

CARDENOLIDE AND BUFADIENOLIDE LIGANDS OF THE SODIUM PUMP. HOW THEY WORK TOGETHER IN NaCl SENSITIVE HYPERTENSION

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1. ABSTRACT

For the past 50 years biomedical scientists have been in quest of an unidentified factor (hormone) that elevates blood pressure and regulates renal sodium transport, i.e., natriuretic hormone. Recent discoveries have led to the identification of such factors which are present in humans, rodents and amphibians, and which, in a complex manner, interact with each other and with the other regulatory systems. In experimental NaCl sensitive hypertension brain endogenous ouabain, via activation of renin-angiotensin system and of sympathetic nervous system, stimulates adrenocortical production of marinobufagenin, a natriuretic and a vasoconstrictor. The combined effects of these endogenous factors may account for the classical properties attributed by Dahl, deWardener and others to the hypothetical “natriuretic hormone”.

2. INTRODUCTION. CONCEPT OF DIGITALIS-LIKE NATRIURETIC HORMONE

The NaCl-dependent hypertension is thought to be due, at least in part, to a compromised ability of the kidneys to excrete sodium, and this is mediated by genetic and environmental factors (1). Many factors have been considered that might modulate the complex relationship between dietary NaCl intake and hypertension. One such factor is Na/K-ATPase and its endogenous inhibitors, digitalis-like substances.

Lewis Dahl hypothesized that salt-induced hypertension is mediated by a humoral factor which raises the blood pressure (2). Based on numerous experimental observations, deWardener and others (3, 4) came to the conclusion that a humoral prohypertensive factor implicated in the pathogenesis of NaCl-sensitive

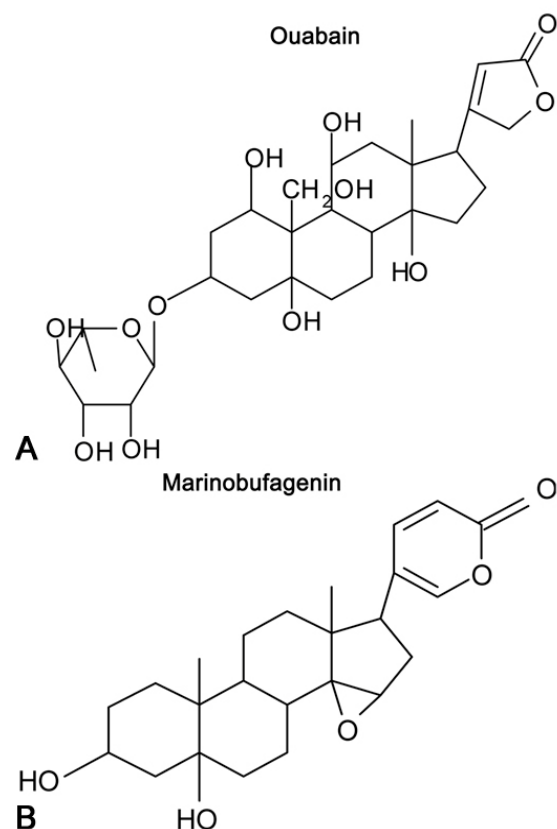


Figure 1. Chemical structure of (A) cardenolides (ouabain) and (B) bufadienolides, (marinobufagenin).

hypertension is an endogenous natriuretic. Since a membrane enzyme, Na/K-ATPase, comprises a major sodium transporting mechanism in the renal tubules, and since digitalis glycosides act as specific ligands of the Na/K-ATPase, it has been postulated that the putative natriuretic hormone is similar to digitalis (3-5). According to the "concept of natriuretic hormone", endogenous digitalis-like sodium pump ligands (SPL) were thought to link sodium/fluid retention to regulation of blood pressure. In other words, although the primary role of endogenous SPL is to promote natriuresis via inhibition of sodium reabsorption in the renal proximal tubules, increased plasma concentrations of SPL can contribute to vasoconstriction via inhibition of the Na/K-pump, coupled with activation of $\text{Na}^+/\text{Ca}^{2+}$ exchange in vascular smooth muscle (6). In support of this view, plasma levels of SPL were shown to be sensitive to plasma volume expansion (5) and to correlate with the level of blood pressure in patients with essential hypertension (7).

3. ENDOGENOUS OUABAIN

The concept of natriuretic hormone was based on a presumption that the primary role of SPL is to induce natriuresis via inhibition of the sodium pump in renal tubules. The discovery of an endogenous ouabain-like substance (EO) (8) and demonstration of its role in the development and maintenance of several forms of experimental hypertension (9) was a major step in the

development of views of digitalis-like natriuretic factors (Figure 1A). However, ouabain exhibits high affinity for the α -2/ α -3 isoforms of Na/K-ATPase, while tubular cells of the mammalian kidney express mainly the α -1 isoform, which is relatively insensitive to ouabain (10). Moreover, clinical and experimental studies demonstrated that EO is not stimulated by acute saline plasma volume expansion (11, 12) or by chronic administration of a high NaCl diet (13). Rather, levels of circulating EO become elevated following NaCl restriction (13).

Although ouabain is not a natriuretic and peripheral levels of EO are not stimulated by high NaCl intake, a body of evidence is emerging indicating that brain EO plays a key role in the pathogenesis of NaCl-sensitive hypertension. First, in a series of experimental studies performed by Takahashi's group, central administration of low concentrations of ouabain were shown to elicit pressor responses and to induce natriuresis (14, 15). The pressor responses elicited by central administration of ouabain were shown to be dependent on the activation of the renin-angiotensin system (RAS) (16). Subsequently, in Dahl salt-sensitive (DS), Wistar, and spontaneously hypertensive rats, brain EO was found to be responsive to acute and chronic NaCl loading (17). Pressor responses to central administration of ouabain and to NaCl induced increases in brain EO levels were found to be mediated via activation of RAS and by the resultant activation of the sympathetic nervous system (18, 19). In parallel with a NaCl induced activation of brain EO, plasma Na/K-ATPase inhibitory activity also substantially increased (20). However, this increase was not associated with the elevations of plasma ouabain-like immunoreactivity (21). Thus, additional endogenous SPL must have existed but remained to be identified.

4. ENDOGENOUS BUFADIENOLIDES

For decades it had been known that Amphibia are capable of producing cardioactive steroids of a Bufadienolide nature. Bufadienolides differ from cardenolides in having a doubly unsaturated, six-membered lactone ring (22). The highest levels of bufadienolides occur in those amphibian species which migrate from dry to aquatic environment. Considering that skin is a major organ for regulation of water/electrolyte homeostasis in Amphibia, it was hypothesized that the sodium pump and bufadienolides in the skin represent a system which regulates water/electrolyte balance (23). Brain and skin levels of bufadienolides in the toads change according to the changes in environmental salinity (24). This observation suggests that bufadienolides represent a natriuretic hormone for Amphibia, i.e. an endogenous digitalis-like immunoreactive Na/K-ATPase inhibitor which is responsible for sodium excretion.

These observations prompted a search for a mammalian bufadienolide. First, Kieval, et al. and Goto, et al. discovered bufalin-immunoreactive material in human bile and plasma (25, 26). Subsequently, presence of bufalin-like immunoreactivity in human plasma was

reported by two other groups (27, 28). Lichtstein, et al. demonstrated the presence of bufalin derivatives in the eye lenses of several mammalian species and proposed a role for these compounds in cataract formation (29). Sich, et al. reported that human plasma and bovine adrenals contain material which cross-reacts with an antibody against a plant-derived bufadienolide, proscillaridin A (30). In 1996, Hilton and co-workers identified a bufadienolide compound in human placenta via mass-spectrometry (31). Later, the same groups identified the presence of bufadienolides(s) in plasma of several volume-expanded subjects (32).

A series of experimental and clinical studies have indicated that at least one of the circulating mammalian SPLs is marinobufagenin (MBG), a steroid previously described in several amphibian species. MBG emerged as a candidate SPL based on the studies of pharmacological properties of the venom of *Bufo marinus* toad (33, 34). Bagrov, et al. showed that the theoretical requirements for a mammalian SPL were met in a series of studies that demonstrated that the venom from *Bufo marinus* toad contains digoxin-like immunoreactive material which evokes vasoconstriction, inhibits the Na/K pump from several tissues, and induces a positive inotropic effect (33). Subsequently, this digoxin-like substance was identified as a previously described bufadienolide, marinobufagenin (Figure 1B) (34). An antibody against MBG was found to cross-react with material from human, canine and rat plasma and/or urine (12, 35-37). In normotensive rats, plasma MBG was stimulated by acute plasma volume expansion and by chronic administration of a high NaCl diet (37, 38). Enhanced production of MBG-immunoreactive factor was observed in patients with preeclampsia (39), and in several hypertensive volume-expanded states, essential hypertension, primary aldosteronism, and end-stage renal disease (40). In hypertensive patients with congestive heart failure, plasma MBG levels exhibited progressive increases, varied with the severity of cardiac failure, and correlated with LV systolic function similarly to alpha-human atrial natriuretic peptide (41). Moreover, MBG-immunoreactive material purified from human urine, mass-spectrometrically was indistinguishable from amphibian MBG (42). Recently, the presence of MBG in human plasma has been confirmed by Takahashi's group (43).

At concentrations comparable to *in vivo* plasma levels of this hormone, MBG exhibited vasoconstrictor properties in isolated human pulmonary and mesenteric arteries (37, 45). Na/K-ATPase inhibitory effects of MBG and ouabain were compared in two membrane fractions isolated from rat thoracic aorta by sucrose density gradient centrifugation. One fraction contained predominantly the alpha-3 isoform of Na/K-ATPase and represented membranes from the perivascular nerve endings. The other membrane fraction, containing predominantly the alpha-1 isoform, was derived from the plasmalemma. The IC_{50} for inhibition of the Na/K-ATPase by ouabain and MBG were 1 nmol/L and 0.5 μ mol/L in the neuronal membrane fraction, and 0.1 μ mol/L and 10 nmol/L in plasmalemmal fraction, respectively (45). Thus, MBG acted as a selective

inhibitor of the alpha-1 isoform of the sodium pump, an exclusive isoform in renal tubules. Accordingly, in a subsequent experiment MBG potentially inhibited the ouabain-resistant Na/K-ATPase from rat kidney (46).

5. DIGITALIS-LIKE FACTORS IN DAHL-S RATS ON A HIGH NaCl INTAKE

Dahl performed his experiments that pointed to the existence of a prohypertensive natriuretic hormone on DS, an inbred strain of rats which while on a high NaCl intake develops progressive hypertension. In several aspects, NaCl-induced hypertension in DS resembles salt-sensitive hypertension in humans. DS placed on an 8% NaCl diet, exhibited a progressive increase in the plasma concentration and renal excretion of MBG immunoreactive material in parallel with the increases in blood pressure (47). When hypertensive DS were acutely treated with anti-MBG antibody, blood pressure markedly decreased, while anti-ouabain antibody did not exert such an effect (47). Therefore, elevated levels of MBG contributed to the maintenance of high blood pressure in this genetic form of NaCl sensitive hypertension. When MBG immunoreactive material was purified from urine of hypertensive DS, it was chromatographically identical to amphibian MBG; and acted as a potent inhibitor of renal (alpha-1 isoform) versus brain (alpha-3 isoform) Na/K-ATPase (48). Thus, MBG satisfies both major criteria for a circulating natriuretic hormone, i.e., it acts as a selective inhibitor of renal Na/K-ATPase and raises the arterial pressure.

When central and peripheral levels of EO and MBG were compared in both chronically and acutely NaCl-loaded DS, the responses of both substances exhibited similar patterns. Levels of EO in the pituitary, adrenals and plasma exhibited a transient peak response which was followed by a sustained rise in plasma levels and renal excretion of MBG (46, 47). Such a consequence of events suggests that a causative relationship exists between an acute EO response and a sustained elevation in MBG, i.e., that brain EO triggers MBG production. In accordance with this hypothesis, pretreatment of the acutely NaCl-loaded rats with anti-MBG antibodies did not attenuate the EO response, while pretreatment with anti-ouabain antibody markedly decreased NaCl-induced renal MBG excretion (47).

6. RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM LINKS CENTRAL AND PERIPHERAL Na/K-ATPase INHIBITORS

For many years, high NaCl intake was considered to reduce activity of the RAS, and the resultant hypertension was, therefore, termed "low renin hypertension". Although activity of circulating RAS, i.e., plasma renin activity, indeed decreases following NaCl loading, growing evidence indicates that NaCl induces activation of local tissue RAS. First, results of several studies clearly demonstrated efficacy of ACE inhibitors and angiotensin receptor blockers in hypertensive DS, although plasma renin activity in these animals is suppressed. Thus,

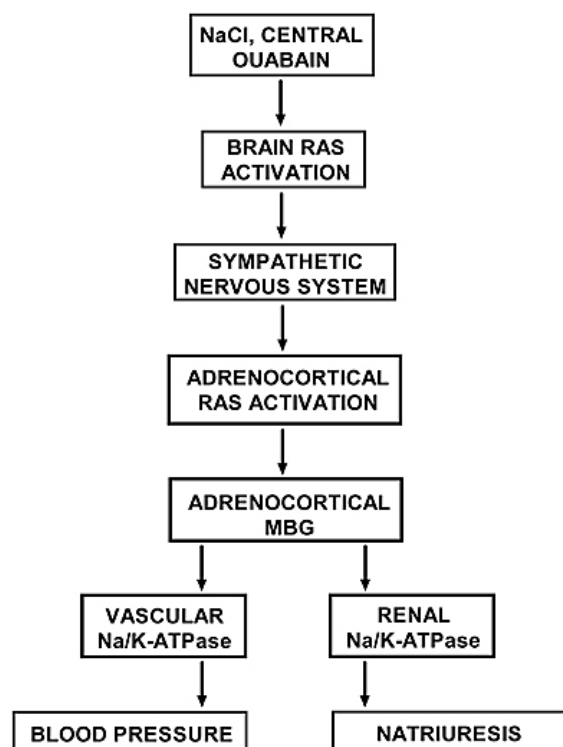


Figure 2. Renin-angiotensin system mediates interaction between central and peripheral digitalis-like sodium pump ligands in NaCl sensitive hypertension.

pharmacological antagonism of RAS in DS results in the reduction of arterial pressure and improvement in both cardiac performance and renal function (49, 50). In the same experimental model, a high NaCl diet, in the absence of changes in plasma levels of angiotensin II (ATII), markedly increases aortic levels of angiotensinogen which occurred in parallel with a 3.5-fold increase in the number of aortic ATI receptors in the aorta (51). NaCl loading of DS induced substantial elevation of renal levels of ATII (52). Finally, as demonstrated by Leenen's group, EO-induced activation of brain RAS was shown to mediate the NaCl-induced pressor responses in DS and in the spontaneously hypertensive rats (17). Thus, it appears that NaCl-induced suppression of the circulating RAS may be associated with an activation of local tissue RAS. Although the clinical relevance of the above findings remains to be elucidated, clinical literature exists that supports the view that high NaCl intake activates RAS in cardiovascular tissues (53).

Considering that, in previous experiments, NaCl loading of DS induced transient response of brain EO followed by a sustained rise in MBG levels (46, 47), and that administration of anti-ouabain antibody to DS prevented NaCl induced increases in MBG and blood pressure (47), we hypothesized that brain RAS may be a factor linking central (EO) and peripheral (MBG) SPL. Recently, it was demonstrated that MBG is produced by cultured adrenocortical cells (54). We hypothesized that activation of RAS in the brain and adrenal cortex, and

activation of sympathetic nervous system may link NaCl-induced elevation of EO to peripheral MBG production. The effects of acute NaCl loading on blood pressure, central and peripheral EO, central and adrenal ATII, plasma norepinephrine and MBG, and Na/K-pump in renal medulla were investigated in 10-week-old DS (55). NaCl loading induced transient peak increases of EO in the hippocampus and pituitary followed by a transient increase in pituitary ATII, increases in plasma NE and adrenocortical ATII levels, a sustained increase in MBG excretion, a 45% inhibition of the renal Na pump, and a 35 mmHg rise in arterial pressure. Pretreatment of rats with anti-ouabain or anti-MBG polyclonal antibodies prevented the NaCl-induced pressor response and renal Na pump inhibition. Anti-ouabain antibody pretreatment also prevented the increases in pituitary and adrenal ATII, and reduced MBG production. Anti-MBG antibody, in contrast, did not affect levels of central EO or ATII. In adrenocortical cells from DS, 1 nmol/L ATII, in a losartan-sensitive manner, doubled MBG production. Thus, in response to NaCl loading, brain EO stimulated adrenocortical MBG via AT1 receptor signaling, and MBG inhibited the renal Na pump and elevated blood pressure, i.e., it behaved like a natriuretic hormone. This consequence of events is schematically presented in Figure 2. The relevance of this hypothesis to the pathogenesis of the other forms of NaCl-induced hypertension remains to be proven.

7. CONCLUSIONS

Despite many controversies and periods of skepticism, the development of the "concept of natriuretic hormone" as a factor in pathogenesis of hypertension has not only fulfilled the initial intuition of deWardener, Blaustein and the others (3, 4, 56) but has substantially exceeded it. As concerns natriuretic hormones per se, it is becoming clear that there are several endogenous factors which, in a complex manner, interact with each other on multiple levels and account for the effects of the putative natriuretic hormone. The most recent studies indicate that both cardenolides and bufadienolides are involved in the regulation of tissue growth (57, 58), apoptosis (59), and immune function (60). Thus, the roles of natriuretic hormones clearly go far beyond regulation of fluid volume, natriuresis and vascular tone. Investigation of these novel functions of the endogenous digitalis-like inhibitors of the sodium pump hopefully will help to resolve the controversial issues on the "concept of natriuretic hormone"

8. ACKNOWLEDGEMENTS

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