in the hypothalamus,<sup>234</sup> but 11 $\beta$ -hydroxy steroid dehydrogenase-2 is barely detectable there.<sup>235,236</sup> Thus, when circulating cortisol reaches its sites of actions in the CNS, it may directly affect MRs, increasing sympathetic activity and hypertension when circulating cortisol reached at the site of actions in the CNS. If the response was caused by stimulation of MR by cortisol, renin-angiotensin system may be at the down-stream of mineralocorticoid. Although no one has tested, EDLF may also be involved in this cascade.

Conjugated estrogen also elicits centrally induced vasopressor responses, which can be abolished by blocking actions of AngII,<sup>237</sup> which is very similar to the central action of cortisol and aldosterone.

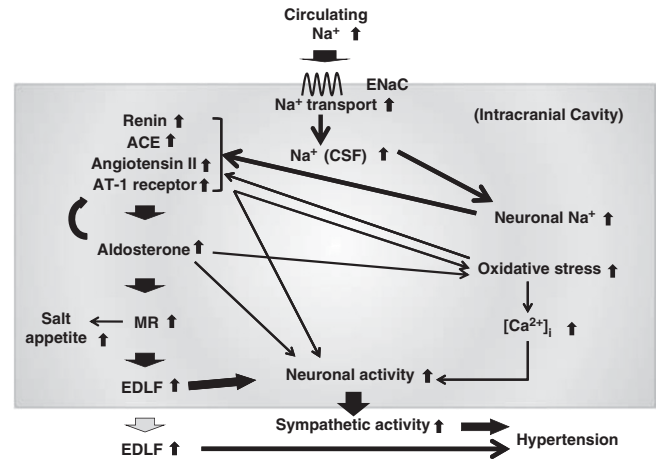
Collectively, not only aldosterone but also glucocorticoids and sex hormones may be acting at the CNS to cause hypertension, corresponding to Cushing's syndrome and pregnancy-induced hypertension in a clinical setting, respectively.

### A HYPOTHESIS IN GENESIS OF HYPERTENSION

When we considered these epidemiological and experimental facts mentioned earlier, a hypothesis may appear. The human who evolved in the salt-deficient environment, has strong salt appetite together with a powerful mechanism to retaining sodium. The RAAS and the sympathetic nervous system made it possible for humans to survive during the Stone Age, because higher blood pressure levels allowed humans to respond and fight enemies and wild animals by increasing the blood supply to the heart and skeletal muscles.

The renal RAAS developed so that changes in the sodium balance could be corrected quickly using negative feedback. In addition, another powerful positive feedback system, possibly the brain EDLF/RAAS, controls long-term maintenance of sodium retention and maintains elevated blood pressure. The sodium balance is thus controlled by opposing forces in the CNS and in the periphery.

Humans may have evolved to be prone to elevated blood pressure via excitation of sympathetic nerve activity in response to stimuli such as sodium loading. The switch in modern times to a diet that includes a lot of salty food has led to elevated blood pressure, which may cause essential hypertension. What, then, is the cause of secondary hypertension? In renovascular hypertension, activation of the RAAS in the CNS causes hypertension directly via Ang II and aldosterone. In patients with primary aldosteronism, increased aldosterone directly affects the CNS. Sodium retention because of hyperaldosteronism may



**Figure 3** A proposed cascade showing sympathetic activation in the brain and the development of hypertension, which is triggered by elevated circulating sodium ion levels. Sodium ions are absorbed via epithelial sodium channels, further activating the brain renin–angiotensin–aldosterone system. Aldosterone stimulates the production of endogenous digitalis, possibly ouabain, in the paraventricular and supraoptic nuclei to trigger sympathetic activation. Angiotensin II and aldosterone cause oxidative stress, which also stimulates sympathetic outflow in the central nervous system. A full color version of this figure is available at the *Hypertension Research* journal online.

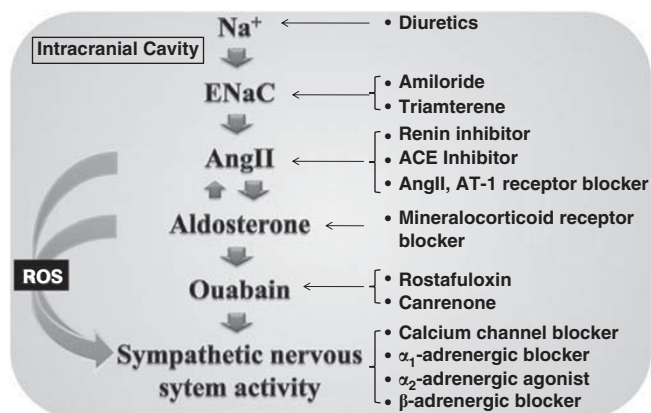
also contribute to centrally induced hypertension. Sodium retention because of impaired renal function may account for the response to sodium loading in patients with renal hypertension and in older hypertensive patients with impaired renal function.

In conclusion, CNS control of sodium metabolism underlies every type of hypertension, including essential hypertension, primary aldosteronism, pheochromocytoma, renovascular hypertension, renal hypertension and pregnancy-induced hypertension (Figure 3).

### ANTI-HYPERTENSIVE AGENTS AND THE CENTRAL MECHANISM OF BLOOD PRESSURE REGULATION

The key factors in the sodium-induced mechanisms of action of hypertension include Na<sup>+</sup>, ENaC, the RAAS, EDLF, oxidative stress and the CNS sympathetic nervous system. Agents that influence these factors can either induce or decrease hypertension (Figure 4). Antihypertensive agents in particular must affect these factors; otherwise, treatment with antihypertensive agents would be accompanied by adverse circulatory events. That is, if the baroreceptor reflex set point is not lowered, the CNS will struggle to restore blood pressure to a higher level. For example, vasodilators such as hydralazine<sup>238</sup> and short-acting dihydropyridine CCBs<sup>239</sup> often cause angina pectoris by increasing sympathetic tone. In fact, although excellent antihypertensive agents are now available, many antihypertensive agents have been screened during the long clinical history of hypertension treatment, only to be rejected because of adverse events.<sup>240,241</sup> A number of large-scale clinical trials have evaluated the prognosis of current antihypertensive agents and provided evidence of their efficacy.<sup>242–245</sup>

Diuretics lower blood pressure by affecting the central mechanism of hypertension. Specifically, diuretics lower the concentration of circulating Na<sup>+</sup>, decreasing sympathetic outflow. However, decreased venous return to cardiopulmonary region because of circulating volume contraction decreases the low-pressure baroreceptor reflex tone (the sensors are located in the cardio-pulmonary region) and increases sympathetic activity.<sup>246</sup> As a result, sympathetic activity



**Figure 4** The cascade that causes sympathetic hyperactivity in the brain and the antihypertensive agents that target components in this cascade. Currently available antihypertensive agents appear to act at the level of the central nervous system to decrease sympathetic outflow. Therefore, reflex tachycardia is absent when blood pressure is lowered with these agents, that is, the baroreceptor set point is reset to a lower level in patients treated with these antihypertensive agents. A full color version of this figure is available at the *Hypertension Research* journal online.

increases after treatment with diuretics in a clinical setting,<sup>247</sup> but blood pressure is decreased and sympathetic hyperactivity is minimal probably due to the central actions of decreased  $\text{Na}^+$  concentrations. Thus, diuretics end up decreasing sympathetic activity and do not result in reflex tachycardia when blood pressure is significantly decreased.<sup>248</sup>

Local blockade of ENaCs in the CNS should reduce blood pressure in humans as well as in animal models. In fact, amiloride and triamterene, which are used to treat hypertension,<sup>249,250</sup> are thought to act at the distal renal tubules to suppress  $\text{Na}^+$  and  $\text{K}^+$  exchange in the kidney and thereby lower blood pressure. It is also possible that these agents act directly on the central site.

Blockade of the RAAS with a direct renin inhibitor, ACEI, ARB or MRB is powerful treatment for almost all types of hypertension.<sup>251–254</sup> These agents most likely act on the most essential part of the central mechanism of hypertension and are considered the best antihypertensive agents because of positive results from a number of large-scale clinical trials.<sup>242–245,255</sup> All are used worldwide. Systemic administration of ARB in rats prevents sympathetic hyperactivity and hypertension caused by ICV injection of hypertonic saline<sup>256</sup> or Ang II.<sup>257</sup> Possibly because these agents act on the central mechanism of hypertension, they have few adverse effects. The prognosis of hypertensive patients treated with these agents is also good as the agents prevent cardiovascular complications.<sup>258–262</sup>

Agents that block EDLF actions may be candidates for antihypertensive agents, with PST2238 (rostafuroxin) serving as a prototype drug.<sup>88</sup> Although there were no significant hypotensive effects in a clinical trial in a general population,<sup>92</sup> very effective hypotension was achieved via increased plasma ouabain levels in patients with genetic variants such as those in adducin 1, lanosterol synthase, hydroxyl-*d*-5-steroid dehydrogenase and ATP-binding cassette sub-family B member 1.<sup>90,263</sup> Therefore, agents that selectively affect EDLF activity may be worth developing.

Sympatholytic agents such as  $\alpha_2$ -adrenergic agonists and imidazoline receptor agonists are also useful antihypertensive agents.<sup>264,265</sup> Those are acting at the vasomotor center in the lower brain stem to decrease sympathetic outflow, which again suggest that sympathetic nervous system activity is a key factor for regulation of blood pressure.

However, adverse effects such as drowsiness and dry mouth limit the use of these agents, particularly  $\alpha_2$ -adrenergic agonists.

The  $\alpha_1$ -adrenoceptor blocker is thought to act on  $\alpha_1$ -receptors in the peripheral arterial wall to dilate vessels, which is the principal effect of this agent.<sup>266</sup> If this blocker works purely as a vasodilator, reflex tachycardia will occur in response to hypotension and the antihypertensive actions will be limited. In fact, pulse rate does not increase in response to hypotension caused by prazosin, doxazosin and bunazosin.<sup>267</sup> When injected intravenously in anesthetized rats, bunazosin lowers blood pressure by suppressing sympathetic outflow.<sup>268,269</sup> Therefore, we assume that these  $\alpha_1$ -receptor blockers act directly at central  $\alpha_1$ -receptors to suppress sympathetic outflow. This may be why treatment with these agents does not result in reflex tachycardia.

CCBs are widely used because they are potent antihypertensive agents with minimal adverse effects. Again, this kind of agent does not induce reflex tachycardia regardless of its hypotensive effect, except when it acts rapidly.<sup>270,271</sup> Some agents act directly on cardiac muscle to suppress pacemaker activity.<sup>272,273</sup> Even with dihydropyridine CCBs, which have less of an effect on the pacemaker, reflex tachycardia is usually absent and the heart rate may decrease.<sup>271</sup> Of course, reflex tachycardia occurs when a potent CCB like nifedipine is administered and rapidly lowers blood pressure.<sup>274</sup> However, when blood pressure is lowered gradually with slow-release nifedipine or amlodipine, reflex tachycardia is absent; instead, there is bradycardia.<sup>275,276</sup> Thereby, peripheral sympathetic tone is decreased.<sup>276</sup> Therefore, when CCBs are administered, there are effects that are very similar to those induced by  $\alpha_1$ -blockers. Diltiazem given intravenously causes hypotension and bradycardia without peripheral sympathetic excitation.<sup>277</sup> ICV injections of diltiazem elicit vasodepression with decreases in abdominal sympatholytic activity, which is attenuated by electric ablation of the hypothalamic anteroventral third ventricle area.<sup>277</sup> Similar findings have been reported with nifedipine in rats on a high-salt diet.<sup>278</sup>

Beta-adrenergic blockers cause centrally induced vasodepression: ICV injections of propranolol, a representative  $\beta$ -blocker, elicits vasodepressor responses accompanied by suppression of sympathetic outflow.<sup>279,280</sup> Intravenous injections of propranolol act on the brain to raise the local concentrations to levels similar to those observed after ICV injection to induce hypotension.<sup>281</sup> Those will be the reason why  $\beta$ -blockers are widely used for treatment of patients with congestive heart failure, who have elevated peripheral sympathetic tone.

The blood–brain barrier blocks the entry of most substances into the brain. Therefore, except for centrally acting  $\alpha_2$ -adrenergic agonists, antihypertensive agents do not generally reach CNS sites. On the other hand, circumventricular organs such as the subfornical organ and the organum vasculosum of the laminae terminalis are critical centers for blood volume and blood pressure regulation.<sup>282–286</sup> The arterial architecture differs in the organum vasculosum of the laminae terminalis and in the area postrema, so there are also differences in terms of the solutes that permeate them.<sup>287</sup> These areas may serve as overall sensors for body fluid and circulatory regulation. The anteroventral third ventricle area, which includes the organum vasculosum of the laminae terminalis, has been implicated in the genesis of several types of experimental hypertension<sup>288–293</sup> and may include a network of neurons that regulate electrolyte balance and sympathetic tone. Circumventricular organs have a less selective blood–brain barrier and may directly (chemically) sense the circulatory environment. When the subfornical organ is electrically ablated, pressor responses to chronic subcutaneous infusion of Ang II are attenuated<sup>27</sup> and the



antihypertensive effects of ARB are decreased. Thus, Ang II and ARB may act on the subfornical organ.

The common characteristic of the first and second choice of antihypertensive agents recommended by clinical guidelines<sup>294,295</sup> is that they do not result in reflex tachycardia during hypotension. This means that a baroreflex system senses that the blood pressure level because of antihypertensive agents is the correct one for the patient. Current antihypertensive agents may reset the baroreceptor reflex threshold to a lower level, a process that must be controlled by the CNS rather than by peripheral barosensors. As noted, antihypertensive agents would have suppressed sympathetic nervous system activity in which level blood pressure converge to restore the sympathetic activity.

Thus, the ENaC–RAAS–EDLF system in the CNS may elicit sympathetic hyperactivity in the sodium-loaded state, leading to an elevation in the blood pressure set point. Sodium retention occurs not only because of excessive intake of sodium salt but also because of decreased excretion of sodium because of impaired renal function in renal hypertension, primary aldosteronism, insulin resistance and senile essential hypertension with renal impairment. Sodium retention is the common underlying cause of almost all types of hypertension. Therefore, treatments for hypertension, including diuretics, ACEI, ARB, MRB,  $\alpha_1$ -blocker,  $\alpha_2$ -agonist and  $\beta$ -blocker, all interfere with this cascade to break the chain that leads to hypertension.

The antihypertensive agents screened during the long history of antihypertensive therapy allow us to lower blood pressure comfortably and improve prognosis. This may indicate that these antihypertensive agents act at the origin of hypertension, that is, the CNS. Therefore, with this understanding of the genesis of hypertension, efforts should focus on novel antihypertensive agents that selectively target this cascade.

## FUTURE TASKS

Despite the long history of hypertension research, some of the possible mechanisms of action of antihypertensive agents have been ignored. Although the brain controls the sympathetic nervous system (and thus hypertension), it seemed unlikely to researchers that antihypertensive therapies targeted the brain. This may be why it took so long to elucidate the pathogenesis of hypertension. We can now forget that such agents can change patients' personalities and may have other serious side effects. Physiological variables such as respiration and body temperature are controlled by the CNS, and the CNS is the overall regulator of many physiological functions, including blood pressure.<sup>29</sup> Sympathetic activation represents a hallmark of the essential hypertensive state and its complications.<sup>296</sup> Some issues remain to be clarified, including the following:

- (1) Does ouabain, digoxin or another Na<sup>+</sup>,K<sup>+</sup>-ATPase inhibitor function as an EDLF in the CNS?
- (2) The evidence establishing the connections in the Na–ENaC–RAAS–EDLF network remains incomplete.
- (3) The expression of genes associated with the Na–ENaC–RAAS–EDLF network must be determined.
- (4) Most evidence supporting the central role of this network in blood pressure regulation was obtained in animal models or cultured cells. We must determine whether there is a similar series of responses in humans.

Despite these remaining questions, the paths that lead to hypertension are becoming clearer, and a full understanding of the mechanisms underlying hypertension may be close at hand.

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